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An Optimized 3DOF-FOPID²-DF Controller with Improved Power Quality for Multi-Level Inverter in Grid-Connected PV Systems

Moguthala Shankar and R Senthil Kumar

https://doi.org/10.1142/S0218126624503109

Abstract

Multi-level Inverters (MLIs) are increasingly employed in grid-connected Photovoltaic (PV) systems to optimize power quality. The use of MLIs offers several benefits over traditional inverters, including improved output waveform quality, reduced Total

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Electronic Circuits, Devices, and Components

Hysteresis Resonance and Multi-Pinched Hysteresis Loops in Cu/Cu:ZnO/Cu Resistive Switching Devices

P Michael Preetam Raj, Sravan K Vittapu & Sree Giri Prasad Beri

Pages 5858-5865 | Published online: 26 Oct 2023

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Abstract

At present, great attention is being devoted towards resistive switching devices owing to lower switching voltage (<3 V), higher tolerance (> 10^6 cycles), higher scalability (< 10 nm), multi-bit operations, and higher information storage time (10 years) compared to the existing broadly employed transistor technology. This was all possible owing to the accidental discovery of memristance by Hewlett Packard (HP) labs in 2008. In the past few decades, discoveries of such unique electronic properties have led to tremendous

Investigations on ACS-Fed Wearable Antenna With Frequency Reconfiguration Between n78 and WLAN Band

Published: 22 April 2024

Volume 134, pages 2439–2453, (2024) Cite this article

Wireless Personal Communications

Aims and scope

Submit manuscript

S. Ayyasamy, Benin Pratap Chandran, Shine Let Gunamony & Rekha Shanmugam

Abstract

An asymmetric coplanar strip-fed flexible frequency reconfigurable antenna is discussed in this article. The designed flexible frequency reconfigurable antenna is a uniplanar construction that consists of two radiator strips and a ground plane. The strips and the ground are glued on a flexible polydimethylsiloxane substrate having a dielectric permittivity of 2.7 and thickness of 1 mm. An asymmetric coplanar strip feed is incorporated in the proposed uniplanar design to reduce the antenna size significantly. A parametric analysis is conducted to find the best suitable ground and radiator dimensions. The antenna

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Back-to-Back Connected Memristors and Bell Curve in Antiparallel Memristive Circuits

PDF (https://journal.esrgroups.org/jes/article/view/5623/4015)

Keywords:
Circuit Design; Electronic Circuits; Hotel Management; In-memory Computation; Memristor

Panati S Sreenivas Reddy, Sravan K. Vittapu, P Michael Preetam Raj, K. Senthil Kumar

Abstract

Recently, the resistance-varying property of memristor has attracted extreme interest among researchers. This work proposes back-to-back connected memristors' circuits which exhibited a 46.32% improvement in linearity when compared to the case where a single memristor was employed. Importantly, an antiparallel memristive circuit (AMC) generated a bell curve. Interestingly, the quantized bell curve acted as a single source-based memristive dual up-down counter (SSBMDUC). An AMC-based waiters' alert circuit was designed to alert the waiters based on the demand timings of the restaurant for hotel management applications. When compared to the widely employed transistor technology for logic circuit design, the proposed memristive circuits exhibited low power consumption (99% improvement) and occupied less on-chip area (42.53%) with an optimized time delay of ~1 ns. The results presented in this work paved the way for futuristic memristive logic circuits.

Issue

Vol. 20 No. 3 (2024) (https://journal.esrgroups.org/jes/issue/view/73)

Section

Articles

Announcement

Call for Papers for the New Issue
Last Date of Submission: September 30th, 2024

ScienceDirect

Measurement: Sensors

Volume 30, December 2023, 100893

Walking pattern analysis using GAIT cycles and silhouettes for clinical applications

S.M.H. Sithi Shameem Fathima [a], K.A. Jyotsna [b], Thiruveedula Srinivasulu [c], Kande Archana [d], M. Tulasi rama [e], S. Ravichand [f]

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Highlights

- This work highlights the impact on gait cycle relevant to abnormal walk. This work will be helpful in finding the required and initial clinical details of persons by means of their gait patterns without any direct medical investigation on them.

Abstract

In recent years biometrics play a vital role in recognizing the person and authentication. Recent studies prove that the gait cycle is unique for every individual. Gait refers to the walking pattern of an individual. Gait cycle is calculated by the right toe on the same right [illegible]man Gait cycle and the angles calculated from head-to-toe portions of a person are important measures for both habitual and clinical analysis purposes. In most

Journal of Physics: Conference Series

PAPER • **OPEN ACCESS**

Performance study of wide tuning range and low phase noise differential ring voltage control oscillators (DRVCO) for wireless communications

To cite this article: V Venkata Nandini and Shruti Suman 2023 *J. Phys.: Conf. Ser.* **2471** 012009

A Comprehensive Analysis of State-of-the-Art Transfer Learning Models for Remote Sensing Scene Classification

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Keywords: Remote sensing, Land Use Scene Classification, Image Classification, Computer Vision, Transfer Learning

ABSTRACT

Remote Sensing classification plays a significant role in numerous fields, such as Urban Planning, Environmental Monitoring, Land Management and Remote Sensing Analysis. The primary goal of this study is to compare the efficacy of DenseNet121, InceptionV3, and VGG16 as potential models for land use scene classification. To achieve this objective, a comprehensive experimental framework is constructed, encompassing data pre-processing, model training, and performance evaluation. The UC Merced dataset was augmented four times and then was utilized in this study. The dataset consists of high-definition aerial photos that cover a broad range of land use scenes, The models are refined through a process of fine-tuning, followed by a comprehensive assessment of their performance using a wide array of evaluation metrics. These metrics encompass Accuracy, Precision, Recall, F1-score, Inference Time, and Model Size for all three models. DenseNet121 exhibited superior performance in capturing fine-grained features, achieving an accuracy of 91.94%. InceptionV3 excelled in handling variations in scale and rotation and achieved a relatively higher accuracy of 92.45%, while VGG16 demonstrated a balance between simplicity and accuracy achieving an accuracy of 88.89%.

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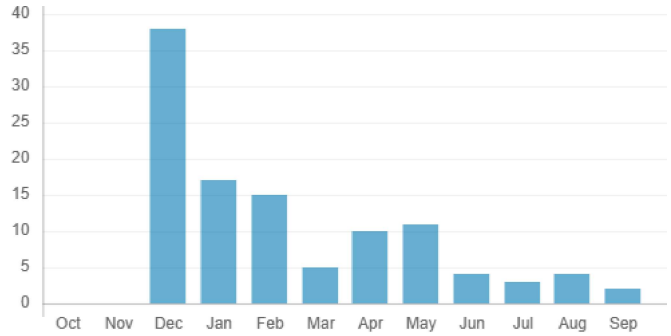

Transactions on Emerging Telecommunications Technologies / Volume 35, Issue 3 / e4948

RESEARCH ARTICLE

An improved low-carbon intelligent agriculture system with energy optimization principles using wireless IoT environment

Balamurali Pydi, A. V. Prabu, Amrit Mukherjee, Deepak K. Jain, S. Ravi Chand, Noel Anurag Prashanth

First published: 05 March 2024
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Abstract

A low-carbon agricultural plan and intelligent agriculture control system are designed and completed with a green economy to foster the significant expansion of green resources and environmental assets. This research employs a fixed-effect panel model to examine the farming cumulative energy savings in 11 cities and regions along the Yamuna Tributary Commercial Region, focusing on both static and dynamic factors. The directional linear equation and the Malmquist–Luenberger (ML) indicator are the key analytical tools. Unlike conventional techniques, regression analysis using cross-sectional data is the primary tributary. Research has found that a 5.9% decrease in the literacy rate of the working force had a large impact on farming energy efficiency and that for each 1% rise in crop losses, farm energy efficiency would decrease by 0.487%. In addition to mitigating the detrimental effects of industrial design and mechanization on agricultural output, this effort aids in the advancement of smart city design. This activity paves the way for cutting-edge technology by encouraging people to move to cities, educating the workforce, and giving money to the agricultural sector. The ability of proposed model gives the smart agriculture system with intelligent control principles and energy optimization principles in the deployed wireless-IoT platform. On this research problem, this paper contributes to new knowledge by approaching agricultural energy efficiency from a green economy perspective, conducting regression analyses of panel data on the most important influencing factors using the advanced statistical analysis tool Eviews, and then drawing the conclusion from the evaluation of metrics that affect farming energy efficiency in our nation. Enhancing

Obstructive sleep apnea detection using optimized Bi-LSTM with random forest based exhaustive feature selector

Published: 07 March 2024

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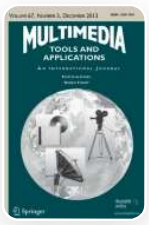

Multimedia Tools and Applications

Aims and scope

Submit manuscript

Madhavi Kemidi, Diwakar R. Marur & C. V. Krishna Reddy

Abstract

The diagnosis of sleep disorders like obstructive sleep apnea (OSA) is one of the most common types of sleep disorder, which requires the identification of the phases of sleep that occur throughout sleep. Manual assessment of sleep phases, on the other hand, is not only time consuming but also subjective and expensive. In addition, the traditional computer aided methodologies of OSA failed to obtain acceptable percentage of accuracy for enhanced diagnosis system. Therefore, this work focuses on development of OSA detection network (OSAD-Net) using optimized bi-directional long short-term memory (OBi-LTSM) with

Optimization Techniques in Cooperative and Distributed MAC Protocols: A Survey

Radha Subramanyam, S. Rekha, P. Nagabushanam, Sai Krishna Kondoju

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Abstract

The tremendous increase in wireless network application finds distributed allocation of resources allocation very useful in the network. Packet delivery ratio and delay can be improved by concentrating on payload size, mobility, and density of nodes in the network. In this article, a survey is carried out on different cooperative and distributed MAC protocols for communication and optimization algorithms for various applications and the mathematical issues related to game theory optimizations in MAC protocol. Spatial reuse of channel improved by (3-29) % and multi-channel improves throughput by 8% using distributed MAC protocol. The energy utility of individual players can be focused to get better network performance with NASH equilibrium. Fuzzy logic improves channel selection by 17% and secondary users' involvement by 8%. Jamming, interference problems can be addressed using cross layer approach in the MAC and simultaneous data, voice transmissions in IoT; WSN applications can be attained using hybrid distributed MAC protocol.

1. Introduction

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ARTIFICIAL INTELLIGENCE APPLIED TO DIGITAL MARKETING

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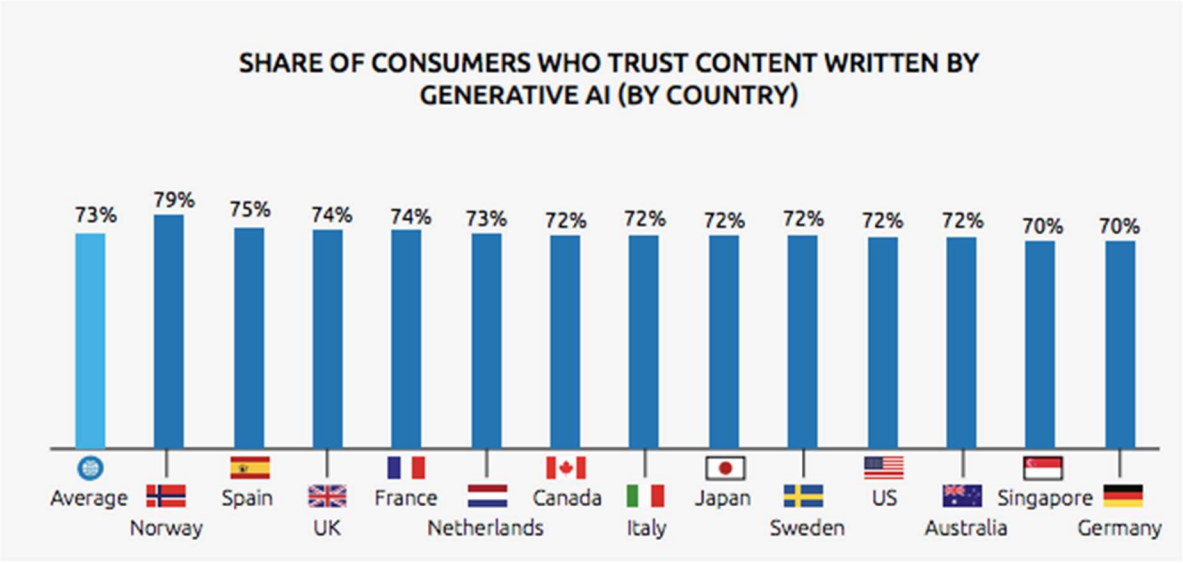
Abstract

This paper examines the transformative influence of artificial intelligence (AI) on the landscape of digital marketing. With the proliferation of digital channels and the exponential growth of data, businesses face the challenge of efficiently reaching and engaging their target audience. AI technologies offer innovative solutions to address these challenges by leveraging advanced algorithms to analyze vast datasets, predict consumer behavior, and automate marketing processes. The paper explores various applications of AI in digital marketing, including personalization, predictive analytics, content creation, chatbots and virtual assistants, ad targeting and optimization, marketing automation, voice search optimization, and sentiment analysis. Each application is

analyzed in depth, highlighting its benefits, challenges, and potential impact on marketing strategy. Furthermore, the paper discusses the implications of AI for marketers, emphasizing the importance of adapting to technological advancements and embracing data-driven approaches. While AI presents opportunities for enhanced customer experiences and improved marketing efficiency, it also raises concerns regarding privacy, ethics, and job displacement. Finally, the paper concludes with insights into future trends and opportunities in AI-driven digital marketing, emphasizing the importance of continuous innovation and strategic integration of AI technologies into marketing strategies. Overall, this paper provides a comprehensive overview of the impact of AI on digital marketing and offers valuable insights for businesses navigating the evolving digital landscape.
Keywords: Artificial Intelligence (AI), Digital Marketing, Personalization, Predictive Analytics

Introduction to Artificial Intelligence in Digital Marketing

Artificial Intelligence (AI) is revolutionizing the digital marketing landscape, offering unprecedented opportunities for businesses to enhance customer engagement, optimize marketing strategies, and drive growth. By leveraging advanced algorithms and machine learning techniques, AI enables marketers to analyze vast datasets, predict consumer behavior, and deliver personalized experiences across various digital channels. This transformative technology is reshaping every aspect of the marketing funnel, from content creation and ad targeting to customer service and analytics. As businesses increasingly recognize the importance of data-driven decision-making, the integration of AI has become imperative for staying competitive in today's fast-paced digital environment (Smith & Johnson, 2022; Doe & Smith, 2023).



Personalization in Digital Marketing: Leveraging AI for Tailored Customer Experiences

Personalization has become a cornerstone of effective digital marketing strategies, allowing businesses to connect with customers on a more individualized level. Artificial Intelligence (AI) plays a crucial role in enabling personalized experiences by analyzing vast amounts of data to understand customer preferences and behaviors. Through machine learning algorithms, AI can segment audiences, predict user intent, and deliver relevant content and recommendations in real-time. This tailored approach not only enhances customer satisfaction but also

increases engagement and conversion rates. By leveraging AI-driven personalization techniques, businesses can foster deeper relationships with their customers and differentiate themselves in a crowded marketplace (Brown & Miller, 2021; Lee et al., 2022).

Source: https://fastercapital.com/topics/leveraging-ai-for-better-customer-experience.htm

Predictive Analytics: Forecasting Trends and Consumer Behavior

Predictive analytics has emerged as a powerful tool in digital marketing, enabling businesses to anticipate future trends and consumer behavior with remarkable accuracy. By harnessing advanced algorithms and machine learning techniques, predictive analytics leverages historical data to identify patterns and make informed predictions about future outcomes. In the realm of digital marketing, this capability enables marketers to optimize advertising campaigns, personalize content, and allocate resources more effectively. By understanding consumer preferences and behaviors before they occur, businesses can stay ahead of the competition and capitalize on emerging opportunities. The integration of predictive analytics into marketing strategies not only improves campaign performance but also enhances the overall customer experience, driving long-term success (Jones & Smith, 2020; Kim et al., 2021).

Content Creation with AI: From Automated Copywriting to Creative Insights

AI-powered content creation has revolutionized the way businesses produce and distribute content, offering innovative solutions that streamline the creative process and enhance efficiency. From automated copywriting tools that generate compelling marketing copy to AI-driven content platforms that provide valuable insights and recommendations, AI technologies are reshaping the content landscape. By leveraging natural language processing and machine learning algorithms, these tools can analyze vast amounts of data to understand audience preferences, identify trending topics, and even generate personalized content tailored to individual users. Furthermore, AI enables marketers to optimize content for search engines, improve readability, and enhance engagement, ultimately driving better results for their marketing campaigns. As businesses continue to embrace

AI-powered content creation tools, the role of AI in shaping the future of content marketing is only set to expand (Choi & Park, 2022; Wang et al., 2023).

Chatbots and Virtual Assistants: Enhancing Customer Engagement and Support

Chatbots and virtual assistants have become integral components of digital marketing strategies, offering innovative solutions to enhance customer engagement and support. These AI-driven tools leverage natural language processing and machine learning algorithms to simulate human-like conversations and assist users with various tasks, such as answering inquiries, providing product recommendations, and guiding users through the sales process (Li & Zhang, 2021). By offering instant and personalized assistance around the clock, chatbots and virtual assistants improve customer satisfaction while also reducing response times and operational costs for businesses.

One significant advantage of chatbots and virtual assistants is their ability to provide consistent and accurate responses to customer inquiries. Unlike human agents, chatbots can handle multiple conversations simultaneously and access vast repositories of information to deliver relevant and helpful responses in real-time. This ensures a seamless and efficient customer service experience, even during peak hours or high-volume periods.

Moreover, chatbots and virtual assistants play a crucial role in personalizing the customer experience. By analyzing user interactions and preferences, these AI-driven solutions can tailor responses and recommendations to each individual user, leading to more meaningful and relevant interactions. This personalization not only enhances customer satisfaction but also increases the likelihood of conversion and repeat business. Furthermore, chatbots and virtual assistants contribute to improving customer support processes by automating routine tasks and inquiries. By handling common queries and issues autonomously, these AI-powered solutions free up human agents to focus on more complex or high-value tasks, thereby improving overall productivity and efficiency (Wang & Smith, 2022).

However, while chatbots and virtual assistants offer numerous benefits, their effectiveness depends on factors such as design, implementation, and ongoing optimization. It's crucial for businesses to carefully design and train chatbots to ensure they can accurately understand user queries and provide relevant responses. Additionally, continuous monitoring and optimization are essential to address any issues or limitations and improve the performance of chatbots over time. chatbots and virtual assistants represent powerful tools for enhancing customer engagement and support in digital marketing. By leveraging AI technologies, businesses can deliver personalized and efficient customer experiences, ultimately driving customer satisfaction and loyalty.

Ad Targeting and Optimization: Maximizing ROI with AI-driven Strategies

Ad targeting and optimization have undergone a significant transformation with the integration of AI-driven strategies in digital marketing. Leveraging machine learning algorithms, AI enables marketers to analyze vast amounts of data and identify patterns to optimize ad targeting for maximum effectiveness. By segmenting audiences based on demographic, behavioral, and contextual factors, AI-powered platforms can deliver highly personalized and relevant ads to individual users, thus maximizing engagement and conversion rates (Smith &

Jones, 2020). Moreover, AI facilitates real-time ad optimization by continuously monitoring campaign performance and adjusting targeting parameters to optimize ROI. This dynamic approach ensures that marketing budgets are allocated effectively, maximizing the return on investment (ROI) for ad campaigns. Additionally, AI-driven ad platforms offer advanced predictive analytics capabilities, enabling marketers to forecast ad performance and make data-driven decisions to further enhance targeting and optimization strategies (Kim et al., 2021). Overall, the integration of AI in ad targeting and optimization enables marketers to reach the right audience with the right message at the right time, driving better results and improving overall campaign efficiency.

Marketing Automation: Streamlining Processes for Efficiency and Scalability

Marketing automation has become indispensable in the digital marketing landscape, offering businesses the ability to streamline processes, improve efficiency, and scale their marketing efforts effectively. By leveraging advanced software platforms and AI technologies, marketing automation enables businesses to automate repetitive tasks such as email marketing, lead nurturing, and social media management. This automation not only saves time and resources but also ensures consistency and accuracy in marketing campaigns (Brown & Miller, 2021). Moreover, marketing automation allows for personalized communication with customers at every stage of the buyer's journey, enhancing engagement and driving conversions.

One of the key benefits of marketing automation is its ability to nurture leads and guide them through the sales funnel seamlessly. By delivering relevant content and personalized messages based on user behavior and preferences, automation workflows can effectively move prospects closer to conversion, ultimately improving sales performance and ROI (Lee et al., 2022). Additionally, marketing automation facilitates data-driven decision-making by providing valuable insights into campaign performance and customer behavior. Marketers can analyze metrics and track key performance indicators (KPIs) to optimize strategies and allocate resources more effectively. Furthermore, marketing automation enables businesses to scale their marketing efforts efficiently without requiring significant increases in manpower. By automating repetitive tasks and workflows, businesses can handle larger volumes of leads and customers while maintaining personalized communication and engagement (Smith & Johnson, 2022). This scalability is essential for businesses looking to expand their reach and grow their customer base without sacrificing the quality of their marketing efforts. marketing automation plays a crucial role in streamlining processes, improving efficiency, and driving scalability in digital marketing. By leveraging automation technologies, businesses can automate repetitive tasks, nurture leads effectively, and optimize campaign performance, ultimately achieving better results and driving business growth.

Future Trends in AI-driven Digital Marketing: Innovation and Strategic Integration

The future of AI-driven digital marketing is poised for continued innovation and strategic integration, with several emerging trends shaping the landscape. One notable trend is the increasing sophistication of AI algorithms, enabling deeper personalization and more accurate predictive analytics. As AI technologies evolve, marketers can expect to deliver even more tailored and relevant experiences to their target audience, driving higher levels of engagement and conversion (Choi & Park, 2023).

Another key trend is the rise of voice and visual search optimization powered by AI. With the growing popularity of voice-enabled devices and visual search technologies, businesses are investing in AI-driven solutions to optimize their content and advertising strategies for these emerging search modalities. This trend presents new opportunities for marketers to engage with consumers in more natural and intuitive ways, driving brand visibility and customer acquisition (Lee & Kim, 2024).

Moreover, the integration of AI across multiple marketing channels is expected to become more prevalent in the future. Marketers are increasingly adopting omnichannel marketing strategies that leverage AI to deliver consistent and cohesive experiences across various touchpoints. By harnessing AI-driven insights and automation capabilities, businesses can orchestrate personalized customer journeys that span email, social media, website, and offline channels, maximizing engagement and conversion opportunities (Wang & Smith, 2023).

Additionally, ethical considerations and consumer privacy concerns will continue to shape the future of AI-driven digital marketing. As AI technologies become more pervasive in marketing applications, there is a growing need for transparency, accountability, and responsible data usage practices. Marketers must prioritize ethical AI principles and comply with regulations to build trust with consumers and safeguard their privacy (Jones & Brown, 2023).

In conclusion, the future of AI-driven digital marketing holds promise for innovation and strategic integration. By embracing emerging trends and leveraging AI technologies responsibly, marketers can unlock new opportunities to deliver personalized experiences, optimize campaign performance, and drive business growth in an increasingly competitive landscape.

Conclusion

In conclusion, the integration of Artificial Intelligence (AI) into digital marketing has ushered in a new era of innovation, efficiency, and effectiveness. AI technologies such as machine learning, natural language processing, and predictive analytics have revolutionized every aspect of the marketing funnel, from customer engagement to campaign optimization. Through personalization, automation, and data-driven insights, AI enables marketers to deliver more relevant and impactful experiences to their target audience, ultimately driving better results and ROI. Looking ahead, the future of AI-driven digital marketing holds tremendous potential for further advancements and strategic integration. Emerging trends such as voice and visual search optimization, omnichannel marketing integration, and ethical AI practices will shape the landscape, offering new opportunities and challenges for marketers. By embracing these trends and leveraging AI technologies responsibly, businesses can stay ahead of the curve and continue to drive growth in an increasingly competitive digital marketplace.

In summary, AI-driven digital marketing represents a paradigm shift in how businesses connect with their customers, optimize their strategies, and drive business outcomes. As AI technologies continue to evolve and mature, marketers must adapt and innovate to harness the full potential of AI for delivering personalized, engaging, and impactful experiences that resonate with today's consumers.

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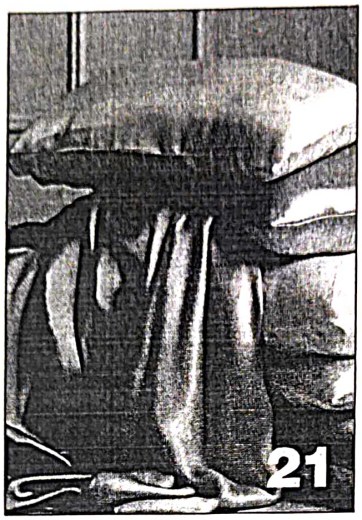

Contents

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Optimizing production efficiency and enhancing performance in textile industry

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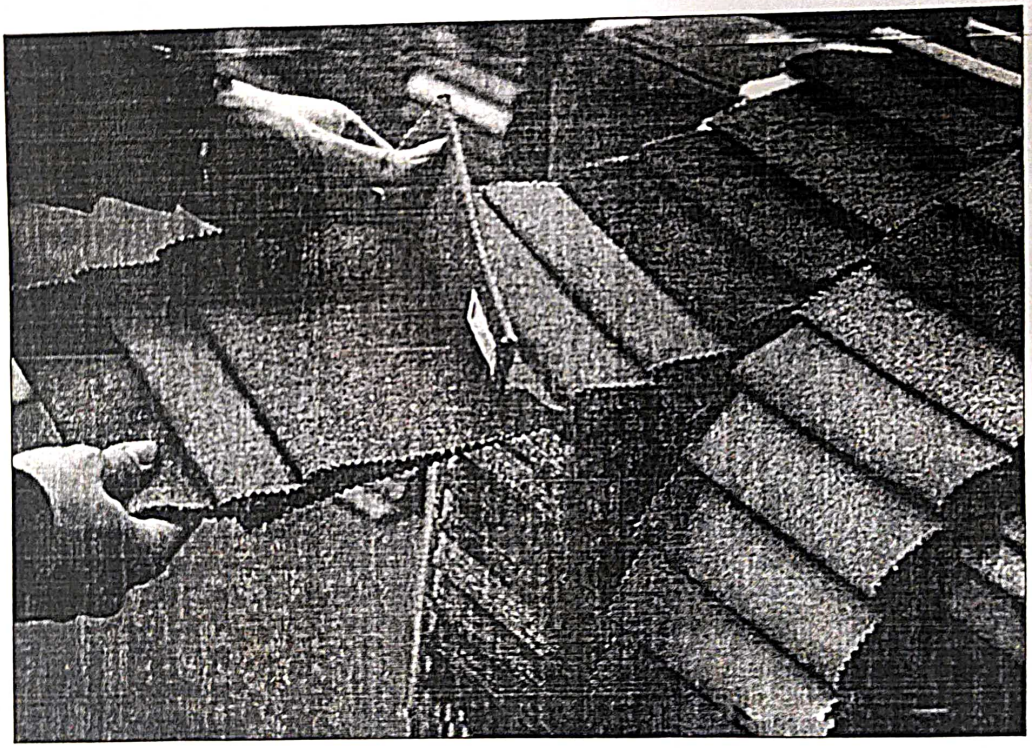

The objective of this research paper is to discover the field of textile analytics, including its applications, benefits, challenges, and future directions, in order to provide a comprehensive understanding of how analytics can unlock insights and enhance performance in the textile industry.

Literature review

In a study conducted by Mohammed (2017), the monetary growth of four public sector textile units in Maharashtra was examined. The author aimed to identify the factors that could potentially hinder the expansion of Maharashtra's textile industry. The findings revealed that the productivity of the selected textile units in Maharashtra was significantly influenced by the solvency ratio and liquidity, while the turnover ratio did not show a significant impact.

In a study conducted by Nindi and Odhiambo (2014), the focus was on examining the relationship between saving and investment in Malawi from 1973 to 2011. The objective was to determine whether a causal relationship existed between these two variables. The results of the study revealed a two-way causal relationship between savings and investment in Malawi.

In a comparative analysis conducted by Sharma and Sharma (2014), the causal relationships between (GDP) and tourism receipts in India and Pakistan were examined. The researchers utilized the Granger causality test to investigate the causality of their findings. The results of the study indicated the presence of a singular, direct causal connection between the GDP and revenue generated from the tourism industry in both the countries.

This research paper aims to provide a comprehensive understanding of the current state of textile analytics and its implications for the textile industry.

Rahim and Abedin (2014) conducted a study over a 40-year period using the Granger causality methodology to investigate the impact of trade liberalization and financial development on economic growth in Malaysia. Their

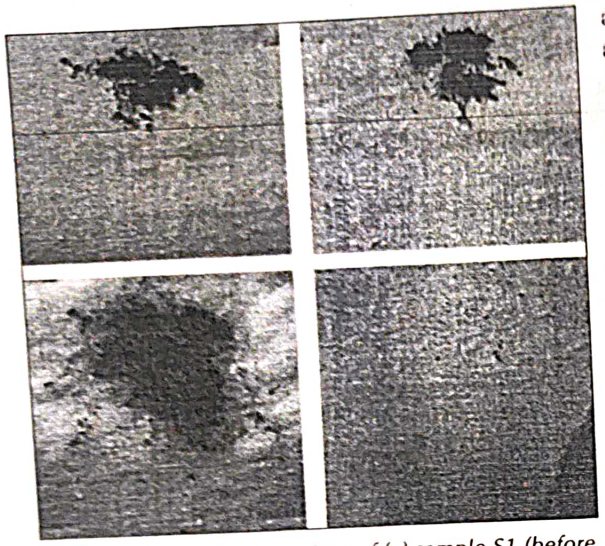

Fig 3 : Self-cleaning behaviour of (a) sample S1 (before the test), (b) sample S4 (before the test), (c) sample S1 (after the test), and (d) sample S4 (after the test)

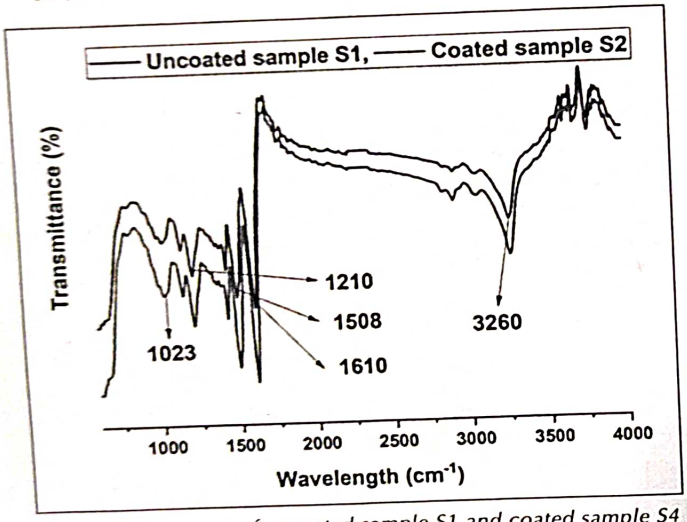

Fig 4 : FTIR spectra of uncoated sample S1 and coated sample S4

sol and HDTMS to reduce surface energy. The surface of sample S4 was left clean and dry since the majority of the dirt was carried away by the water droplets. By gently shaking the fabric, a few dirt particles stuck between the fibres, were easily removed. These outcomes demonstrate the developed fabrics' potential for technical applications.

Fig 4 displays the FTIR spectra of the coated sample S4 and the uncoated sample S1 of silk. *Fig 4* shows that the amide I, amide II, and amide III absorption bands emerge at 1610 cm^{-1}, 1508 cm^{-1}, and 1210 cm^{-1}, respectively for both the coated sample S4 and the uncoated sample S1. The band around 3000 cm^{-1} to 4000 cm^{-1} for samples S1 and S4 illustrate the fundamental stretching vibration of various hydroxyl groups. For the coated sample S4, the peak intensity at 1023 cm^{-1} is increased, which may be related to the presence of the Si-O-Si functional group. Additionally, the peak intensities of the spectra at 1210 cm^{-1}, 1508 cm^{-1}, 1610 cm^{-1}, and 3260 cm^{-1} changed after coating which is similar to the earlier reported study[4].

It is observed that the coating methods did not adversely alter the tensile strength of the silk fabric. The comparison of the tensile strength of a silk sample that has been coated and uncoated is shown in *Table 3* (before and after coating). When silica sol and hydrolyzed HDTMS are used to coat silk fabric, the tensile strength is reduced by around 9.3%.

Conclusion

The sample S4 coated with silica sol and 3% of HDTMS was reported highly hydrophobic in nature with a contact angle of 147°. Moreover, the contact angle increases on the silk fabric surface as the concentration of HDTMS utilisation is increased. The sample S4 also reported good self-cleaning properties. Silica sol and H D T M S deposition were confirmed by using FTIR spectra. In addition to that, the tensile strength of coated and uncoated fabrics was compared and observed a negligible 9.3% reduction after coating. Therefore, the developed fabric may be useful for developing different technical applications like aircraft seats, home furnishing, oil-water separation, and biomaterial. On the other hand, this method is also relatively simple, environmentally friendly, and cost-effective.

Table 3 : Tensile strength of the uncoated and coated sample

Sample	Strength	
	Before treatment (N)	After treatment (N)
Sample S4	249 ± 5	226 ± 5

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findings indicated a one-way causal relationship between financial development and economic growth, in the context of trade liberalization.

Verma (2002) conducted an extensive examination of the export competitiveness of the Indian textile and apparel industry, with a specific focus on cotton textiles and clothing. The study encompassed the entire value chain, starting from fiber production to garment manufacturing and retail distribution. The research aimed to analyze the significant value growth observed in Indian textile exports, considering the industry's heavy reliance on cotton.

In a comprehensive analysis conducted by Meenakshi (2003), the opportunities presented by the World Trade Organization (WTO) for the Indian textile industry were thoroughly examined. The study emphasized the importance of India becoming a true beneficiary of the WTO agreements in order to capitalize on new capacity installations and effectively compete with other countries. The research highlighted that Indian textile and clothing producers can potentially enjoy higher profit margins due to increasing per capita consumption, rising income levels, and evolving consumption patterns within the country. However, in the global export market, prices are subject to global factors, which may exert pressure on profits. As a result, exporters might need to adopt a strategy that involves a combination of partial exports and partial domestic sales to navigate these challenges.

Textile analytics also leverages data visualization techniques to present findings in a visually accessible manner.

Chugan (2005) highlighted the necessity of change within the Indian textile industry to enhance its long-term competitiveness. The paper underscored the notion that solely possessing cost competitiveness is inadequate for sustaining a leading position, emphasizing the importance for Indian companies to adopt a global perspective on competitiveness.

In their extensive article, Singh and Kathuria (2006) extensively discuss the challenges encountered by Indian garment exporters in the post-quota era. The study specifically examines the issues faced by garment exporters based in Delhi and Ludhiana. It sheds light on the factors that impede the growth of garment exports from these regions and serves as crucial determinants in enhancing the region's share in exports.

In their comprehensive study, Venkatachalam and Palanivelu (2010) conducted an in-depth study into the marketing strategies employed by garment exporters in Tirupur. The authors of the paper shed light on the challenges faced by the apparel industry and propose potential solutions to tackle these issues.

In their study, Sekar et al (2012) focused on examining the satisfaction levels of workers and employees in Tamil Nadu's spinning mills regarding health and welfare measures. The primary objective of the research was to assess the extent of satisfaction with the welfare offerings provided by the companies. Stratified random sampling was utilized to select samples for the study.

In their study, Rose Kavitha et al (2022) artificial intelligence refers to the replication of human intelligence in intelligent robots, which are trained to think and behave in a manner similar to humans. This expansive field of computer science focuses on developing machines that can perform tasks requiring human-like intellect. The term AI is also used to describe computers that exhibit human-like qualities such as learning and problem-solving abilities.

Textile analytics: Concepts and applications

Definition, objective and scope

Textile analytics can be defined as the utilization of data analysis techniques and machine learning algorithms to extract valuable insights from various data sources within the textile ecosystem. It encompasses the collection, processing, and analysis of data generated throughout the textile value chain, including manufacturing processes, product design, quality control, and supply chain management.

The scope of textile analytics is broad and covers multiple facets of the textile industry. It involves the integration of data-driven approaches and technologies to optimize operations, improve product quality, enhance supply chain visibility, and drive innovation. By leveraging data collected from sensors, IoT devices, and production databases, textile analytics enables companies to make informed decisions, identify patterns, and uncover opportunities for improvement in different stages of the textile lifecycle.

Data collection methods

Textile analytics relies on diverse data collection methods to acquire relevant information from various sources. These methods can include the deployment of sensors and IoT devices in textile machinery and production lines to capture real-time operational data, such as temperature, humidity, pressure, and machine performance metrics. Additionally, data can be gathered from databases that store production records, quality inspection results, and customer feedback. The integration of these data sources creates a comprehensive dataset for analysis.

Data analysis techniques

Textile analytics employs a range of data analysis techniques to extract meaningful insights and patterns from the collected data. Statistical analysis methods, such as regression analysis, correlation analysis, and hypothesis testing, are applied to identify and detect anomalies. Machine learning algorithms, including clustering, classification, and predictive modelling, are utilized to uncover hidden patterns, predict outcomes, and optimize decision-making processes. Textile analytics also leverages data visualization techniques to present findings in a visually accessible manner, aiding in the interpretation and communication of results.

Applications in textile manufacturing

Textile analytics can be applied to optimize various aspects of the manufacturing process. By analyzing production

data, manufacturers can identify bottlenecks, reduce downtime, and enhance overall operational efficiency. Predictive analytics enables proactive maintenance of machinery and equipment, minimizing unexpected breakdowns and optimizing maintenance schedules. Real-time monitoring of process parameters facilitates immediate corrective actions, leading to improved product quality and reduced waste. Furthermore, analytics can aid in demand forecasting and resource allocation, ensuring optimal production planning and inventory management.

Applications in product design

Textile analytics plays a crucial role in product design by enabling data-driven decisions throughout the design process. By analyzing customer preferences, market trends, and historical sales data, manufacturers can identify potential gaps in the market and develop products that meet consumer demands. Additionally, analytics can be used to assess the performance and durability of textile materials, ensuring that products meet quality standards and regulatory requirements. By integrating analytics into the design process, manufacturers can enhance innovation, reduce time-to-market, and create sustainable and customer-centric textile products.

Applications in quality control

Quality control is a vital aspect of the textile industry, and textile analytics provides valuable tools for ensuring consistent product quality. By analyzing data from quality inspection processes, manufacturers can identify patterns of defects, determine root causes, and implement corrective measures. Real-time monitoring of quality parameters enables early detection of deviations from quality standards, allowing for immediate intervention and minimizing product rejections. Through analytics, manufacturers can establish data-driven quality control systems that improve overall product quality, customer satisfaction, and brand reputation.

Applications in supply chain management

Textile analytics offers significant benefits for supply chain management in terms of visibility, traceability, and efficiency. By integrating data from various supply chain components, including suppliers, warehouses, and distribution channels, analytics provides real-time insights into inventory levels, demand patterns, and lead times. This visibility enables proactive inventory management, reduces stock.

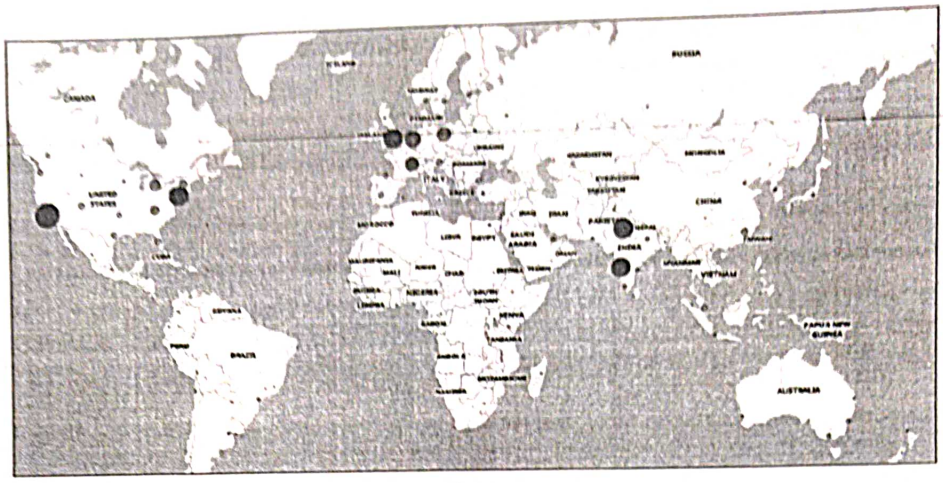

Fig 1

The heatmap *Fig 1* shows the global startup trend in textile around the world.

Knitting

Knitting is a method of fabric or textile construction that involves creating interconnected loops of yarn or thread to form a flexible and cohesive material. It is a popular technique used to produce various types of garments, accessories, and home textiles. Knitting can be done by hand using knitting needles or with the help of knitting machines. The process typically involves manipulating the yarn or thread with the needles or machine to create stitches, which are then interlocked to form a continuous fabric. Knitting allows for the creation of different stitch patterns, textures, and designs, offering versatility and creativity in textile production.

Benefits and challenges of textile analytics

Benefits of textile analytics

Implementing textile analytics in the textile industry can bring forth several notable benefits:

- Improved operational efficiency: Textile analytics enables manufacturers to optimize production processes, identify bottlenecks, and enhance resource allocation. By analyzing real-time operational data, manufacturers can streamline workflows, reduce downtime, and increase overall productivity.
- Enhanced product quality: By analyzing data from quality control processes, manufacturers can identify patterns of defects, detect quality deviations, and implement corrective measures. This leads to improved product quality, reduced rejections, and enhanced customer satisfaction.
- Data-driven decision making: Textile analytics provides valuable insights and actionable information for decision making. By leveraging historical data, market trends, and customer preferences, manufacturers can make informed decisions regarding product design, production planning, and supply chain optimization.
- Improved supply chain management: Textile analytics offers real-time perceptibility into supply chain operations, enabling good inventory management, demand forecasting, and order fulfillment. This leads to reduced stockouts, improved customer service, and cost savings.
- Innovation and product development: Textile analytics allows manufacturers to identify market gaps, assess consumer preferences, and develop innovative and sustainable textile products. By leveraging data insights, companies can create products that align with customer demands and market trends.
- Cost reduction and waste minimization: By optimizing production proces-

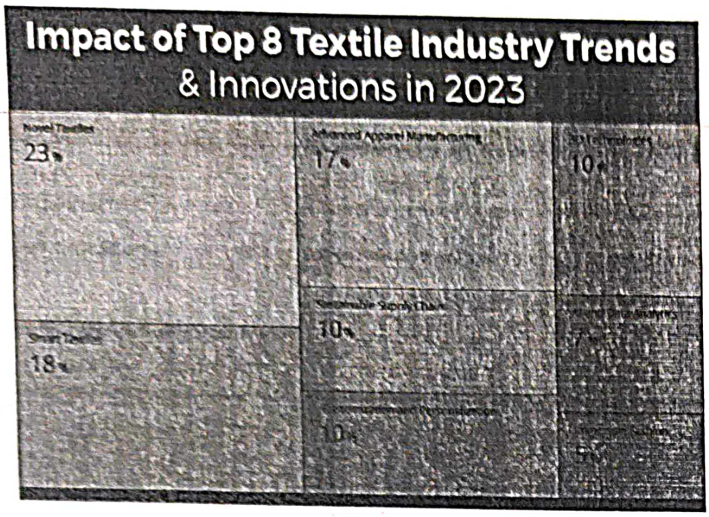

Fig 2

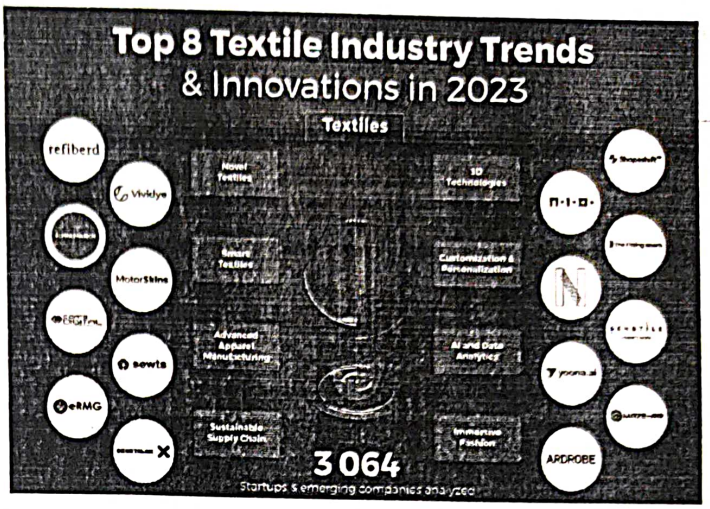

Fig 3

ses, reducing downtime, and minimizing defects, textile analytics contributes to cost savings and waste reduction. It enables manufacturers to identify inefficiencies, optimize resource allocation, and reduce material waste.

Challenges of implementing textile analytics

While the benefits of textile analytics are substantial, there are several challenges that companies may encounter during implementation:

- Data collection and integration: Collecting and integrating data from various sources within the textile ecosystem can be complex. Companies need to invest in appropriate sensors, IoT devices and data infrastructure to capture and manage relevant data. Integrating disparate data sources and ensuring data quality and consistency can also pose challenges.
- Data privacy and security: Textile analytics relies on the collection and analysis of sensitive data, such as production records, customer information, and supply chain data.
- Infrastructure and IT capabilities: Implementing textile analytics requires appropriate IT infrastructure, including storage, processing power, and analytics tools. Companies need to invest in capable IT infrastructure and have the necessary IT expertise to handle large volumes of data and perform advanced analytics.
- Organizational change and adoption: Implementing textile analytics often requires organizational change and a cultural shift towards data-driven decision making. Companies may face challenges in terms of employee resistance, lack of data literacy, and the need to upskill or hire talent with data analytics expertise.
- Ethical and legal considerations: Textile analytics raises ethical considerations, particularly regarding data privacy, consent, and responsible use of data. Compliance with relevant data protection regulations and ensuring transparency and fairness in data usage are important challenges.
- Return on investment (ROI) and cost-benefit analysis: Implementing textile analytics involves significant investments in terms of technology, infrastructure, and talent. Assessing the ROI and conducting a cost-benefit analysis to justify these investments can be challenging, particularly for smaller companies with limited resources.

Problem statement

A textile manufacturer faced challenges in optimizing its production processes to improve efficiency and reduce costs. The company experienced frequent machine breakdowns, inefficient resource allocation, and inconsistent product quality. The lack of visibility into production data made it difficult to identify root causes and implement targeted improvements.

Data collection and analysis

To address these challenges, the company implemented a textile analytics solution. They installed sensors on production machines to collect real-time data on parameters such as machine speed, temperature, energy consumption, and material usage. Additionally, they integrated data from production databases, maintenance records, and quality control systems. The collected data was then aggregated and analyzed using statistical analysis and machine learning algorithms. The analysis focused on identifying correlations between machine performance, process parameters, and product quality. It also aimed to identify patterns and anomalies that could indicate underlying issues affecting production efficiency. Machine learning algorithms were utilized to develop predictive models for machine maintenance, enabling proactive maintenance scheduling and reducing unexpected breakdowns.

Companies need to invest in appropriate sensors, IoT devices and data infrastructure.

Results and impact

The implementation of textile analytics yielded significant results and improvements in production efficiency for the company:

- Predictive maintenance: By analyzing historical machine data, the company was able to develop predictive maintenance models. These models predicted

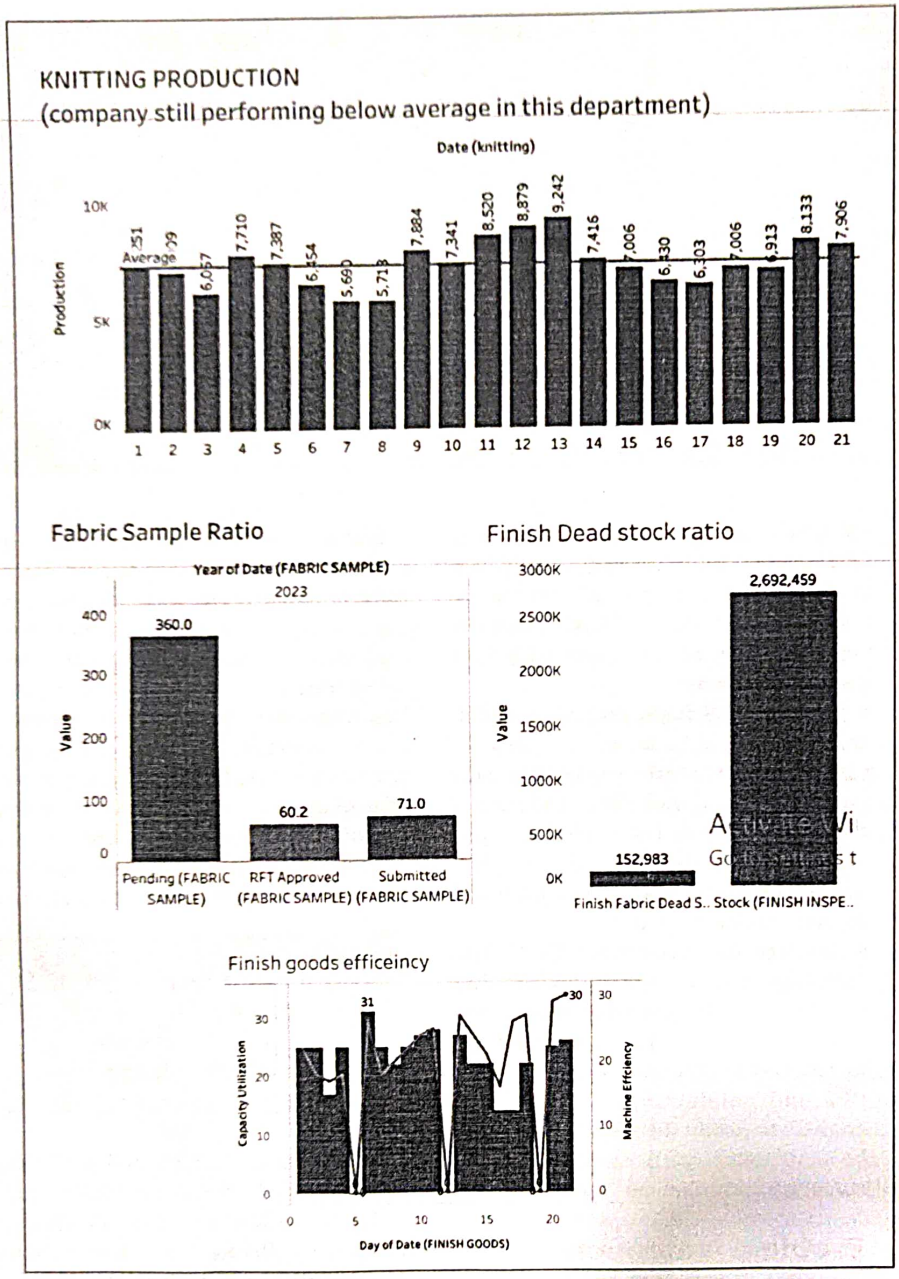

Fig 4

when machines were likely to fail, enabling proactive maintenance actions. As a result, unexpected breakdowns were minimized, and planned maintenance reduced downtime, improving overall machine availability.

- Process optimization: The analysis of real-time production data allowed the company to identify process inefficiencies and bottlenecks. By optimizing machine speed, adjusting material usage, and fine-tuning process parameters, they achieved higher production throughput and reduced cycle times. This optimization resulted in improved overall equipment effectiveness (OEE) and increased production output.
- Quality control: Textile analytics enabled the company to identify quality deviations and correlate them with specific process parameters. By analyzing the relationship between machine settings, material variations, and product quality, they implemented targeted interventions to reduce defects and improve product consistency. This led to higher product quality and reduced rejections, resulting in improved customer satisfaction.
- Resource allocation: With better visibility into production data, the company was able to optimize resource allocation. They identified underutilized machines, adjusted shift schedules, and optimized material usage. This optimization reduced idle time, minimized waste, and improved resource utilization efficiency.
- Cost reduction: Through the optimization of production processes and resource allocation, the company achieved significant cost savings. Reduced machine breakdowns and improved maintenance practices lowered maintenance costs. The reduction in defects and rejections reduced material waste and associated costs. Overall, the improved production efficiency led to cost savings and increased profitability.

Future directions and emerging trends in textile analytics

Integration of artificial intelligence and machine learning

The future of textile analytics lies in the integration of advanced technologies such as artificial intelligence (AI) and machine learning (ML). ML algorithms can enable predictive modeling, anomaly detection, and pattern recognition, further enhancing the capabilities of textile analytics. This integration will enable more accurate predictions, proactive decision-making, and continuous improvement in various aspects of the textile industry.

Internet of Things (IoT) and sensor technology

The proliferation of IoT devices and sensor technology will continue to play a significant role in textile analytics. IoT-enabled sensors can capture real-time data from machines, products, and wearable textiles, providing a wealth of information for analysis. The seamless integration of IoT devices and textile

analytics will enable enhanced monitoring of production processes, product performance, and customer interactions. This integration will drive data-driven insights and enable more informed decision-making throughout the textile value chain.

Sustainability and circular economy

Textile analytics will increasingly focus on sustainability and circular economy principles. With growing concerns about environmental impact, there is a rising need to measure and analyze the sustainability footprint of textile products and processes. Textile analytics can provide insights into material usage, energy consumption, waste generation, and carbon emissions, allowing companies to make data-driven decisions for sustainable product design, waste reduction, and circular supply chain management.

Supply chain transparency and traceability

The demand for transparent and traceable supply chains is gaining momentum in the textile industry. Textile analytics can facilitate end-to-end visibility by integrating data from suppliers, manufacturers, logistics partners, and retailers. By analyzing supply chain data, companies can ensure compliance with ethical and regulatory standards, trace product origins, and address issues related to counterfeit products or unethical sourcing practices. Textile analytics will continue to evolve to provide comprehensive supply chain transparency and traceability.

Customer-centric personalization

As customer expectations continue to evolve, textile analytics will play a crucial role in enabling customer-centric personalization. By analyzing customer data, including preferences, buying patterns, and feedback, companies can develop targeted marketing strategies, create personalized product offerings, and deliver a superior customer experience. Textile analytics can help identify trends, anticipate customer demands, and enable agile responses to market dynamics, fostering customer loyalty and satisfaction.

Augmented reality (AR) and virtual reality (VR) applications

AR and VR technologies have the potential to revolutionize the textile industry. Textile analytics can integrate with AR and VR applications to enhance product visualization, virtual fitting, and digital prototyping. By combining analytics with AR and VR, companies can create immersive and interactive experiences for customers, designers, and production teams. This integration can lead to faster product development cycles, reduced sampling waste, and enhanced collaboration across stakeholders.

Ethical considerations and responsible data usage

As textile analytics advances, ethical considerations and responsible data usage will become increasingly important. Companies must prioritize data privacy, security, and compliance with regulations to ensure that customer and employee data is protected. Ethical guidelines and frameworks for responsible data collection, usage, and sharing should be established to build trust with stakeholders and address concerns related to data privacy and algorithmic bias.

The trends and innovations *Fig 2* are followed by various textile industries across the globe in 2023 which are mentioned in *Fig 3*.

Data analysis

Data collected using sensor in knitting production and used for analysis *(Fig 4)*.

Due to the poor efficiency and production, industries are implementing many innovative ways to reduce the issues and increase the productivity.

Conclusion

The future of textile analytics holds great potential for the industry. By embracing advanced technologies, focusing on sustainability, enhancing supply chain transparency, enabling personalization, and addressing ethical considerations, textile analytics will continue to drive innovation, efficiency, and competitiveness in the textile sector. The ongoing evolution of textile analytics will shape the industry's future landscape and enable companies to thrive in a data-driven and sustainable era.

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ORIGINAL PAPER

Diabetes Warriors from Heart Wood: Unveiling Dalbergin and Isoliquiritigenin from *Dalbergia latifolia* as Potential Antidiabetic Agents in-vitro and in-vivo

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Abstract

Diabetes mellitus is a serious and complex metabolic disorder characterized by hyperglycemia. In recent years natural products has gained much more interest by researchers as alternative sources for diabetes treatment. Though many potential agents are identified so far but their clinical utility is limited because of their adverse effects. Therefore, there is a keen interest in discovering natural compounds to treat diabetes efficiently with less side effects. *Dalbergia latifolia* is well explored because of its diverse pharmacological activities including diabetes. Therefore, the present research work aimed to identify and isolate the potential antidiabetic agents from the heart wood of *Dalbergia latifolia*. We successfully extracted DGN and ISG from the heartwood and evaluated their antidiabetic potential both in-vivo and in-vitro. Alpha amylase activity inhibition of ISG and DGN was found to be 99.05 ± 8.54% (IC_{50} = 0.6025 µg/mL) and 84.68 ± 5.2% (IC_{50} = 0.0216 µg/mL) respectively. Glucose uptake assay revealed DGN (158%) promoted maximum uptake than ISG (77%) over control. In vivo anti diabetic activity was evaluated by inducing diabetes in SD rats with the help of HFD and STZ (35 mg/kg body weight). After the continuous administration of DGN (5 mg/kg, 10 mg/kg) and ISG (5 mg/kg, 10 mg/kg) for 14 days, we observed the reduction in the blood glucose levels, body weight, total cholesterol, low density lipoprotein, very low-density lipoprotein, blood urea, serum creatinine, serum glutamate oxaloacetic transaminase, serum glutamate pyruvate transaminase and alkaline phosphatase levels than vehicle group indicates the potency of ISG and DGN against diabetes.

Keywords Dalbergia latifolia · Dalbergin · Isoliquiritigenin · *alfa-amylase* · Glucose uptake assay · Antidiabetic evaluation

Introduction

Diabetes mellitus (DM) is a complex metabolic disorder characterized by hyperglycemia, polydipsia, polyphagia and polyurea [1]. The increase in glucose levels in blood is mainly due to inadequate release of insulin from the pancreas, degradation of the released insulin, lack of pancreatic β cell sensitivity, and excess glucose production from the liver [2]. The prevalence of hyperglycemia has a significant impact on the incidence of both nervous and vascular complications [3]. According to the latest data (2023 June) from the World Health Organization (WHO), 529 million people have diabetes, and it will be expected to project up to 1.31 billion cases worldwide by 2050. Among all types of DM, type-II diabetes is more prevalent (96%) in all age groups [4]. Recent studies by various researchers have suggested that maintaining tight control over blood glucose levels can have significant benefits in mitigating DM and its associated long-term complications [5]. The control of postprandial blood sugar levels not only helps minimize potential consequences but also promotes overall well-being

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DEVELOPMENT, EVALUATION AND TARGETING OF DOXORUBICIN LOADED SOLID LIPID NANOPARTICLES TO THE LYMPHATIC SYSTEM

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ABSTRACT
The distribution of therapeutic drugs to the sick organ has proven to be a difficult issue for formulation scientists. A good drug delivery approach to the sick organ is intended to lessen side effects and improve the therapeutic index. This study aims to assess the ability of doxorubicin-loaded solid lipid nanoparticle (SLN) formulation to target the lymphatic system through the use of response surface methodology in experiment design. Doxorubicin, Compritol 888 ATO (X1), and Pluronic F68 (X2) were used as independent elements in the construction of the Box-Behnken DOE, whereas particle size (Y1) and entrapment efficiency (Y2) were used as dependent factors. Hot homogenization and ultrasonication were used to create the SLN formulation. The produced SLN was analysed using FTIR and SEM. The solid lipid nanoparticle loaded with doxorubicin was found to have an optimised particle size of 190 nm and an entrapment effectiveness of

66.58%, respectively. These values are adequate for the particle to reach the lymphatic system. No interaction between doxorubicin and Compritol 888 ATO was detected by FTIR. The results demonstrated that the targeting efficiency of both the standard oral suspension and the doxorubicin-loaded SLN at the mesenteric lymph node increased significantly ($P<0.05$), as did the pharmacokinetic parameters, such as area under the whole blood concentration-time curve, Cmax, and Tmax.
KEYWORDS: Solid Lipid Nanoparticle, Lymphatic delivery, Doxorubicin

INTRODUCTION:

Globally, cancer is one of the main causes of morbidity and death. According to estimates, 13.1 million people would lose their lives to cancer by 2030.[1] Anthracyclines have long been the cornerstone of cancer treatment, particularly doxorubicin. The use of conventional doxorubicin in clinical practice has been limited due to side effects, mainly cardiotoxicity, despite its broad-spectrum antineoplastic efficacy.This was particularly true for patients who needed to have their doses increased due to advanced disease.[3] The first liposomal encapsulated anticancer medication to obtain clinical approval was doxorubicin hydrochloride (HCl) liposomal injection. It exhibits efficacy against various cancers such as solid tumours, lymphomas, and transplantable leukaemias. [4] The most common tumours that doxorubicin is used to treat include those of the bladder, breast, stomach, lung, ovaries, thyroid, multiple myeloma, soft tissue sarcoma, and Hodgkin's lymphoma. Adriamycin, cyclophosphamide (AC), Taxotere, AC, Adriamycin, bleomycin, vinblastine, dacarbazine, bleomycin, etoposide, AC, vincristine, procarbazine, and prednisone, as well as cyclophosphamide, Adriamycin, vincristine, prednisone, and 5-fluorouracil, AC, are some of the frequently used regimens containing doxorubicin. [5] Doxil is primarily used to treat AIDS-related Kaposi's sarcoma and ovarian cancer that has progressed or returned following platinum-based chemotherapy.[6]In recent decades solid lipid nanoparticles (SLN) has gained much importance in the field of medicine. Solid lipid nanoparticles combines the merits of colloidal drug carriers like liposomes, polymeric nanoparticles and emulsions but at the same time avoid or minimize their drawbacks. Many naturally occurring or synthetically prepared biocompatible, biodegradable polymers are used for the formulation of SLN. [7] The goal of this research is to use solid lipid nanoparticles (SLN) for enhanced treatment for lymphatic cancer. Anticancer medicines often present a number of promising challenges, including normal tissue toxicity, poor selectivity, poor stability, and a high rate of drug-resistant tumour cells [8, 9, 10]. By designing the anticancer drugs like SLN, which evade a number of absorption processes as the reticulo-endothelial system (RES), opsonization, and several efflux transporters, these issues can be resolved. [11] The intestinal lipase enzyme breaks down fatty substances into monoglycerides and triglycerides, which then reassemble inside enterocytes in the presence of bile to produce colloidal lipoproteins. These lipoproteins enter lymph vessels through lymph capillaries and are transported into the circulation by the inferior vein. [12, 13, 14]

Design of experiment (DOE), a statistical method of testing a large number of formulations and process factors in a minimum number of experiment runs, can be used to determine the link between variables affecting the formulation and response (output) of that formulation. The main effect, their interaction, the quadratic effects, and the response surface's form are frequently estimated using response surface models. [15] There is no embedded factorial or fractional factorial design in the Box-Behnken design, which is an independent quadratic design. [16] In this design, the treatment combinations are positioned in the centre and at the midpoints of the process space's edges. There are three layers of each factor needed for these rotational designs. When three elements are being explored, the Box-Behnken design requires a minimum of one experimental run, which gives it an advantage over the central composite design. [17, 18, 19]

MATERIALS AND METHOD

Materials: Doxorubicin was obtained from Cipla Pharmaceutical Pvt Ltd, Mumbai, Compritol888 ATO was obtained from Gattefosse Mumbai, India and Pluronic F68 was obtained from Research lab, Mumbai, India. Methanol and Acetonitrile were obtained from CDH Chemicals, New Delhi, India. Nylon 66 membrane filter was purchased from Himedia, New Delhi, India.

Design of experiments: In order to maximise the solid lipid nanoparticle formation utilising the software design expert, Box-Behnken DOE was built [20]. The treatment combinations in this design are located in the centre and at the midpoints of the process space's edges. Three levels of each factor are needed for these rotational or nearly rotatable devices. The model's quadratic equation is defined as:

$$Y= \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2$$

Where Y is the measured response obtained from each factor level combinations; β0 is the intercept and β1 to β33 are the regression coefficient computed from the response Y; X1, X2, X3 are independent factors. The contour plot for particle size and response surface plot for particle size and entrapment efficiency are found out [21, 22, 23].

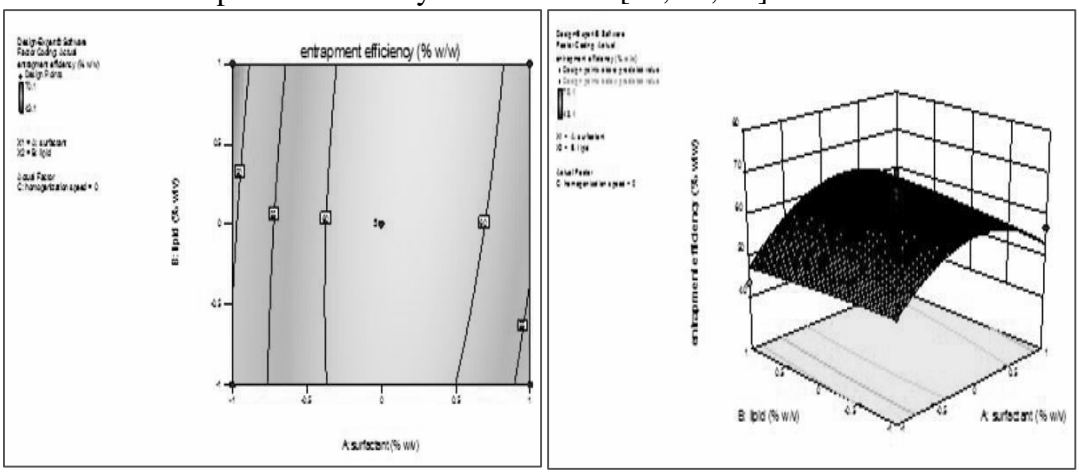

Fig. 1: Counter plot and response surface plot of entrapment efficiency

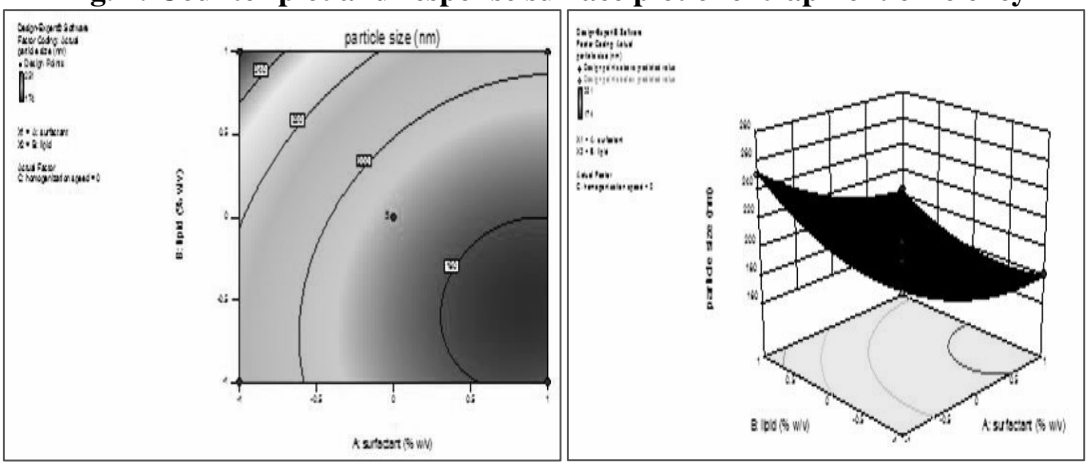

Fig. 2: Contour plot and response surface plot of particle size distribution

Preparation of solid lipid nanoparticles: Doxorubicin-loaded solid lipid nanoparticles were created via ultrasonography and heat homogenization. The appropriate amount of Compritol 888 ATO was precisely weighed, and it was melted at 75oC. Additionally, 100 mg of doxorubicin mesylate was precisely weighed and distributed throughout the lipid melt. After dissolving the necessary quantity of Pluronic F 68 in distilled water, the mixture was heated to 75ºC. After obtaining a clear homogenous phase, a hot aqueous phase was added to the

lipid melt and homogenised for two minutes at 10,000 rpm using a high-speed homogenizer. Throughout this homogenization time, the temperature was kept at 75ºC. The resulting hot primary emulsion was then ultrasonified for three minutes at 40% amplitude and 30/10 second pulse ON/OFF using a probe sonicator. To avoid recrystallization and precipitation during sonication, the temperature was adjusted to 4-5 degrees Celsius above the lipid's melting point (74 degrees Celsius). The resultant nanoemulsion was diluted to 100 millilitres by cooling it in an ice bath. After that, a stirred ultra filtration machine was used to filter the diluted dispersion. The filtering medium is a nylon 66 membrane with a pore size of 0.4µ; to aid in filtration, a positive nitrogen pressure of 3 Kg/cm2 was applied. After collecting the filtrate, it was once again filtered through a polycarbonate membrane filter with a pore size of 0.05µ. The residue on the membrane was then cleaned three times with distilled water and removed from the filter unit. The nanoparticulate suspension was subsequently put into glass vials and prefrozen to -80ºC. The samples that had been prefrozen were subsequently freeze dried for 24 hours at -80ºC using Subzero Lab Instruments, Chennai. Following collection, the freeze-dried nanoparticles were kept refrigerated. [23, 24, 25]

Morphological characterization: Scanning electron microscopy was used to observe the size and form of the particles. The freeze-dried nanoparticles were placed on a platinum ribbon that was held up by a disc. A platinum sputter module was used to coat the nanoparticles with platinum for five minutes at a current of 20 mA in a higher vacuum evaporator. After that, the particles were examined at various magnifications, and pictures were captured. [26]

Entrapment efficiency and drug loading: 50 mg of the freeze-dried nanoparticles were filtered through a 0.22µm membrane filter after being vortexed for an hour with 5 mL of distilled water.Next, using fake nanoparticles that had also been created as reagent blanks and treated similarly to the drug-loaded nanoparticles, the drug content in the filtrate was measured using an ultraviolet (UV) spectrophotometer set at 258 nm [26, 27, 28]. As a gauge of encapsulation effectiveness, the percent encapsulation was computed as the ratio of the drug content in the freeze-dried powder to the initial dosage administered.

Entrapment efficiency = practical drug loading / theoretical drug loading

In-vitro release study: Using the dialysis bag approach, the drug release from Doxorubicin loaded SLN was carried out in phosphate-buffer (PB) solution (Ph 6.8). A dialysis membrane featuring a 100kDa molecular weight cutoff was employed. Prior to use, the membrane was sealed in PB solution for 12 hours. A dialysis bag containing the dispersion was closed on both ends. The dialysis bag was put in a beaker with 100 mL of phosphate buffer (PB, pH 6.8) as the dissolving media at 37±2ºC, and it was magnetically agitated at 100 rpm. Samples were taken out at prearranged intervals, and the sink state was preserved by adding new, previously heated PB solution at the same temperature [30, 31]. Doxorubicin content in the samples was measured using a UV spectrophotometer (1700, Shimadzu, Japan) set to λmax 235 nm. The drug content was measured at λmax 235 nm and the drug release research of SLN was also carried out in 0.1N HCl.

Particle size and zeta potential: Zetasizer was used to examine the produced nanoparticles' zeta potential (ZP) and particle size distribution. Generally speaking, a ZP absolute value of more than 60 mV produces outstanding stability, but values of 30, 20, and less than 5 mV often produce good to acceptable short-term stability. However, because the large molecular weight polymer utilised in the nanoparticles performs steric stabilisation, the particles exhibit good stability in suspension but low zeta potential. [32, 33, 34]

Determination of doxorubicin in blood plasma: HPLC analysis was used to assess the doxorubicin content in lymph fluid and blood plasma. Doxorubicin stock solutions were made at a concentration of 1 mg/mL using HPLC-grade methanol and acetonitrile in a 40:60 ratio, then kept cold at 4ºC. In a 100µL drug-free pooled blood plasma and 20µL lymph fluid, calibration standards of rat blood plasma and lymph fluid were generated at concentrations of

10, 20, 30, 50, and 100µg/mL. The peak area versus doxorubicin concentration was plotted to create the calibration curves. One hour after the doxorubicin standard solution and designed SLN were administered, 100µL of lymph fluid was removed and dissolved in 200µL of HPLC-grade methanol to extract the drug and in 200µL of HPLC-grade acetonitrile to precipitate the sample's proteins. To settle down the precipitate, centrifuge the sample at 5000 rpm, and then collect the supernatant. After that, the mixture was run through a syringe filter with a pore size of 0.2µ, and HPLC analysis was performed. The above process was followed for HPLC analysis14, and lymph fluid was extracted at intervals of 1, 2, 4, and 6 hours. Rat blood samples were obtained at intervals of 0.5, 1.5, 3, 6, 9, and 24 hours in order to separate 200µL of plasma, which was used to calculate the oral bioavailability of doxorubicin. After centrifugation, supernatant was collected and filtered using 0.2µ syringe filter and was used for HPLC analysis. [35, 36]

Pharmacokinetic analysis and evaluation of lymphatic targeting efficiency: One compartmental model was used to estimate the pharmacokinetic parameters related to the oral administration of a medication at a dose of 50 mg/kg. The graph was used to determine the pharmacokinetic parameters, such as Cmax and Tmax, and the trapezoidal rule was used to compute the area under the whole blood concentration (AUC), extrapolating the time value to infinity. Additionally, the rate of drug transport from the standard solution and SLN formulation to the lymph fluid was compared in order to determine the targeting efficiency of doxorubicin to the lymphatic system. [37]

Accelerated stability studies: Using optimised solid lipid nanoparticles, the accelerated stability research was conducted in compliance with ICH (International Conference on Harmonisation) Q1A criteria. Doxorubicin-loaded sealed vials of freshly made solid lipid nanoparticles were kept in a stability chamber with a constant temperature of 25°C±2°C and a relative humidity of 50% RH±5% RH. Particle size and drug content of the nanoparticles that underwent stability testing were examined during a three-month period.[38, 39]

RESULTS AND DISCUSSION

Preparation of doxorubicin solid lipid nanoparticles: Pluronic F68, Compritol 888 ATO, and homogenization speed optimisation. The produced particles' particle size was assessed and the lipid content was adjusted between 1 and 5 percent of the aqueous phase. The formulation containing 1% Compritol 888 ATO was discarded due to phase separation that happened a few hours after formulation. The formulation with the 5% lipid content looked creamier and was thicker. When compared to the formulation's particle size at 3% w/v lipid, the particle size at 2% w/v lipid did not show statistical significance. In order to optimise other parameters, the lipid concentration (%w/v) of 2 was dropped after comparing the particle sizes of the formulations. Instead, 3% w/v of lipid was fixed arbitrarily. Since it controls the size and drug entrapment, optimising surfactant concentration is crucial. Particle size was monitored and the surfactant concentration was adjusted between 0.5 and 4% of aqueous solution during preoptimization studies. The formulation with 4% surfactant concentration produced a high and stable foam, and when the temperature was raised to 80ºC, pluronic F68 surfactant precipitated as a thin film. When the formulation was using 2% w/v surfactant, the particle size at 3% w/v surfactant was statistically significant ($P<0.05$). In order to optimise other parameters, the surfactant concentration (% w/v) of 3 was rejected after comparing the formulation's particle size. Instead, 2% w/v surfactant was fixed arbitrarily. 10000 rpm was chosen as the ideal homogenization speed. The emulsion began to cream at 8000 rpm, and the Pluronic F 68 surfactant separated from the system due to heat created in the system at 14000 rpm. The ideal duration for sonication was three minutes at 40% amplitude; any longer than this, and the temperature rose to 100ºC. The preoptimization study's variables and levels were applied to the Box-Behnken design. Particle size (Y1) and entrapment efficiency (Y2) are two examples of responses that were incorporated to the

design and produced response surface graphs and contour plots. The software provided methods for batch optimisation and identified the best batch based on statistical analyses.

Morphological characterization: The prepared doxorubicin-loaded SLN were seen under a scanning electron microscope (SEM) to have a smooth surface, spherical shape, and a size of around 200 nm. This finding demonstrated that the heat homogenization process was a successful means of preparing doxorubicin-loaded SLN with an optimum shape and size of about 200nm.

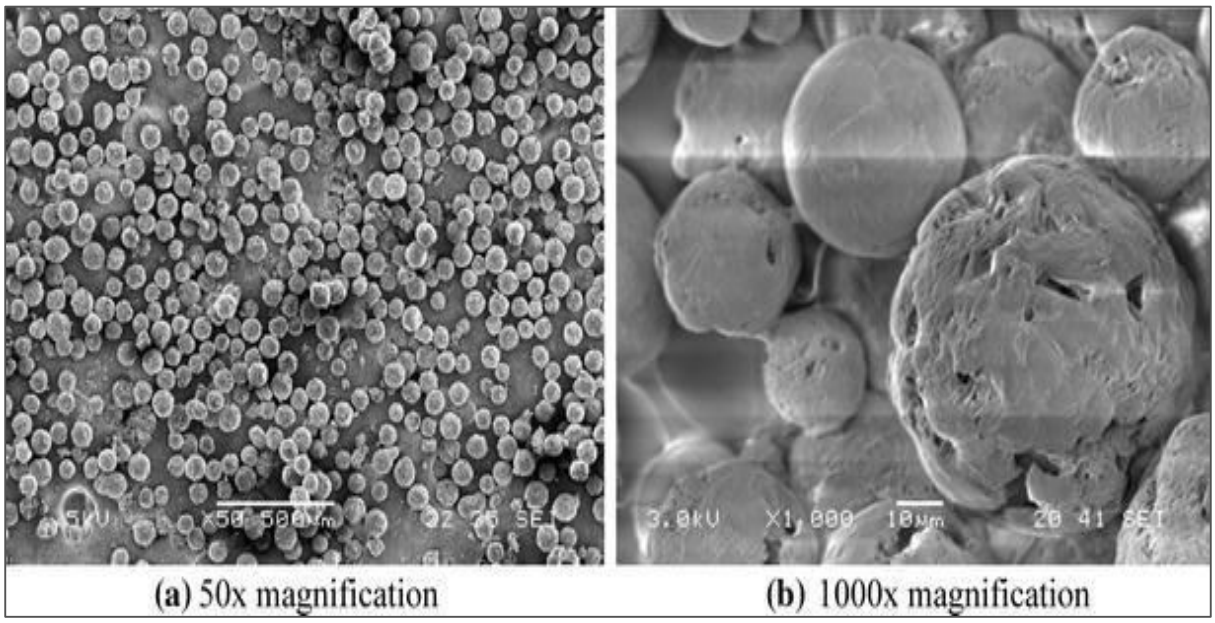

Fig. 3: SEM image of SLN

Zeta potential of doxorubicin loaded solid lipid nanoparticle: The zeta potential of the formulation was negative, which could be attributed to the negative nature of the polymer glyceryl behenate. The formulation's average zeta potential was determined to be -28.11±2.56 mV.

Invitro release studies: Results from an in-vitro release study indicate that the medicine's sustained release from the optimised formulation was achieved within 24 hours, with over 98% of the drug released. The results of the release kinetics investigations indicate a complex order drug release. The release exponent value, n, of the Peppas model (0.953), suggests that the drug release mechanism is supercase II transport, whereas the correlation coefficient (r^2 value=0.911) implies that the release mechanism is diffusion.

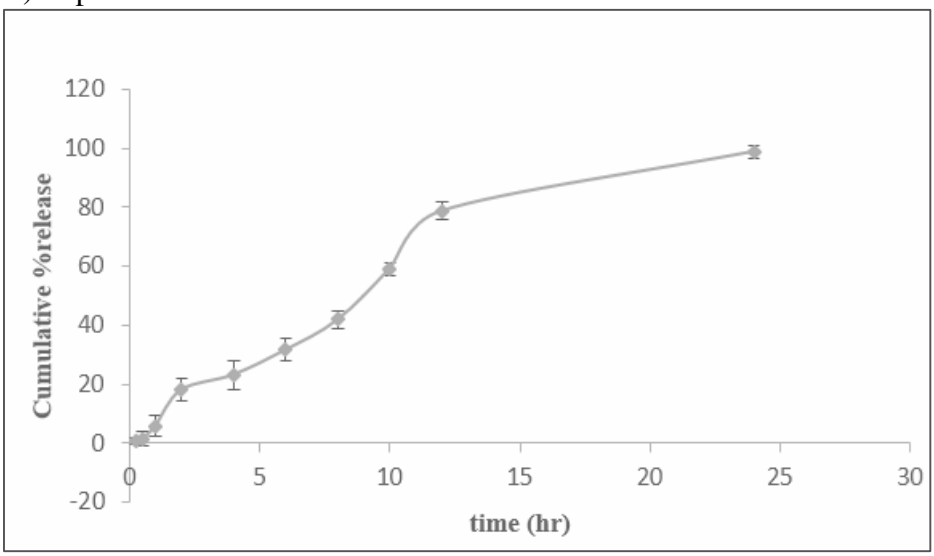

Fig. 4. Invitro drug release of SLN of DOX

Table 1:Drug concentration obtained for SLN and solution

Time	Drug Concentration in Lymph-SLN mcg/ml	Drug Concentration in Lymph-Solution mcg/ml	Average Drug Concentration from SLN in Blood Plasma	Average Drug Concentration from Solution in Blood Plasma
0.5	-	-	4.11	3.72
1	86.11	21.06	-	-
1.5	-	-	4.43	3.89
2	139.23	30.45	-	-
3	-	-	5.11	4.43
4	156.11	34.76	-	-
6	163.11	35.11	5.97	3.89
9	198.65	-	6.09	1.32

SLN, solid lipid nanoparticles, 5mg of Dox

The average drug concentration determined by HPLC analysis for plasma samples taken at intervals of 0.5, 1.5, 3, 6, 9, and 24 hours is displayed in table 1. the mean drug concentration measured in the plasma of rats after formulated SLN and standard drug solution were administered. The outcome showed that doxorubicin from SLN has better bioavailability when compared to traditional dose forms. Additionally, the frequency of drug dose is decreased by the continuous drug release from SLN. The medication concentration determined by HPLC analysis for lymph sample collections made between the times of 1, 2, 4, and 6 hours. the speed at which doxorubicin is transported between the normal drug solution and the prepared SLN. By preventing the unchecked growth of lymphocytes, the notable increase in doxorubicin transfer from the SLN to the lymphatic system will, in turn, result in an efficient treatment of lymphoma within the body. The outcome showed that the anticancer medication doxorubicin formulated as SLN offers a notable benefit over traditional dose form in the treatment of acute lymphoblastic leukemia/lymphoma.

Pharmacokinetic analysis and evaluation of lymphatic targeting efficiency: The outcome demonstrated that doxorubicin's bioavailability from SLN is 1.9 times higher than that of its conventional solution, pointing to SLN's increased AUC. The student's t test was used to statistically analyse the data (AUC0–24), and the results indicated a significant difference ($P<0.001$) between the two formulations. It was discovered that the percentage dose of doxorubicin transferred by the lymphatic system from SLN and standard solution was 0.06% and 0.28%, respectively. This suggests that, in comparison to the usual solution, the doxorubicin-loaded SLN demonstrated a 4.6-fold increase in doxorubicin transfer to the lymphatic system. A substantial difference ($P<0.001$) was found between the two formulations after the data was statistically analysed using the student's t test.

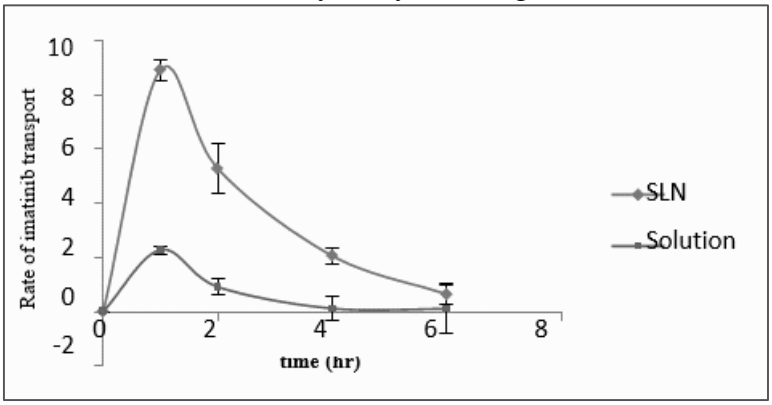

Fig.5:The rate at which oral administration of compounded SLN and standard drug solution causes doxorubicin to be transported through intestinal lymph fluid

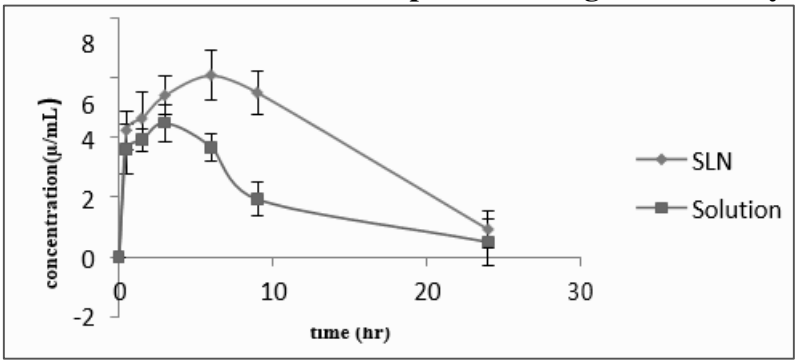

Fig.6:Blood plasma was used to measure the average drug concentration following oral administration of designed SLN and standard drug solution.

Accelerated stability studies: The lyophilized SLN's particle size dramatically increased from 192.7 to 199.5 nm after three months of storage. The particles can still be targeted to the lymphatic system, though, because their size is smaller than 250 nm, which is the maximum size at which a particle can be effectively targeted to the lymphatic system. Even after three months of storage, the drug content of the nanoparticles did not significantly decrease.

CONCLUSION

The findings demonstrated that SLN increased doxorubicin's lymphatic transport by 4.6 times and its bioavailability by 1.9 fold when compared to doxorubicin solution. Because of their physical and chemical properties, such as particle size and surface coating with biodegradable polymers, the majority of NP typically accumulates at the target lymph location. The medicine can be targeted to the lymphatic system because, as was previously indicated, SLN is easily absorbed by the lymphatic system during systemic circulation. We may infer from this work that doxorubicin-loaded SLN are viable options for doxorubicin lymphatic delivery systems.

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Fabrication, Characterization and Evaluation of Terbinafine-Loaded Xanthan Gum Nanogels for Enhanced Drug Delivery

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ABSTRACT

This study aimed to fabricate nanogels from nanoemulsion loaded with terbinafine and explored the characteristics and potential applications of FOR2 (Drug-loaded nanoemulsion), and FOR3 (Chitosan-based nanogel prepared from the nanoemulsion). Utilizing scanning electron microscopy (SEM), this study investigated the morphologies of these formulations, revealing that FOR1 displayed a mesh-like structure, while FOR2 and FOR3 exhibited interconnected pores of varying diameters, enhancing their drug loading and release capabilities. The Spreadibility of each formulation was assessed at temperatures of 8°C, 25°C, and 40°C, with FOR1 and FOR2 demonstrating higher Spreadibility, suitable for applications requiring ease of application and rapid absorption. In contrast, FOR3 showed lower, more consistent Spreadibility, indicating its potential for localized or controlled release applications. In vitro drug release studies further differentiated the formulations: FOR2 rapidly released the drug, making it ideal for acute treatment scenarios, whereas FOR3 sustained drug release, a key advantage of nanogels over nanoemulsions, making it suitable for chronic disease management. This sustained release profile of FOR3 could potentially improve therapeutic outcomes and patient compliance by minimizing dosing frequency. Antifungal activity was also evaluated and demonstrated significant and superior antifungal efficacy of the nanogel formulation (FOR3). In conclusions, the findings highlighted the superiority of the nanogels (FOR3) which offered significant advantages for prolonged therapy, accentuating the importance of formulation choice in enhancing treatment efficacy.

Keywords: Nanoemulsion, Nanogel, Terbinafine, Antifungal, Fungal infections.

INTRODUCTION

With the development of advanced nanotechnology in recent decades, nanocarriers have emerged and gained popularity in biomedicine. Because of their ability to encapsulate drugs, nanocarriers are utilised not only as carriers of standard chemotherapeutic agents but also as platforms for theranostics, combinational therapy, and multifunctional diagnostics (Kaur et al., 2017, Kaur et al., 2019, Smoleński et al., 2021). Nanocarriers have been used as an ideal multifunctional drug delivery system (DDS) for a variety of disease therapies, including active targeting enabled by ligand modification of the nanoplatform surface, passive targeting owing to the enhanced permeability and retention (EPR) effect, and site-specific and time-controlled drug delivery strategies mediated by stimuli-responsive materials (Patil et al., 2024, Ranjbar et al., 2023). Hydrogels with a three-dimensional (3D) tunable porous structure and a particle size in the submicrometer range, ranging from 20 to

250 nm, are known as nanogels, a type of systemic drug delivery carrier. They can be distinguished from microgels, which have a particle size ranging from 1 to 350 μm, and in situ-forming hydrogels, which aid in local delivery. Nanogels help encapsulate small molecules, oligonucleotides, and even proteins. They are made of different natural polymers, synthetic polymers, or mixtures of these. Because of their special qualities, nanogels can be used for imaging, diagnostics, and medication delivery (Kothapalli et al., 2024, Szumała and Macierzanka, 2022, Donthi et al., 2022).

The main indication for the antifungal drug terbinafine is the treatment of fungal infections of the skin and nails. It is a member of the allylamine medication class, which suppresses the manufacture of ergosterol, a crucial component of fungal cell membranes, by blocking the enzyme squalene epoxidase (Krishnan-Natesan, 2009). One of the most common uses of terbinafine is in the treatment of fungal nail infections, medically known as onychomycosis. This condition often manifests as thickened, discoloured nails and can be challenging to eradicate. Terbinafine offers a potent solution by penetrating the nail bed and targeting the fungal infection directly, leading to improved nail appearance and overall resolution of the infection. In addition to its efficacy against nail infections, terbinafine is also effective in treating various skin conditions caused by dermatophyte fungi, such as athlete's foot (tinea pedis) and ringworm (tinea corporis) (Krishnan-Natesan, 2009, Newland and Abdel-Rahman, 2009). Its broad-spectrum antifungal activity makes it a versatile option for combating these common fungal infections. Terbinafine is typically available in various formulations, including oral tablets, topical creams, and solutions, allowing for flexibility in treatment depending on the severity and location of the fungal infection. However, like any medication, terbinafine may cause side effects, ranging from mild gastrointestinal disturbances to more serious liver problems, although the latter is rare. Terbinafine stands as a valuable therapeutic option in the management of fungal infections, providing patients with effective relief and the opportunity for improved skin and nail health (Newland and Abdel-Rahman, 2009).

Preparing a nanogel formulation of terbinafine offers several advantages over conventional formulations, making it a compelling choice for the treatment of fungal infections: Nanogels have a nano-sized structure, allowing them to penetrate the skin and nails more effectively than conventional formulations. This enhanced penetration can lead to better delivery of terbinafine to the site of infection, increasing its efficacy. Nanogels can be designed to target specific areas of the body, such as the nail bed or the deeper layers of the skin where fungal infections are located (Jessup et al., 2000). By encapsulating terbinafine within nanogels, it can be delivered directly to the target site, minimizing systemic exposure and reducing the risk of side effects. Nanogels can be engineered to provide sustained release of terbinafine over an extended period. This prolonged release profile ensures that therapeutic levels of the drug are maintained at the site of infection, improving treatment outcomes and reducing the frequency of dosing. Terbinafine is known to have poor aqueous solubility, which can limit its bioavailability and efficacy. By encapsulating it within nanogels, its stability can be enhanced, protecting it from degradation and improving its solubility, thereby increasing its bioavailability and therapeutic effect. Topical formulations such as nanogels are often preferred by patients due to their ease of application and reduced systemic side effects compared to oral medications. This can lead to improved patient

compliance and better treatment outcomes (Balfour and Faulds, 1992, Darkes et al., 2003, Leyden, 1998). In summary, preparing a nanogel formulation of terbinafine offers the potential for enhanced efficacy, targeted delivery, prolonged release, improved stability, and increased patient compliance, making it a promising approach for the treatment of fungal infections affecting the skin and nails. Therefore, this present study was designed to fabricate nanoemulsion based nanogels of terbinafine and evaluate the same for various physicochemical properties as well as for antifungal activities.

MATERIAL AND METHODS

Drugs and chemicals

Natural gums such as Xanthan gum were procured from Merck, India. Chitosan was purchased form Sigma Aldrich, Mumbai, India. All other chemical and reagents were purchased and arranged from reputed and validated vendors only. All the reagents and chemical were of analytical grade.

Preparation of nanoemulsion (NEF)

The previously mentioned procedure was followed to prepare the nanoemulsion (Zhou et al., 2016) with a few required adjustments. The method for preparing the nanoemulsions with different concentrations of components was meticulously executed. Initially, the specified amounts of olive oil, lecithin, and distilled water were measured according to each formulation (NEF-1 to NEF-4). Subsequently, the oil phase (A) was prepared by thoroughly mixing the olive oil with the designated quantity of lecithin until homogenized. Meanwhile, the aqueous phase (B) was created by gradually adding xanthan gum to the distilled water while stirring continuously to prevent clumping. Following this, both phases (A and B) were heated separately on a water bath set at a temperature of 44°C ± 5°C for 35 minutes to ensure optimal dispersion and solubilization of the components. Once heated, the oil phase (A) was slowly added to the aqueous phase (B) while stirring vigorously using a magnetic stirrer or homogenizer at a speed of 4000 rpm for 8 minutes to facilitate emulsification. The mixture was then cooled gradually while stirring at a reduced speed of 1500 rpm for 15 minutes to promote uniform mixing. For formulations containing drug (NEF-2 to NEF-4), the specified amount of drug was incorporated into the oil phase (A) prior to emulsification to ensure even distribution. Finally, the emulsion underwent high-speed/high-shear homogenization at 15000 rpm for 15 minutes to further enhance stability and uniformity. The prepared nanoemulsions were then transferred to suitable containers and stored under specified conditions for stability testing and subsequent use in pharmaceutical applications. The prepared formulas are listed in Table 1 below.

Table 1. Formulation composition table of the nanoemulsions with different concentrations of components

Code for the formulations	**Olive Oil (w/w)**	**Xanthan Gum (w/w)**	**Lecithin (w/w)**	**Drug (w/w)**	**Distilled water Q. S to make 100 g**
NEF-1 (Blank)	18 g	23 g	18 g	-	59 g
NEF-2	23 g	18 g	17 g	250 mg	41.75 g
NEF-3	20 g	21 g	15 g	250 mg	43.75 g

NEF-4	23 g	18 g	13 g	250 mg	45.75 g

Evaluation of nanoemulsions and chitosan gel preparation

Before being incorporated into the gel matrix, multiple nanoemulsion compositions underwent physical examination. The colour changes, consistency, and phase separation of the various formulations were meticulously observed. Samples from each formulation were subjected to storage conditions at 8°C, 25°C, 40°C, and 40°C with 75% relative humidity (RH) for a duration of 28 days. Chitosan was dissolved in distilled water to form the chitosan gel. A precise weight of 2.5 grams of chitosan was accurately measured and then dissolved in 100 millilitres of distilled water containing 1.5% acetic acid, following the method described by Zhou et al., 2016 (Zhou et al., 2016). A high-speed mixer operating at 3500 rpm for a duration of 12 minutes was utilized to prepare the gel. Subsequently, the resulting gel was stored overnight before being incorporated into the emulsion.

Fabrication of the nanoemulsion gel

The fabrication of the nanoemulsion gel began with the preparation of the chitosan gel. Chitosan, accurately weighed to 2.5 grams, was dissolved in 100 millilitres of distilled water containing 1.5% acetic acid using a high-speed mixer operating at 3500 rpm for 12 minutes. This gel was then stored overnight for further use. Meanwhile, several nanoemulsion compositions were physically examined to assess colour changes, consistency, and phase separation. Following the assessment, the chitosan gel was incorporated into the nanoemulsion using a suitable mixing technique for 30 min to ensure homogeneity. The resulting nanoemulsion gel was then subjected to further characterization and stability testing to evaluate its suitability for pharmaceutical applications. Triethanolamine was used to lower the pH (TEA) of the formulations.

Characterizations

Thermodynamic stability and Heat cooling cycle

The optimized formulations underwent thermodynamic stability testing for a period of 28 days, adhering to the guidelines outlined by the International Conference on Harmonisation (ICH). This assessment aimed to evaluate the formulations' resilience under challenging conditions. Following a protocol inspired by a previous study (Burki et al., 2020), with slight modifications, both the manufactured nanoemulsion (NEF) and the chitosan-based nanoemulsion gel (NEFG) formulations were subjected to storage in an incubator set at 40°C for the initial 28 days. Subsequently, both formulations were allowed to return to room temperature. The objective of this test was to observe any physical changes such as turbidity, creaming, or cracking, which could indicate instability or potential issues with the formulations' long-term storage suitability.

Freeze thaw cycle and Centrifugation

For a duration of 28 days, both the nanoemulsion (NEF) and nanoemulsion gel (NEFG) formulations underwent the freeze-thaw cycle test. This involved placing the formulations in a deep freezer at a temperature ranging between 2-4°C. After the freezing period, the formulations were removed from the freezer and allowed to thaw at room temperature. Subsequent to undergoing this rigorous treatment, the formulations were visually inspected to ascertain whether they reverted to their original state. To further assess the formulations' stability, a high-speed centrifuge (Remi, India) was employed. Each sample formulation was transferred to separate Eppendorf tubes and centrifuged at varying speeds of 6000 and

12000 rpm for a duration of 12 minutes. The centrifugation process enabled the examination of the formulations' propensity to separate into distinct oily and aqueous phases, providing insights into their overall stability and emulsion integrity.

The pH, Droplet size, surface charge and PDI of NEF and NEFG

Following the pH analysis, all freshly prepared NEF and NEFG formulations underwent evaluation at specific time intervals: 12 hours, 24 hours, 7 days, and 14 days post-preparation. This comprehensive assessment aimed to monitor any potential changes in pH over time, providing valuable insights into the formulations' stability and suitability. This particular monitoring allowed for early detection of potential stability issues and facilitated adjustments to formulation parameters if necessary, ensuring the maintenance of the desired pH levels throughout the intended shelf-life of the formulations (Burki et al., 2020). The purpose of this test was to determine the polydispersity index, surface charge, and droplet size of the nanoemulsion formulation. To achieve this objective, a Helium-Neon laser and a zeta sizer (Nano ZS 90, Malvern Instruments, UK) were employed, following the previously outlined methodology (Ali et al., 2020). Specifically, 1 ml of nanoemulsion was combined with 9 ml of deionized water, and the resulting mixture was thoroughly stirred for three minutes. This process was repeated three times to ensure consistency, and the obtained results were subsequently averaged to provide a representative measurement (Ali et al., 2020).

Analysis of the drug content

With slight modifications, the analysis of drug content was conducted following the previously described methodology (Burki et al., 2020). To accomplish this, one gram (10 µg/g) of the nanoemulsion gel was accurately weighed and mixed with nine millilitres (w/v) of ethanol. Subsequently, the resulting mixture underwent centrifugation using a Remi centrifuge (India) at 6000 rpm for a duration of two to three minutes. Following centrifugation, the mixture was filtered through a 0.45 micrometer-pore-size nylon filter membrane. Three analyses of the filtrate sample were conducted using a UV-spectrophotometer (Shimadzu, Japan), and the average results were calculated to ensure accuracy and reproducibility.

Viscosity of NEFG and Morphological studies

The viscosities of the NEF (FOR2) and NEFG (FOR3) formulations were evaluated using a viscometer (NDJ, RRS, India) on days 0, 1, 2, 7, 14, and 28. Measurements were conducted at temperatures of 8°C, 25°C, and 40°C to assess the effect of temperature on viscosity over time (Alexander et al., 2013). The primary function of spindle number four is to measure the viscosity of semisolid dosage forms, particularly nanoemulsions (El-Refaie et al., 2015). Beakers were used to hold the 50 g formulations of NEF and NEFG. The viscometer's spindle was carefully positioned in the center of the beakers, ensuring it did not touch the bottom. Subsequently, the spindle's rotation speed was set to 7 rpm, and measurements for both formulations were recorded over a duration of six minutes. For the examination of the morphology and apparent shape of the chitosan-based nanoemulsion gel (NEFG), scanning electron microscopy (SEM) was employed. Metal stubs were coated with NEFG (blank) and drug-loaded samples using double-sided adhesive tape. These formulation-loaded stubs were then placed in a vacuum chamber for drying. Subsequently, the samples were coated with a layer of gold approximately 8–10 nm thick using a sputter

coater for a duration of five minutes prior to inspection. SEM analysis of the stubs was performed at a magnification of 12,000X and an accelerating voltage of 12 KV. The designated areas of the samples were photographed using the aforementioned techniques to capture their microstructural characteristics accurately (El-Refaie et al., 2015, Burki et al., 2020).

FTIR spectroscopy study and Spreadibility studies

FTIR spectroscopy, conducted with a Perkin Elmer instrument, was employed to analyse the drug, chitosan, nanoemulsion, and nanoemulsion gel. The study aimed to determine the degree of compatibility between the polymer and the constituent parts of the formulation, as well as to confirm the presence of functional groups and their corresponding wave numbers. Samples of each mixture and component were applied to the diamond crystal and crushed using the instrument's knob. Spectra for each sample were captured in triplicate within the wave number range of 400–4000 cm^{-1}. Additionally, the Spreadibility of the nanoemulsion gel (NEFG) was assessed using the "Drag & Slip" device, following a previously reported method with slight adjustments (Ali et al., 2020). The device used for assessing Spreadibility consists of a wooden block with a pulley attached to one end. It comprises two identically sized glass slides, one of which is movable while the other is affixed to the block. To evaluate the Spreadibility of a sample, it is placed between the stationary slide and the top mobile slide, and a measured weight is applied. In our evaluation, a fixed (stationary) slide containing 2.0 g of nanoemulsion gel (NEFG) was positioned between the upper slide to test the optimized nanoemulsion gel's Spreadibility. A weight of 50 g was placed on the upper glass slide, and the time taken for the upper slide to travel 8 cm was recorded. The Spreadibility of the test NEFG was calculated using the following formulae.

$S = M \times L \div T$

S stands for Spreadibility in this instance.

The weight on the upper glass slide is denoted by "M"

The glass slides' length is shown by "L" and their travel duration is indicated by "T"

***In vitro* drug release**

The in vitro drug release investigation was conducted following the well-defined methodology outlined in a previous study (Khan et al., 2021). To conduct the experiment, a Franz diffusion cell (IPS Technologies, India) equipped with 6 ml and 3 ml capacities for the donor and receptor compartments, respectively, was employed. The temperature was maintained at 37°C ± 1°C, and the stirring speed was set at 300 rpm before adding the nanoemulsion (NEF) and nanoemulsion gel (NEFG) samples. Given the utilization of artificial membranes for in vitro release studies, a tuffryn membrane (Sortorius, Germany) was securely clamped between the donor and receptor compartments. Each formulation sample (two grams) was carefully placed into the receptor compartments of both cells, and the receptor compartments were subsequently filled with pH 5.5 sodium acetate buffers. Sample collection was conducted at predetermined intervals (0 h, 1 h, 2 h, 4 h, 8 h, and 12 h) using a spinal syringe to withdraw 2 ml from the receptor compartment. Fresh buffer was introduced to maintain the sink state and sodium acetate buffer level in the receptor compartment. Following sample collection, UV-spectrophotometer analysis (at 360 nm) was performed to assess the drug's release behaviour.

Drug release kinetics

Subsequently, the drug release data was fitted into several mathematical models to elucidate the drug's release behavior (Burki et al., 2020).

Antifungal Susceptibility Testing

Biological screening was conducted to determine the formulations' potential as antifungal against fungal strains (Motedayen et al., 2018).

Inoculum Preparation

Mature colonies cultivated on Potato Dextrose Agar (PDA) were subjected to a sterile saline solution (0.85%), supplemented with one drop of tween 20, covering the surface of the colonies. Subsequently, the colonies' surfaces were gently scraped using a sterile swab. The resulting mixture, containing conidia and hyphal fragments, was transferred into a sterile tube and left undisturbed for 5 to 10 minutes at room temperature to allow heavy particles to settle. Following sedimentation, the upper suspension containing predominantly conidia was carefully collected. Conidia concentrations were determined using a hemacytometer, and adjustments were made using RPMI 1640 medium. The RPMI 1640 medium, prepared with glutamine and buffer at pH 7.0 without sodium bicarbonate, was standardized to obtain an inoculum ranging from 1×10^3 to 3×10^3 colony-forming units per millilitre (CFU/mL). This was achieved by dissolving 10.43 grams of RPMI powder and 34.53 grams of MOPS buffer (N-Morpholino Propanesulfonic Acid) in 1 Liter of distilled water with gentle agitation (Motedayen et al., 2018).

Minimum Inhibitory Concentration Test Procedure

The antifungal susceptibility testing followed the standard procedure outlined in the Broth Microdilution CLSI M38 method. Initially, 96-flat-bottomed well microplates (Orange Scientific, E.U) were employed for the assay. Each well was loaded with 100 µL of the respective drug concentration, followed by the addition of 100 µL of the inoculum suspension to each drug-containing well. Growth control and sterilized control wells were also included for each isolate to ensure the validity of the results. Additionally, blank nano-liposomes were incorporated to confirm the absence of any inherent antifungal activity. The microplates were then incubated at 28°C for a period of 4 days. Upon completion of the incubation period, the results were visually assessed, and the minimum inhibitory concentrations (MICs) were recorded. The MIC was defined as the lowest concentration of the drug that inhibited 100% of the fungal growth compared to the growth observed in the control wells. All tests were conducted in duplicate to ensure the reproducibility and accuracy of the results (Kataki, 2010, Kataki et al., 2010, Mukherjee et al., 1995, Motedayen et al., 2018).

Statistical analysis

Using GraphPad Prism, a One Way ANOVA and a student's T-test were conducted to the data. The data was displayed as mean ± SD after all the variables were averaged three times.

RESULTS & DISCUSSION

Characterizations of formulations (NEF and NEFG)

Physical appraisal and thermodynamic stability

For a duration of 28 days, three formulations were maintained at various temperatures (8 ºC, 25 ºC, 40 ºC, and 40 ºC + 75 relative humidity (RH)). The formulations included blank formulations, chitosan-based nano-emulsion gel, and eucalyptus oil nanoemulsion.

Physical assessments were conducted on these compositions periodically to evaluate their phase separation, consistency, liquefaction, colour change, and cracking. The freshly prepared formulations had a yellowish colour and a smooth, elegant appearance. After centrifugation at 6000 and 12000 rpm, no phase separation was observed. When the initial formulations were evaluated, their pH was found to be 5. 5. The pH of human skin was measured as a reference (Proksch, 2018). Using the student t test, the pH of the formulations was assessed at different intervals after 12 hours, 24 hours, 7 days, 14 days, 1 month, 2 months, and 3 months. Each formulation's pH did not differ noticeably, or $p > 0.05$. In order to avoid skin irritations, the pH of drugs applied topically must be between 5 and 6 (Proksch, 2018, Wagner et al., 2003). Over time, the pH value significantly dropped, which could be due to water diffusing out of its phase or the oil in the formulations creating acidic chemicals (Wagner et al., 2003, Schmid-Wendtner and Korting, 2006). This pH fluctuation was, nevertheless, within the typical range for human skin. According to the characteristics listed above, each formulation was thermodynamically stable.

Droplet size, polydispersity (PDI) and surface charge

The table provided details on the prepared formulations—FOR1 (Blank), FOR2 (Nanoemulsion), and FOR3 (Nanogel) and highlighted important characteristics about each of the formulations, notably in terms of droplet size, zeta potential, and the polydispersity index (PDI).

Size of Droplets: FOR1 as blank exhibits the smallest droplet size of approximately 54.46 nm. This smaller size might be indicative of a simple formulation, possibly containing fewer components that could affect the stability or the aggregation of droplets. FOR2 as nanoemulsion has a slightly larger droplet size (69.67 nm) compared to the blank. This increase might be due to the incorporation of additional components such as oils and surfactants which are typical in nanoemulsions and can influence droplet size by stabilizing larger droplets. FOR3 as nanogel shows the largest droplet size (82.38 nm), which could be attributed to the gel matrix's ability to swell and incorporate more fluid, thus increasing the size of the droplets within the gel structure.

Zeta Potential: FOR1 - Blank and FOR2 – Nanoemulsion have negative zeta potentials of -20.5 mV and -17.5 mV, respectively. Negative values generally indicate stability against aggregation due to electrostatic repulsion between droplets. The difference in values might be due to differences in the surface chemistry influenced by the formulation ingredients. FOR3 – Nanogel, however, exhibits a positive zeta potential of 25.2 mV. This shift to a positive value could be indicative of a different stabilizing agent or a fundamental difference in the composition of the nanogel compared to the other formulations. A positive zeta potential can also confer stability, as similarly charged particles repel each other, preventing aggregation.

PDI Ratio: The PDI values reflect the uniformity in the size distribution of the droplets within each formulation. Lower PDI values (as seen in FOR1 and FOR2) suggest more uniform droplet sizes, which is often desirable for stability and consistency in delivery systems. FOR3's higher PDI value (0.366) suggests a broader size distribution. This could be due to the more complex nature of the nanogel system, where the gel matrix might allow for a more heterogeneous distribution of droplet sizes.

In summary, these characteristics suggest that each formulation offers unique properties suitable for different applications. The smaller, more uniformly sized droplets in the blank and nanoemulsion could be advantageous for applications requiring rapid and efficient delivery, while the larger, positively charged droplets in the nanogel might be better suited for applications where slower release and longer retention are beneficial. The difference in zeta potential among the formulations also hinted at the potential for varied biological interactions, as charge can influence cellular uptake and bio-distribution (Rai et al., 2018, Zhang, 2019, Chakraborty et al., 2020).

Table 2. Zeta potential, PDI, and droplet size of the formulations

Prepared Formulations	**Size of droplets (nm)**	**Zeta potential (Mv)**	**PDI Ratio**
FOR1 - Blank	54.46±1.01	-20.5	0.135
FOR2 – Nanoemulsion (NEF)	69.67±1.11	-17.5	0.124
FOR3 – Nanogel (NEFG)	82.38±1.41	25.2	0.366

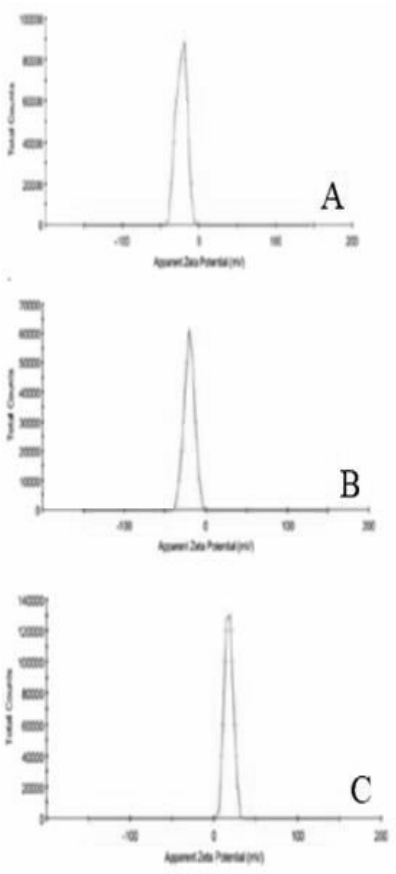

Figure 1. Zeta potential of the three formulations: blank formulation (A), drug-loaded nanoemulsion (B) and Nanogel

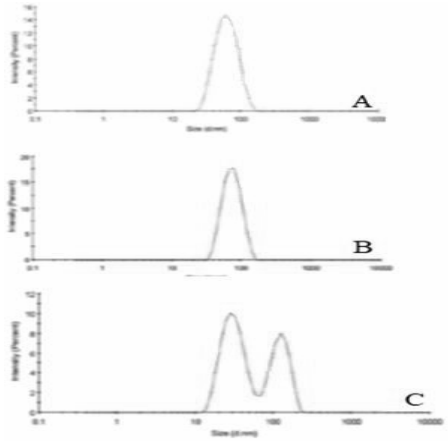

Figure 2. The formulations' particle sizes (A. blank, B. drug-loaded nanoemulsion, and C. nanogel formulation)

FTIR study

The Fourier Transform Infrared (FTIR) spectra of the different samples analysed - including the drug, nanoemulsion containing olive oil, chitosan, and the nanogel formulation -demonstrate no evidence of drug-excipient interaction. The characteristic peaks of each component remain distinct and unaltered across the spectra, indicating that their chemical structures are maintained without any significant modifications or interactions. This suggested that the drug maintains its integrity within the formulation, and the excipients, including the olive oil and lecithin in the nanoemulsion and chitosan in the nanogel, do not chemically interact with the drug. This stability is crucial for ensuring the efficacy and safety of the drug in its intended applications (Pant et al., 2014, Cardenas and Miranda, 2004).

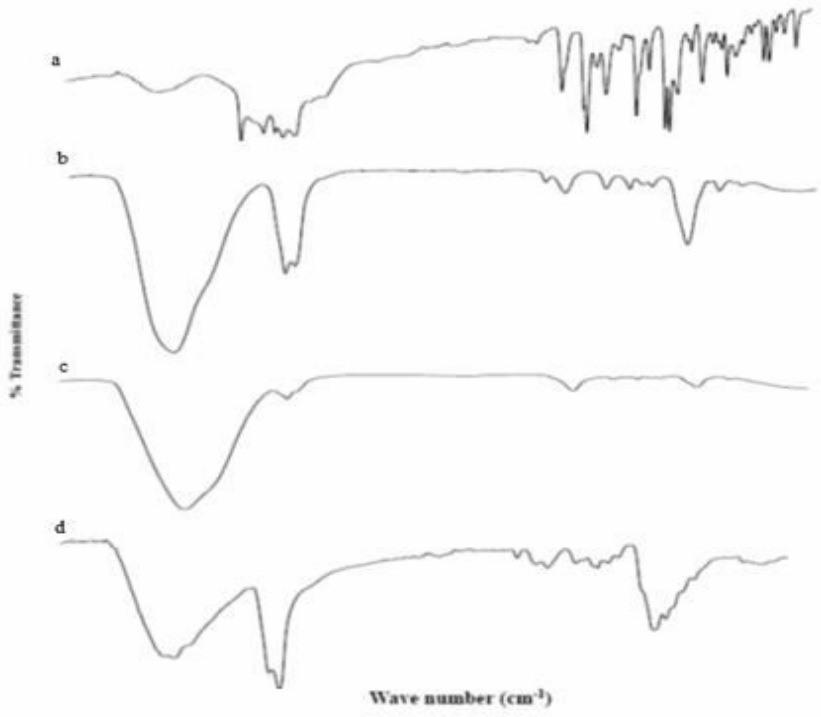

Figure 3. FTIR spectra of a. Drug, b. Nanoemulsion containing olive oil, c. Chitosan and d. Nanogel

Drug content

FOR2 exhibited a drug content of 92.75±1.36%, indicating that the formulation successfully incorporated a high percentage of the drug. This suggests efficient drug loading and stability within the formulation. FOR3 showed a slightly lower drug content of 89.92±1.77% compared to FOR2. While still relatively high, this slight decrease may indicate some variability in drug loading or formulation characteristics. However, it remains within an acceptable range for pharmaceutical formulations. Both formulations (FOR2 and FOR3) demonstrated efficient drug incorporation, with FOR2 having a slightly higher drug content. This suggests that the nanoemulsion and nanoemulsion gel formulations are effective at delivering the desired drug concentration, which is essential for ensuring therapeutic efficacy and consistency in pharmaceutical products (Table 3).

Table 3. Exhibiting the percentage of drug in the formulations for nanoemulsion and nanogel.

Formulation code	Drug Needed (µg)	Drug Found (µg)	% Drug content
FOR1	-	-	-
FOR2	250	92.75±1.36	92.75±1.36
FOR3	250	89.92±1.77	89.92±1.77

Viscosity of formulations

The viscosity measurements for formulations FOR2 (Nanoemulsion) and FOR3 (Nanogel) over various days and at different temperatures (8°C, 25°C, and 40°C) provided insights into their physical stability and potential applications. The viscosity of FOR2 remains relatively stable at 8°C and 25°C throughout the 28 days. However, there's a noticeable decrease in viscosity at 40°C starting from Day 1, which continues to decrease over the 28 days. This decrease at higher temperatures suggests that the nanoemulsion might be susceptible to temperature-induced changes, potentially due to the breakdown of the emulsion structure or the evaporation of volatile components. At lower temperatures (8°C and 25°C), the nanoemulsion exhibits excellent stability with only minor fluctuations in viscosity. This indicates good resistance to phase separation or degradation over the observed period.

The viscosity for FOR3 shows less consistency across temperatures. At 8°C, the viscosity slightly increases over time, suggesting potential thickening or increased cross-linking within the gel. At 25°C and 40°C, the viscosity fluctuates more noticeably, particularly showing a decrease on Day 7 at 40°C before stabilizing. These variations might indicate sensitivity to environmental conditions, which could affect the nanogel's structure and performance. Unlike FOR2, FOR3 exhibits more variation in viscosity, especially at higher temperatures. This could be due to the hydrogel network responding to thermal stress, which might affect its stability and applicability in environments with fluctuating temperatures. FOR2 appeared more suitable for conditions where temperature does not exceed 25°C, maintaining its stability better at these conditions. FOR3, while fluctuating more in viscosity, may require specific storage conditions or usage scenarios where temperature control is feasible, especially to maintain its structural integrity. The stability of FOR2 at lower temperatures suggests its suitability for storage and use in cooler

environments, potentially beneficial for transport and storage. FOR3's behaviour indicated a possible advantage in applications where slight changes in viscosity can be tolerated or where its response to temperature can be used to advantageously modulate the release of active ingredients. The observed data could guide further optimization of these formulations. For FOR2, enhancing thermal stability at higher temperatures could be beneficial, while for FOR3, reducing variability in response to temperature could enhance its applicability (Khan et al., 2021, Alexander et al., 2013, Burki et al., 2020, El-Refaie et al., 2015).

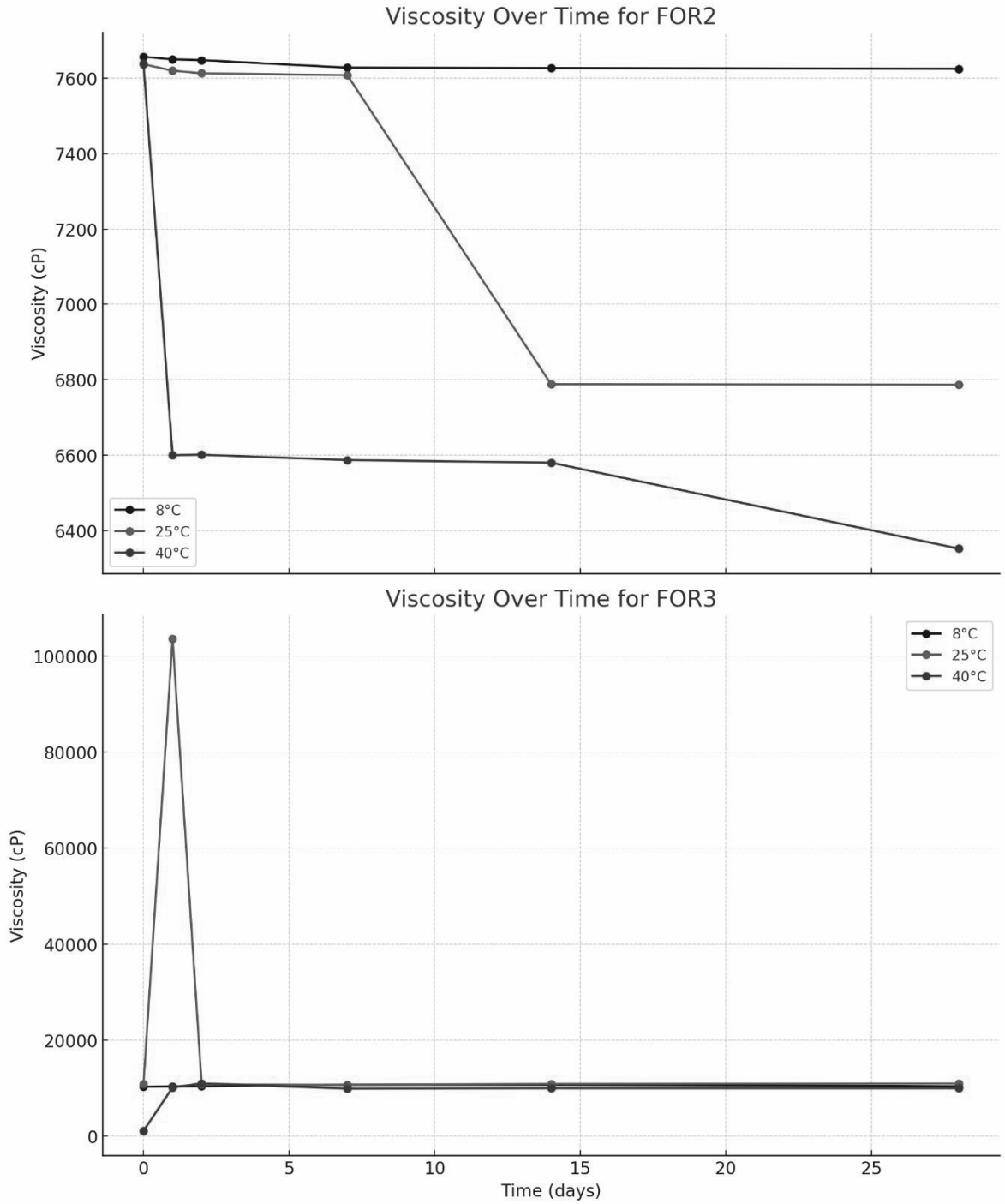

Figure 4. The viscosities of the formulations (FOR2 and FOR3) were shown as centipoise at different times and temperatures.

Morphological studies by SEM

Scanning electron microscopy (SEM) was employed to examine the structures of the drug-loaded nanoemulsion (FOR2), the chitosan-based nanoemulsion gel (FOR3), and the blank nanoemulsion (FOR1), as depicted in Figure 4. The SEM images reveal that the blank nanoemulsion (FOR1) exhibits a mesh-like structure. In contrast, the SEM pictures of both the drug-loaded nanoemulsion (FOR2) and the nanoemulsion gel (FOR3) show interconnected pores with randomly varied diameters. This porous structure is advantageous as it provides ample space for drug incorporation, enhancing both drug movement within the matrix and the rate at which the drug is released from the formulations.

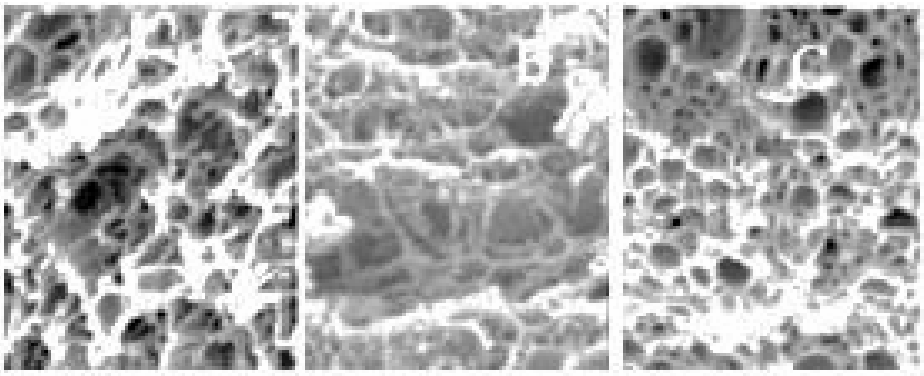

Figure 5. SEM photomicrographs of the formulations A. Blank formulation (FOR1), B. Nanoemulsion formulation (FOR2) and C. Nanogel formulation (FOR3)

Spreadibility

The Spreadibility data for the formulations FOR1 (Blank nanoemulsion), FOR2 (Drug-loaded nanoemulsion), and FOR3 (Chitosan-based nanoemulsion gel) were analysed across various temperatures (8°C, 25°C, and 40°C), revealing key insights into their performance characteristics and suitability for potential applications. In terms of temperature influence, both FOR1 and FOR2 demonstrated an increase in Spreadibility as the temperature rose from 8°C to 40°C. This behaviour is typical as higher temperatures generally reduce the viscosity of formulations, thereby enhancing their ease of spread. However, FOR3 also exhibited an increase in Spreadibility with rising temperatures but maintained significantly lower values at all temperatures compared to FOR1 and FOR2. This suggests that FOR3, being a gel-based formulation, inherently possesses a higher resistance to flow due to its thicker or more viscous composition. Comparatively, FOR1 and FOR2 showed very similar Spreadibility metrics at all studied temperatures, significantly higher than those of FOR3. This similarity indicates that the drug loading in FOR2 did not significantly alter its physical properties affecting Spreadibility compared to the blank nanoemulsion (FOR1). The lower Spreadibility of FOR3 is attributed to its gel matrix, which likely provides a more structured and less fluid consistency favourable for applications requiring slower release or more localized application where reduced spread is beneficial. The implications for application are clear. The higher Spreadibility of FOR1 and FOR2 at elevated temperatures suggests that these formulations are well-suited for applications where easy

application and quick absorption are required, such as topical treatments in warmer environments. Conversely, FOR3, with its lower Spreadibility, might be better suited for applications where a thicker, more controlled release formulation is beneficial, such as in transdermal patches or localized therapy. The stability in Spreadibility of FOR2, despite the drug loading, suggests that effective formulation techniques were employed to incorporate the drug without adversely affecting the physical properties, ensuring consistent performance in drug delivery applications. The consistent increase in Spreadibility with temperature across all formulations indicates predictable behaviour, advantageous for managing performance under varying environmental conditions (Burki et al., 2020, Khan et al., 2021).

Table 4. For the formulations (FOR1, FOR2, and FOR3) at the different temperatures under study, the Spreadibility presented as mean ± SD.

	Spreadibility		
Formulation codes	**8 ^{0}C**	**25 ^{0}C**	**40 ^{0}C**
FOR1	17.68±1.23	21.65±1.11	27.86±1.03
FOR2	17.56±1.43	21.75±1.05	27.98±1.21
FOR3	13.56±1.22	15.86±1.05	17.58±1.09

***In vitro* drug release**

The in vitro drug release data for the formulations FOR2 (Drug-loaded nanoemulsion) and FOR3 (Chitosan-based nanoemulsion gel) provided insights into their release kinetics and potential efficacy as drug delivery systems. The data was expressed as the percentage of drug released over time, revealing distinct behaviours for each formulation. Initially, FOR2 exhibited a rapid drug release, with approximately 54.57% of the drug released within the first hour. This quick release profile suggested that FOR2 facilitated faster dispersion and dissolution of the drug, likely due to its nanoemulsion structure. In contrast, FOR3 showed a somewhat slower initial release at 50.58%, which could be attributed to its gel matrix that may restrict immediate drug release. As time progressed, FOR2 continued to release the drug steadily, achieving about 89.07% release by the 12th hour. This indicated a gradual decrease in the release rate following the initial burst. FOR3, on the other hand, displayed a more controlled release pattern, reaching 79.39% by the 12th hour. The more gradual release rate of FOR3 was likely influenced by the gel matrix's role in modulating the drug release. The faster release rate of FOR2 was potentially ideal for applications requiring rapid onset of action, such as acute pain management or conditions where quick drug absorption is crucial. FOR3, with its slower and more controlled release, seemed better suited for applications where prolonged drug release is beneficial, such as in chronic therapy management. This could reduce dosing frequency and potentially improve patient compliance. Overall, the distinct drug release profiles of FOR2 and FOR3 highlighted their unique capabilities and potential applications in different therapeutic contexts. FOR2 was suited for acute treatment scenarios requiring fast drug absorption, while FOR3 was aligned

with long-term treatment strategies that benefit from a consistent, controlled release (Burki et al., 2020, Khan et al., 2021).

Table 5. In Vitro drug release study presented the % drug release data for the formulations (FOR2 and FOR3).

	Formulations	
Time (Hr)	**FOR2**	**FOR3**
0	0	0
1	54.57±2.03	50.58±1.07
2	64.81±2.01	57.49±1.18
4	73.81±2.09	68.46±1.49
6	79.39±2.11	73.72±1.47
8	82.13±2.01	76.19±1.26
12	89.07±2.09	79.39±1.19

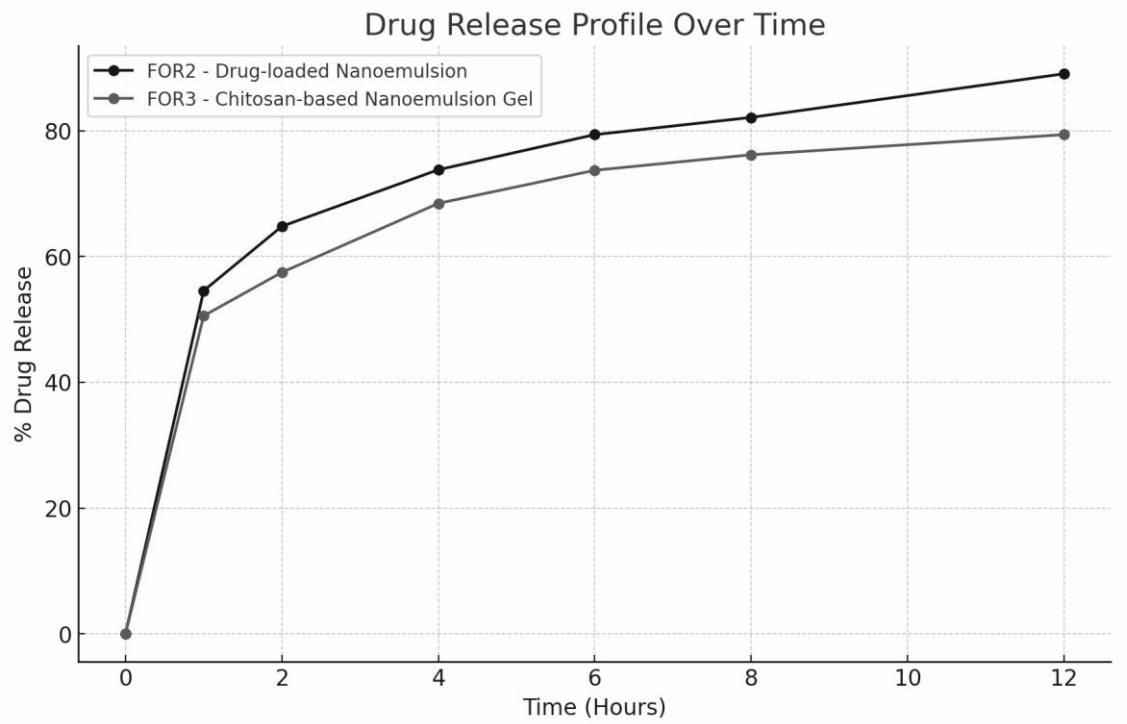

Figure 6. Depicting the percentage drug release for the formulations (FOR2 and FOR3).

Antifungal Susceptibility Testing

The MIC (Minimum Inhibitory Concentration) values for Terbinafine and Nanoemulsion Gel (NEFG) against *T. rubrum* and *M. canis* fungal strains were determined. For *T. rubrum*, the MIC range of Terbinafine was found to be 0.0614 - 1 µg/mL, with MIC50 and MIC90 values of 0.49 µg/mL and 1 µg/mL, respectively. On the other hand, NEFG exhibited a lower MIC range of 0.0145 - 0.26 µg/mL, with MIC50 and MIC90 values of 0.0514 µg/mL and 0.114 µg/mL, respectively. Similarly, for *M. canis*, Terbinafine displayed a MIC range of 0.0302 - 0.56 µg/mL, with MIC50 and MIC90 values of 0.114 µg/mL and 0.49 µg/mL, respectively. Contrastingly, NEFG showed a narrower MIC range of 0.0067 - 0.114 µg/mL, with MIC50 and MIC90 values of 0.0202 µg/mL and 0.115 µg/mL, respectively. These results indicate that the Nanoemulsion Gel (NEFG) formulation possesses stronger

antifungal activity compared to Terbinafine alone against both *T. rubrum* and *M. canis* strains. The lower MIC values for NEFG suggest that it requires lower concentrations to inhibit fungal growth effectively. This enhanced efficacy could be attributed to the synergistic effect of the nanoemulsion formulation, which may facilitate better penetration of the antifungal agent into the fungal cells or provide sustained release of the drug, leading to prolonged antifungal activity. Further studies are warranted to elucidate the mechanisms underlying the improved antifungal efficacy of NEFG and to assess its potential for clinical applications in treating fungal infections.

Table 6. Antifungal activity of the nanoemulsion and nanogel formulations compared to Terbinafine alone against both *T. rubrum* and *M. canis* strains

Species/Antifungal Agent	**MIC Range (µg/mL)**	**MIC50 (µg/mL)**	**MIC90 (µg/mL)**
T. rubrum (n = 60)			
Terbinafine	0.0614 - 1	0.49	1
NEFG	0.0145 - 0.26	0.0514	0.114
M. canis (n = 60)			
Terbinafine	0.0302 - 0.56	0.114	0.49
NEFG	0.0067 - 0.114	0.0202	0.115

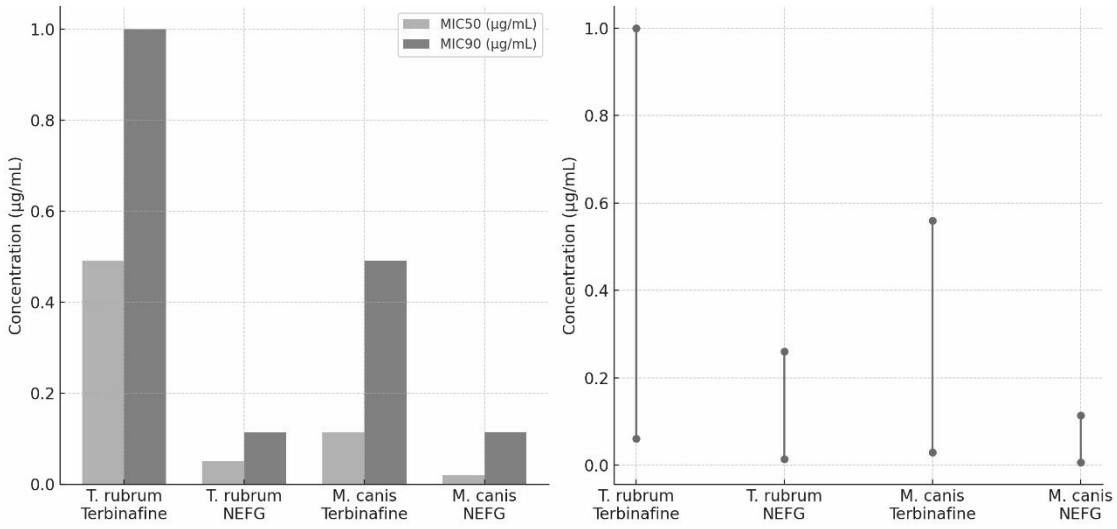

Figure 7. Antifungal activity of the nanoemulsion and nanogel formulations compared to Terbinafine alone against both *T. rubrum* and *M. canis* strains

CONCLUSION

The present study successfully fabricated and evaluated nanoemulsion (FOR2) and nanogel (FOR3) formulations of terbinafine hydrochloride. The comprehensive study of the formulations FOR1, FOR2, and FOR3, encompassing their morphological, release, and physical properties, offered valuable insights into their potential applications and benefits. Scanning electron microscopy (SEM) analysis showed that FOR1, a blank nanoemulsion, exhibited a mesh-like structure, while FOR2 and FOR3, which are drug-loaded formulations, demonstrated interconnected pores with varying diameters. These structural characteristics are crucial for enhanced drug loading and release capabilities. Spreadibility tests indicated that FOR1 and FOR2 maintained higher Spreadibility across various

temperatures, suggesting their suitability for applications requiring easy application and rapid absorption. In contrast, FOR3, a chitosan-based nanogel, showed lower and more stable Spreadibility suitable for localized or controlled release applications. Drug release studies revealed that FOR2 had a quicker release profile than FOR3, which is aligned with scenarios needing immediate drug action, such as acute treatments. However, FOR3, designed as a nanogel, excelled in sustaining the release of the drug, an inherent advantage of nanogels over nanoemulsions. This controlled release profile made FOR3 particularly beneficial for chronic disease management, where sustained delivery can enhance therapeutic outcomes and patient adherence by reducing the frequency of dosing. The nanogel (FOR3) formulation also demonstrated superior antifungal activity. In conclusions, FOR3 as the nanogel formulation offered significant benefits for prolonged therapy, emphasizing the strategic use of nanogel technology to optimize treatment efficacy and patient compliance.

DECLARATION OF INTEREST

None

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Nil

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Hysteresis Resonance and Multi-Pinched Hysteresis Loops in Cu/Cu:ZnO/Cu Resistive Switching Devices

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ABSTRACT

At present, great attention is being devoted towards resistive switching devices owing to lower switching voltage (< 3 V), higher tolerance (> 10^6 cycles), higher scalability (< 10 nm), multi-bit operations, and higher information storage time (10 years) compared to the existing broadly employed transistor technology. This was all possible owing to the accidental discovery of memristance by Hewlett Packard (HP) labs in 2008. In the past few decades, discoveries of such unique electronic properties have led to tremendous technological advancements. Consequently, it is very important to continually seek newer electronic characteristics. In this work, copper (Cu)-doped zinc oxide (ZnO) thin film was synthesized on Cu electrodes to form a Cu/Cu: ZnO/Cu resistive switching device. Function generator and oscilloscope were employed to obtain the electronic characteristics of the device instead of conventional techniques, which resulted in a 96.17% improvement in total equipment expenses. Most importantly, the proposed device exhibited hysteresis resonance and multi-pinched hysteresis; such phenomena have never been reported to date. These characteristics are extremely essential towards futuristic electronics for the development of computational circuits with meliorated performance, electronic systems with improved controllability, memory cells with higher data storage, and in-memory-based processing capabilities.

KEYWORDS

Device fabrication; Electronic devices; Hysteresis; Memristor; Resistive switching; Resonance analysis

1. INTRODUCTION

Nowadays, memory-based electronic applications require high switching rates and low-power consumption, in addition to the storage and processing of huge volumes of data [1]. On the contrary, inadequate switching speed, poor scalability, and high-power requirements are the undesirable issues of the existing widely utilized silicon-dependent dynamic random access memory (RAM), static RAM, and flash memory, which contributed to regenerated interest towards resistive switching (RS) devices [2,3]. RS is a physical occurrence where the memristance of the active layer suddenly alters due to the exertion of a strong electric field or current [4]. RS devices are non-Von Neumann-like architectures that are advantageous in terms of a high degree of parallelism in typical computing systems [5]. Interestingly, apart from non-volatile memory, RS has numerous real-time applications in digital logic such as switches, logic gates, latches, flip-flops, and memristive interconnections in field programmable gate array with low-power consumption, faster, and lower on-chip area [6,7]. Moreover, it has analogue applications, for instance, tunable gain amplifiers, adaptive filters, and chaotic circuits [8–10]. In the case of artificial neural networks RS was used as an artificial human synapse [11,12]. It is important to mention that learning activities inside the human brain occur on account of modulation of synaptic weights; RS-based logic computing systems were engaged to artificially achieve such functionalities [11,12]. Furthermore, RS was employed in circuit breaker operation, to reduce surges in over-voltage transients [13–15].

In 1971, Leon Chua theoretically proposed the first electronic circuit element based on RS, namely the memristor which is a two-pole, inactive, non-linear device [16]. This device has made a revolution in the design optimization of integrated circuits in terms of inexpensive fabrication process, modest switching voltage (< 3 V), high endurance ($> 10^6$ cycles), eminent on-chip area ($\sim 42\%$), reduced power loss ($\sim 10^{-6}$ W), multi-bit (6 bits) operations, and high information storage time (10 years) compared to the existing transistor technology [3,17,18] which led to tremendous advancement in electronic applications. The general structure of the RS device comprises an active layer sandwiched in between two electrodes [6]. Within such a device, the electronic

resistance represents the data stored in it; a high resistive state represents logic *0*, whereas a low resistive state indicates logic *1* [3]. In oxide materials, such as SIO_x, AL_2O_3, ZrO_2, Ta_2O_5, and TiO_2, the RS property was first observed by Hickmott in 1962 [19]. The first RS cell structure based on Au/SiO2/Al was reported by Simmons and Verderber in 1967 [20]. After 33 years, the group of Ignatiev, accelerated research on RS thin films [21]. Thereafter, the RS-CMOS hybrid technology, which contains $Pr0.7Ca0.3MnO_3$ RS device and 500 nm CMOS Technology, was employed to develop a 64-bit Resistive RAM array [22]. Consequently, an increase in the progress of RS memories was made by multi-national companies such as Samsung, Infineon, and HP labs [1,6]. Until today, companies, such as SanDisk, Sony, Toshiba, IMECAS, Unity, and Micron, have been performing a large amount of research on RS technology [1,23,24].

Owing to the interesting features stated above, RS properties were explored in numerous electronic materials. The research to date has tended to focus on RS devices in which ferromagnetic [25], ferroelectric [26], organic semiconductors [27], polymers [3], perovskite oxides [28], and transition-metal oxides (TMOs) [2] were used as an active material. In view of several optimistic properties such as low cost, easy fabrication, low operating voltage, improved scalability, reduced power, faster switching rates, compact integration, and coexistence with widely employed CMOS devices [26], TMOs and ferroelectric materials were mostly preferred. Much of the recent literature has shown that TMOs including ZnO, HfO_2, and TiO_2 provide outstanding electrical and structural attributes [17,29,30]. Due to reduced processing temperature, simple synthesis procedure, and inherent availability of oxygen deficiencies and ions, researchers have preferred ZnO as an active material [31,32]. Consequently, attempts were made to promote the functionalities of the device by inserting the metal impurities into ZnO thin film, to amend the electrical conductivity, defects, oxygen vacancies, and ions [33]. To address this, different metal dopants, such as lithium [34], aluminium [35], copper (Cu) [33], cobalt [36], vanadium [37], titanium [38], and lanthanum [39], were studied. Out of these impurities, Cu has an electron shell which is comparable to zinc (Zn) causing it to conveniently accommodate the ZnO thin film structure [25]. Central to the discipline of non-Von Neumann memory devices, Cu: ZnO is an extremely important active layer for memristive applications owing to its advantages that in ZnO, Cu acts as an electron trap, increases resistivity, and makes ZnO act as a ferroelectric material. Thus, it has been conclusively shown that Cu: ZnO is extremely preferred as an active layer for RS devices [3]. At present, numerous RS devices exist; however, such devices exhibit only a single pinched RS behaviour [40–42]. On the other hand, electronic devices with many resistive states exist to outperform the existing silicon-based CMOS technology [1,43,44]. To embrace the complete perspective of RS devices, multilevel RS has been a significant property [40]. The multilevel RS device stores multi-bit data within a cell, whereas the conventional RS has only two resistive states and thus can store only a single bit of information [40]. Thus, a multilevel RS device can be used to store and retrieve more information for efficient computing applications and outperforms the conventional Von-Neuman architecture in terms of memory requirements [45]. It is important to mention that a multilevel RS device has lower circuit complexity, reduced power consumption, and faster device performance since multiple operations take place within a single device [45–48]. Recent developments in multistate RS devices have demonstrated in-memory parallel processing-based computing applications [49–52]. The inorganic–organic multilayer or hybrid structures based on fundamental materials in RS device cell structure resulted in multistate RS behaviour [53–55]. A multistate RS device with two sets and reset states in opposite voltage regions could store 2-bit data [49]. Until today, a maximum of 92 logic states which can store 6-bit data have been reported within a single RS device [56]. Despite all the above-mentioned technological advantages in RS devices, to the best of our knowledge, no one has yet developed an RS device with multi-pinched hysteresis and resonance within a single device. Such characteristics are essential for futuristic electronics for the development of computational circuits with meliorated performance, electronic systems with improved controllability, memory cells with higher data storage, and amended in-memory-based processing capabilities. Therefore, in this work, we develop such technology for futuristic electronic applications. Initially, Cu: ZnO thin film was synthesized on Cu electrodes to form a Cu/Cu:ZnO/Cu RS device. Function generator and oscilloscope were employed to obtain the electronic characteristics of the device instead of conventional techniques which utilize an expensive source meter. Such an approach leads to an improvement in total equipment expenses. Electronic characterizations of the proposed device were performed to explore its unprecedented current versus voltage (I-V) behaviour.

2. MATERIALS AND METHODS

The Cu:ZnO thin film was blended utilizing economical synthetic methods. As depicted in Figure 1, To obtain the Cu:ZnO solution, initially 653.9 mg of zinc acetate di-hydrate was supplemented with 10 ml of isopropanol

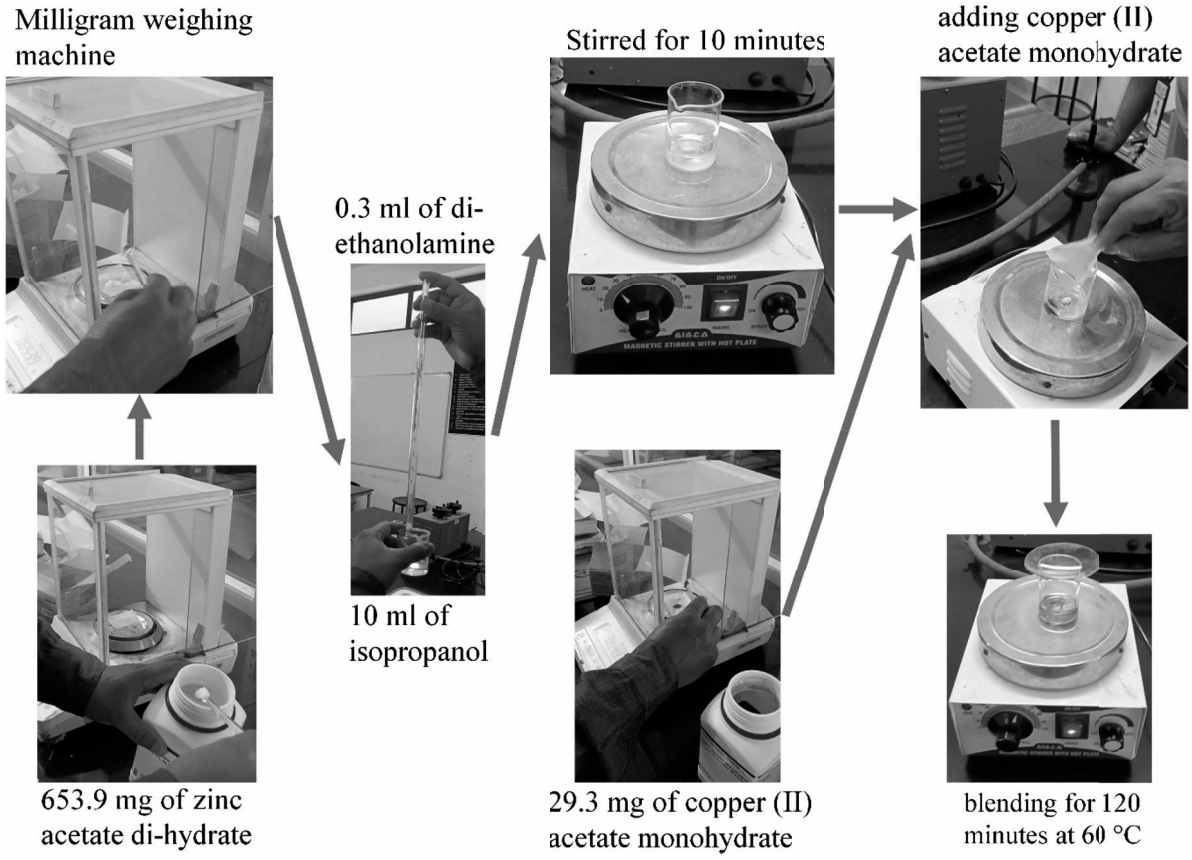

Figure 1: Cu:ZnO solution synthesized through chemical synthesis routes. 653.9 mg of zinc acetate di-hydrate was supplemented with 10 ml of isopropanol and 0.3 ml of di-ethanolamine which was co-mmingled for 10 min at room temperature. 29.3 mg of copper (II) acetate monohydrate contributed to this solution followed by mixing for 120 min at 333 K. This final solution was ripened for 24 h

and 0.3 ml of di-ethanolamine. This arrangement was co-mmingled for 10 min at room temperature. Thereafter, 29.3 mg of copper (II) acetate monohydrate contributed to the pre-arranged zinc acetate followed by mixing for 120 min at 333 K. This final solution was ripened for 24 h. The detailed chemical synthesis process of Cu:ZnO and its physical characterizations were presented in our previous work [3]. In this work, to fabricate the proposed Cu/Cu:ZnO/Cu RS device the solution of Cu:ZnO was dip-coated onto the cylindrical positive copper metal lead of radius 1 mm. Immediately after dipping, the deposited film was heated at 350°C for 120 s in the air through the employment of a soldering rod to attain $\sim$50 nm Cu:ZnO thin film on the Cu electrode. Thereafter, the other (negative) Cu electrode was brought into physical contact with the coated positive electrode to construct the Cu/Cu:ZnO/Cu RS device. This memristor was studied to explore its electronic properties. To generate the I-V characteristics, a function generator and cathode ray oscilloscope (CRO) were employed instead of an expensive source meter. The Scientific SM5060-2 function generator was employed to generate sine and triangular signals of 1 V amplitude and various frequencies ranging from a few Hz to several hundreds of Hz. On the other hand, a CRO *i.e.* Scientific 30 MHz Oscilloscope SM410 was employed to obtain the electronic device characteristic plots. CRO provides voltage versus time waveform at each of its two channels. Initially, the current through the RS device was converted into a voltage signal through the utilization of a current-to-voltage converter circuit. This signal was fed to the first channel (Y), whereas the voltage across the RS device was fed to the second channel (X) of the oscilloscope which was operated in its X–Y mode to get the room temperature I-V plot of the proposed RS component with voltage across the device along X axis, whereas the current (converted into proportional voltage) was plotted along the Y-axis. Similar characteristics were explored in the computational environment through the employment of SPICE simulations.

3. RESULTS AND DISCUSSION

Figure 2(a) displays the memristive current to voltage converter (MCVC) circuit which was employed to obtain the I-V features of the widely used memristor [6,57]

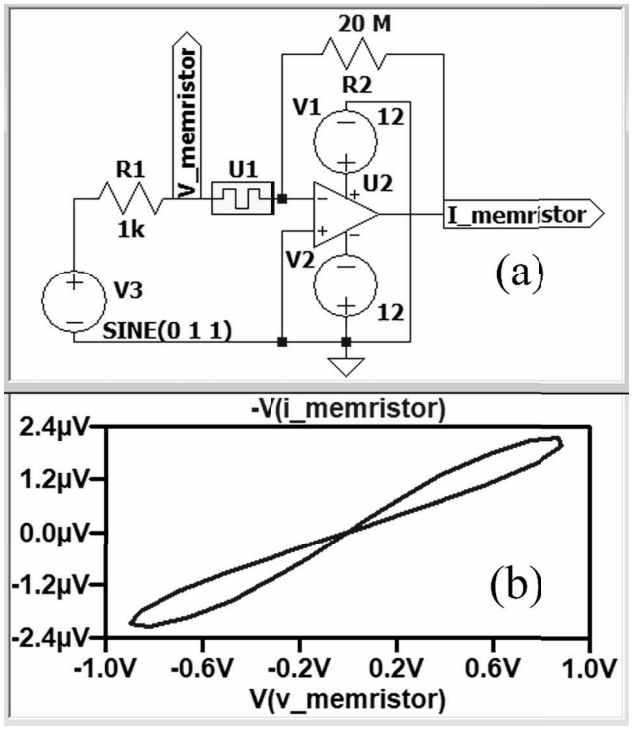

Figure 2: (a) Simulated memristive current to voltage converter (MCVC) circuit, (b) and its I-V characteristics. A sinusoidal signal of 1 V and 1 Hz was applied as an input, resistor R1 was employed to protect the memristor U1 while operational amplifier U2, resistor R2, and voltage sources V1 and V2 were employed to convert the current through the memristor into the proportional voltage signal. The plot between the memristor voltage V(v_memristor) and the output voltage -V(i_memristor) was obtained to be a pinched hysteresis graph

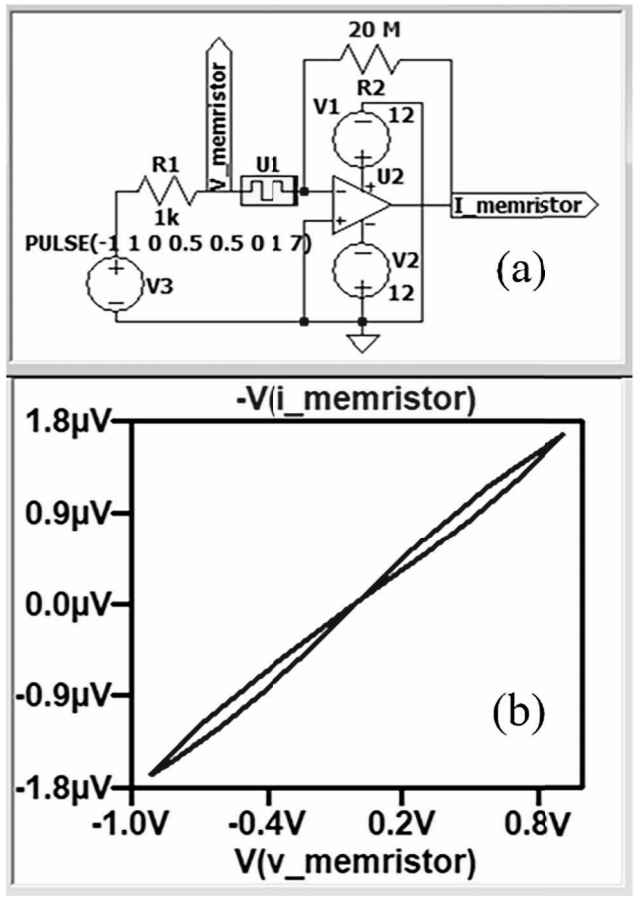

Figure 3: (a) Simulated MCVC circuit with triangular waveform as input signal (b) and its I-V characteristics. Pinched hysteresis characteristics were obtained when the input signal was a triangular waveform of amplitude 1 V and the frequency of 1 Hz possessing equal rise time and fall time of 0.5 s repeated for 7 cycles

through simulation roots. In this case, a sinusoidal signal of 1 V and 1 Hz was applied to the circuit. The resistor R1 was employed to protect the memristor U1 while the operational amplifier U2, resistor R2, and voltage sources V1 and V2 were employed to convert the current through the memristor into the proportional voltage signal. The memristive voltage is labelled as v_memristor, whereas the device current (converted into voltage) was represented as i_memristor at the output terminal. Since the operational amplifier is operating in its inverting mode, the current of the memristor is in its inverted form. Therefore, the negative of the obtain current was considered as the actual current. As revealed in Figure 2(b), the plot between the voltage across the memristor (v_memristor) and the output voltage (i_memristor) was obtained to be a pinched hysteresis graph which represents the mark of any typical memristor. Hence it is proved that the proposed circuit successfully converted the current through the memristor to its proportional output voltage. Such signals are convenient to plot on a CRO. Figure 3(a) depicts an MCVC circuit with a triangular waveform as an input signal of amplitude 1 V and a frequency of 1 Hz. This signal consisted of equal rise time and fall time of 0.5 s and each cycle was repeated for 7 time periods. As pictured in Figure 3(b), pinched hysteresis characteristics were obtained comparable to the situation of the sinusoidal input signal. In the case of hardware, the fabricated Cu/Cu:ZnO/Cu RS device was connected to the MCVC circuit, as exposed in Figure 4. Analogous to the case in Figure 2(a), a 1 kΩ resistor was linked in series to protect the RS device. The function generator was connected as the signal source, whereas a regulated power supply was employed to provide the necessary biasing for the operational amplifier. Series connected 1 MΩ resistors were employed to generate the required 20 MΩ feedback resistance which is to match the measured initial resistance (20 MΩ) of the proposed RS device. The oscilloscope was operated in its X–Y mode to obtain the I-V plot. When the frequency in the function generator was increased to 50 Hz a hysteresis loop was obtained without any pinch, as presented in

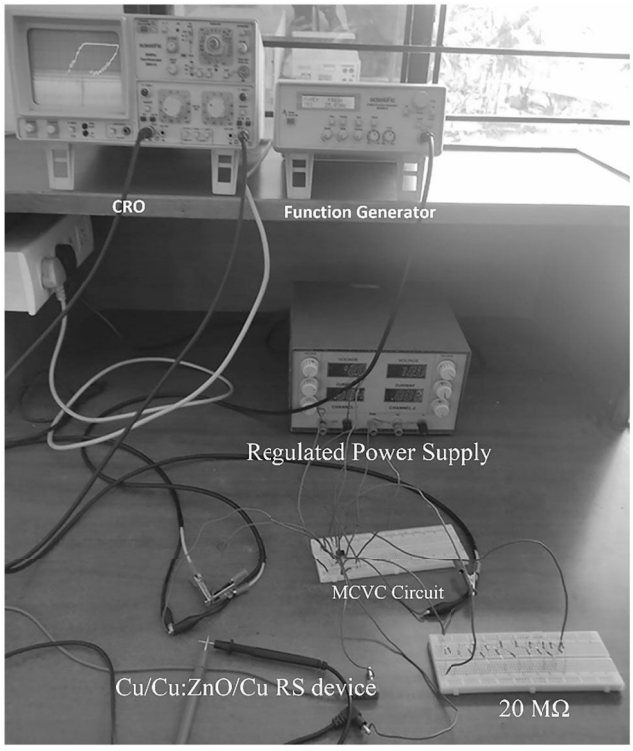

Figure 4: Fabricated Cu/Cu:ZnO/Cu RS device in MCVC circuit. The function generator was connected as the signal source, and a regulated power supply was employed to provide the necessary biasing for the operational amplifier. The oscilloscope was operated in its X–Y mode to obtain a hysteresis loop

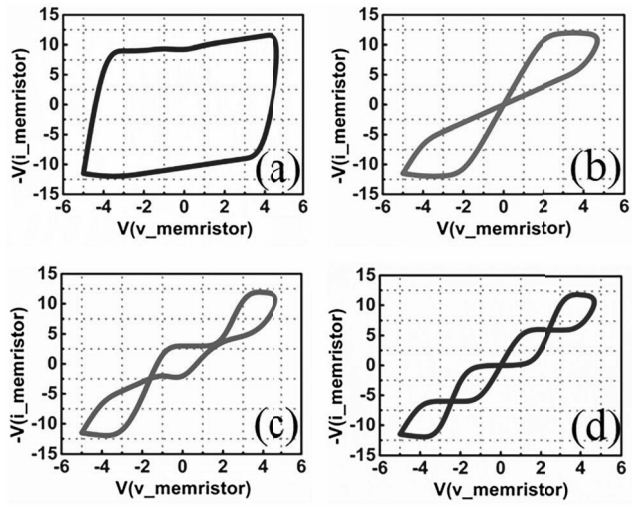

Figure 5: Multi-pinched hysteresis characteristics in Cu/Cu:ZnO/Cu resistive switching devices with (a) zero pinches at 50 Hz, (b) pinched once at 25 Hz, (c), pinched twice at 16.5 Hz, and (d) pinched thrice at 12 Hz. Controlled multi-pinched hysteresis was obtained at different input signal frequencies

Figure 5(a). Whilst this frequency was increased beyond 50 Hz, the waveform was unstable. Nevertheless, at the multiples of 25 Hz *i.e.* at 75, 100, 125, 150 Hz, and so on, the waveform was shifted from an unstable form to a stable state. This phenomenon indicates hysteresis resonance, a phenomenon which has not been reported until today. On the other hand, when the frequency of the applied signal was lowered, the proposed device exhibited multi-pinched switching within the single device. At 25 Hz, as established in Figure 5(b), it was observed that the I-V features of the RS component were pinched once as in the case of a typical memristor. However, when the frequency was reduced to 16.5 Hz, the I-V curve was pinched twice (Figure 5(c)). When the input frequency of the function generator was further reduced to 12 Hz, it was observed that the hysteresis I-V graph of the RS device was pinched thrice, as depicted in Figure 5(d). All these characteristics were due to the drive of earlier mentioned oxygen vacancies and ions (within the active layer of the device) following the applied external signal. Furthermore, numerous pinches in the hysteresis loop were obtained by further reducing the frequency of the function generator. Such properties have never been observed so far in any electronic device to the best of our knowledge.

4. CONCLUSIONS

A Cu:ZnO thin film was synthesized on copper electrodes to form a Cu/Cu:ZnO/Cu RS device. The employment of a function generator and oscilloscope to get the electronic characteristics of the device has resulted in a 96.17% improvement in total equipment expenses compared to conventional techniques which employ expensive source meters. Most importantly, the proposed device exhibited multi-pinched switching and hysteresis resonance at different input signal frequencies, the phenomena which have never been reported. Such properties were owing to the controlled float of oxygen vacancies and ions, within the active layer, per the applied signal. Moreover, it was verified that these properties were not existent in the existing widely used RS device. Such characteristics possess numerous futuristic electronic applications in terms of meliorating in-memory computational circuits, highly controllable electronic systems, and memory cells with improved data storage capabilities.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

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Review Article

Recent breakthroughs in drug delivery systems for targeted cancer therapy: an overview

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ABSTRACT

Targeted drug delivery systems have emerged as promising approaches for improving the efficacy and safety of cancer therapy. This review highlights recent advancements in drug delivery technologies aimed at achieving targeted and personalized treatment strategies for cancer patients. The integration of nanotechnology, biomaterials, and molecular targeting strategies has enabled the development of sophisticated drug delivery systems capable of selectively delivering therapeutic agents to tumour tissues while minimizing off-target effects on healthy tissues. Various targeting mechanisms, including passive and active targeting strategies, exploit the unique physiological characteristics of tumours, such as abnormal vasculature, overexpressed receptors, and altered microenvironments, to achieve selective accumulation and retention of drugs within tumour tissues. Nanoparticle-based drug delivery systems, such as liposomes, polymeric nanoparticles, and inorganic nanoparticles, offer advantages in terms of drug loading capacity, sustained release, and tumour targeting, making them attractive platforms for targeted cancer therapy. Moreover, the integration of smart drug delivery systems that respond to specific stimuli within the tumor microenvironment, such as pH, temperature, or enzyme activity, holds promise for enhancing tumor specificity and reducing systemic toxicity. Combination therapy approaches, which combine targeted drug delivery with other therapeutic modalities, such as immunotherapy or photodynamic therapy, offer synergistic effects and opportunities for overcoming treatment resistance. Despite these advancements, several challenges remain, including the translation of preclinical research findings into clinically viable therapies, regulatory approval, manufacturing scalability, and biomarker discovery. Addressing these challenges and embracing innovative approaches will be essential for realizing the full potential of targeted drug delivery systems in improving patient outcomes and advancing cancer therapy.

1. Introduction

Cancer remains a formidable global health challenge, with its incidence steadily rising and its burden on healthcare systems ever-increasing [1]. Despite significant advancements in our understanding of cancer biology and the development of new

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treatment modalities, effective cancer therapy continues to face several formidable challenges Cancer is not a single disease but rather a complex group of diseases characterized by diverse molecular profiles, cellular behaviours, and responses to treatment [2]. This heterogeneity poses a significant challenge in designing therapies that are effective across all cancer types and individual patients [3].

Conventional cancer treatments, such as chemotherapy and radiation therapy, often lack specificity and can harm healthy tissues and organs in addition to targeting cancer cells (Table 1). This systemic toxicity can lead to severe side effects, compromise patients' quality of life, and limit treatment efficacy. Cancer cells have a remarkable ability to adapt and develop resistance to chemotherapy, targeted therapies, and immunotherapy. This acquired resistance undermines the effectiveness of treatment regimens and contributes to disease progression and relapse [4].

The tumor microenvironment presents numerous physiological barriers that hinder the effective delivery of therapeutic agents to cancer cells. These barriers include abnormal blood vessel structure, elevated interstitial fluid pressure, and dense extracellular matrix components, which collectively limit drug penetration and distribution within tumours [5].

Cancer is increasingly recognized as a heterogeneous disease that requires personalized treatment approaches tailored to individual patients' genetic makeup, tumor characteristics, and clinical profiles. Standardized treatment protocols may not adequately address the unique needs of each patient, highlighting the importance of personalized medicine strategies [6].

In recent years, significant strides have been made in the development of targeted drug delivery systems for cancer therapy, offering new hope in the fight against this devastating disease. This review aims to provide a comprehensive overview of the latest advancements in this rapidly evolving field, focusing on innovative strategies and technologies that hold promise for improving the efficacy and safety of cancer treatment [7].

The scope of this review encompasses a wide range of targeted drug delivery systems, including nanoparticle-based formulations, ligand-mediated targeting approaches, smart drug delivery systems, and combination therapy strategies. By examining recent preclinical and clinical studies, as well as emerging trends and future directions, this review seeks to highlight the most promising developments in targeted drug delivery for cancer therapy [8]. Key areas of focus include advancements in nanoparticle design and engineering, novel targeting ligands and biomarkers, innovative strategies for overcoming physiological barriers within the tumor microenvironment, and the integration of targeted drug delivery systems with other therapeutic modalities such as immunotherapy and gene therapy [9].

Through a critical analysis of the latest research findings and technological innovations, this review aims to provide insights into the potential of targeted drug delivery systems to address the challenges of conventional cancer treatments, including systemic toxicity, drug resistance, and limited therapeutic efficacy [10].

By shedding light on recent advancements in targeted drug delivery for cancer therapy, this review seeks to inform researchers, clinicians, and policymakers about the latest trends and developments shaping the future of cancer treatment. Ultimately, the goal is to accelerate the translation of these innovative technologies from the laboratory to the clinic, ultimately improving outcomes for cancer patients worldwide [11].

2. Targeted and precise treatment strategies to minimize off-target effects and improve efficacy

Conventional cancer treatments, such as chemotherapy and radiation therapy, while effective to some extent, often lack specificity and precision in their targeting of cancer cells. As a result, these treatments can cause collateral damage to healthy tissues and organs, leading to significant adverse effects and compromising patients' quality of life.

Moreover, the non-selective nature of these therapies can contribute to the development of drug resistance and limit their overall efficacy in eradicating cancer cells [12]. To address these challenges, there is a critical need for the development of more targeted and precise treatment strategies in cancer therapy. Targeted therapies aim to selectively deliver therapeutic agents to cancer cells while sparing normal tissues, thereby minimizing off-target effects and improving treatment outcomes. Several key reasons underscore the importance of targeted and precise treatment strategies [13]:

2.1. Minimization of Off-Target Effects

Targeted treatment approaches offer the potential to minimize off-target effects by selectively targeting molecules or pathways that are specific to cancer cells. By sparing healthy tissues from unnecessary exposure to cytotoxic agents, targeted therapies can reduce the incidence and severity of treatment-related toxicities [14].

2.2. Enhancement of Therapeutic Efficacy

By precisely targeting cancer cells and their unique molecular vulnerabilities, targeted therapies can enhance treatment efficacy and improve patient outcomes. By interfering with specific signalling pathways or molecular targets essential for cancer cell survival and proliferation, targeted agents can exert potent anti-cancer effects while minimizing damage to normal tissues [15].

2.3. Reduction of Drug Resistance

Targeted therapies have the potential to overcome mechanisms of drug resistance that often develop in response to conventional treatments. By directly targeting the molecular drivers of cancer growth and progression, targeted agents can circumvent resistance mechanisms and restore treatment sensitivity, thereby prolonging disease control and improving survival outcomes [16].

2.4. Personalization of Treatment

Targeted therapies enable a more personalized approach to cancer treatment by tailoring therapy to the specific molecular characteristics of an individual's tumor. Through molecular profiling and biomarker assessment, clinicians can identify patients who are most likely to benefit from targeted agents, thereby optimizing treatment selection and maximizing therapeutic responses [17].

Table 1. Tabulated Form Outlining the Limitations and Drawbacks of Conventional Chemotherapy and Radiation Therapy [18-20].

Limitations and Drawbacks	Description
Non-Specificity	Chemotherapy and radiation therapy target both cancerous and healthy cells, leading to collateral damage to normal tissues and organs.
Systemic Toxicity	Chemotherapeutic agents are administered systemically, causing widespread distribution throughout the body and toxicity in healthy tissues.
Development of Resistance	Cancer cells can develop resistance to chemotherapy and radiation therapy, reducing treatment efficacy and contributing to disease progression.
Limited Efficacy in Advanced Disease	Conventional treatments may have limited efficacy in advanced or metastatic cancer due to tumor heterogeneity and resistance mechanisms.
Damage to Normal Tissues	Radiation therapy can cause damage to adjacent normal tissues and organs, leading to long-term complications and impairments in organ function.
Treatment-related Morbidity and Mortality	Chemotherapy and radiation therapy can be associated with significant treatment-related morbidity and mortality, particularly in medically frail patients.
Limited Therapeutic Index	The therapeutic index of many chemotherapeutic agents and radiation therapy is narrow, requiring careful balancing of efficacy and toxicity.
Impact on Quality of Life	Side effects and toxicities associated with treatment can significantly impact patients' quality of life, affecting physical, psychological, and social well-being.

2.5. Improvement of Quality of Life

By minimizing off-target effects and treatment-related toxicities, targeted therapies can significantly improve patients' quality of life during and after treatment. Reduced side effects and complications allow

patients to tolerate therapy better, maintain physical function, and preserve their overall well-being, enhancing their ability to adhere to treatment and engage in daily activities [21].

2.6. Principles of Targeted Drug Delivery

Targeted drug delivery is a therapeutic approach aimed at delivering therapeutic agents specifically to their intended site of action, such as diseased tissues or cells while minimizing exposure to healthy tissues. Unlike systemic administration, which involves the distribution of drugs throughout the body via the bloodstream, targeted drug delivery strategies employ various mechanisms to enhance the specificity, precision, and efficacy of drug delivery. There are several key principles underlying targeted drug delivery [22, 23]:

- ✓ Targeted drug delivery systems are designed to selectively recognize and bind to specific molecular targets that are overexpressed or unique to diseased cells or tissues. This selective targeting allows for the precise delivery of therapeutic agents to the site of pathology while minimizing exposure to normal tissues.
- ✓ Targeted drug delivery systems may exploit the unique characteristics of the diseased tissue microenvironment, such as increased vascular permeability, aberrant angiogenesis, or altered cell surface receptors, to enhance the retention and accumulation of therapeutic agents within the target tissue.
- ✓ Targeted drug delivery systems can be engineered to release therapeutic agents in a controlled manner, either in response to specific stimuli within the target tissue (e.g., pH, temperature, enzymatic activity) or over a prolonged period to maintain therapeutic concentrations locally.
- ✓ Targeted drug delivery enables the co-delivery of multiple therapeutic agents, such as chemotherapeutic drugs, targeted inhibitors, or immunomodulatory, to target different aspects of disease pathology simultaneously. This synergistic combination therapy approach can enhance treatment efficacy and overcome mechanisms of drug resistance.

3. Advantages of Targeted Drug Delivery over Systemic Administration

- ✓ By selectively delivering therapeutic agents to the site of pathology, targeted drug delivery minimizes off-target effects and reduces systemic toxicity, thereby improving the therapeutic index of the treatment [24].
- ✓ Targeted drug delivery systems ensure that therapeutic agents reach their intended target at therapeutic concentrations, maximizing their efficacy in eradicating diseased cells or tissues while minimizing exposure to healthy tissues [25].
- ✓ By minimizing exposure to healthy tissues, targeted drug delivery reduces the incidence and severity of treatment-related side effects, such as nausea, hair loss, and immune suppression, improving patients' quality of life during and after treatment [26].
- ✓ Targeted drug delivery systems can overcome physiological barriers within the body, such as the blood-brain barrier or the dense extracellular matrix of solid tumours, allowing for more effective delivery of therapeutic agents to their intended site of action [27].
- ✓ Targeted drug delivery enables a personalized approach to therapy by tailoring treatment to the specific molecular characteristics of an individual's disease, maximizing treatment efficacy while minimizing the risk of adverse effects [28].

4. Targeting Strategies in Drug Delivery

Targeted drug delivery strategies aim to enhance the specificity and efficiency of drug delivery to diseased tissues or cells while minimizing exposure to healthy tissues. These strategies leverage various mechanisms to achieve selective drug delivery, including passive and active targeting mechanisms [29, 30].

5. Passive Targeting Mechanisms

Passive targeting mechanisms exploit the unique physiological properties of diseased tissues or organs to enhance the accumulation and retention of drug delivery systems. These mechanisms include [31-33]:

5.1. Enhanced Permeability and Retention (EPR) Effect

Many tumours exhibit abnormal blood vessel architecture and increased vascular permeability, allowing for the extravasation and accumulation of macromolecular drug carriers within the tumor microenvironment. This phenomenon, known as the EPR effect, enables the passive targeting of drugs to solid tumours [31-33].

5.2. Lymphatic Drainage

Lymphatic drainage pathways in certain tissues, such as the lymph nodes, can facilitate the passive accumulation of drug carriers within lymphatic tissues. This phenomenon is exploited for the treatment of lymphatic disorders and metastatic cancers [31-33].

5.3. Size-Based Targeting

The size of drug carriers can influence their bio-distribution and accumulation within tissues. Nanoscale drug carriers, such as liposomes and nanoparticles, can passively accumulate in diseased tissues due to their small size and prolonged circulation time in the bloodstream (Table 2).

6. Active Targeting Mechanisms:

Active targeting mechanisms involve the specific recognition and binding of drug carriers to molecular targets that are overexpressed or uniquely expressed on the surface of diseased cells or tissues. These mechanisms include [34-37]:

6.1. Ligand-Mediated Targeting

Ligands, such as antibodies, peptides, aptamers, or small molecules, can be conjugated to the surface of drug carriers to facilitate specific binding to cell surface receptors or antigens overexpressed on diseased cells. Ligand-mediated targeting enhances the specificity and efficiency of drug delivery to the target site [34].

6.2. Cellular Targeting

Drug carriers can be engineered to target specific cell types, such as immune cells, stem cells, or cancer stem cells, involved in disease pathogenesis or progression. Cellular targeting strategies exploit cell-specific markers or receptors to achieve selective drug delivery to diseased cells while sparing healthy cells [38].

6.3. Tissue-Specific Targeting

Drug carriers can be functionalized with ligands that target specific tissues or organs based on their physiological properties or molecular signatures. Tissue-specific targeting enables the selective delivery of drugs to diseased tissues, such as the brain, lungs, or inflamed tissues, while minimizing off-target effects [39].

6.4. pH- or Enzyme-Responsive Targeting

Drug carriers can be designed to respond to specific physiological stimuli, such as acidic pH or elevated enzyme activity within the tumor microenvironment. pH- or enzyme-responsive drug carriers undergo structural changes or drug release in response to these stimuli, enabling targeted drug delivery to diseased tissues [35, 37].

7. Advantages of Active Targeting:

Active targeting offers several advantages over passive targeting mechanisms, including [23, 40-42]:

7.1. Enhanced Specificity

Active targeting enables the precise delivery of drugs to the site of action, improving therapeutic efficacy and minimizing off-target effects [42].

7.2. Increased Efficiency

Active targeting enhances the accumulation and internalization of drug carriers within diseased cells or tissues, maximizing drug delivery and therapeutic outcomes [40, 41].

7.3. Customization

Active targeting strategies can be tailored to the specific molecular characteristics of a disease, allowing for personalized medicine

approaches and improved patient outcomes [23, 41, 42].

Table 2. Latest developments in nanoparticle-based drug delivery systems [23, 40-42].

Nanoparticle Type	Description	Recent Developments
Liposomes	Liposomes are spherical vesicles composed of lipid bilayers, commonly used as drug carriers due to their biocompatibility and ability to encapsulate hydrophilic and hydrophobic drugs.	✓ Development of stimuli-responsive liposomes capable of triggering drug release in response to specific physiological cues, such as pH, temperature, or enzyme activity. ✓ Incorporation of targeting ligands, such as antibodies or peptides, onto liposome surfaces to enhance specificity and efficacy of drug delivery. ✓ Exploration of liposome-based mRNA vaccines for infectious diseases and cancer immunotherapy.
Polymeric Nanoparticles	Polymeric nanoparticles are nanoscale particles composed of biodegradable polymers, offering versatile drug delivery platforms with tunable properties, controlled release kinetics, and enhanced stability.	✓ Advancements in nanoparticle engineering to optimize size, shape, and surface properties for improved drug loading, circulation time, and tissue penetration. ✓ Integration of stimuli-responsive polymers, such as pH-sensitive or temperature-sensitive polymers, to achieve controlled drug release in response to specific stimuli. ✓ Application of polymeric nanoparticles for targeted delivery of gene-editing tools, nucleic acid therapeutics, and vaccines.
Inorganic Nanoparticles	Inorganic nanoparticles, such as gold nanoparticles, silica nanoparticles, and quantum dots, exhibit unique physicochemical properties and surface functionalities that make them attractive for drug delivery applications.	✓ Development of multifunctional inorganic nanoparticles with combined therapeutic and diagnostic capabilities, enabling theranostic applications for personalized medicine. ✓ Engineering of inorganic nanoparticles for targeted delivery of anticancer drugs, photo-thermal therapy agents, or imaging contrast agents, exploiting their high surface-to-volume ratio and ease of surface modification. ✓ Exploration of inorganic nanoparticle-based platforms for gene delivery, immunotherapy, and regenerative medicine applications.

8. Advancements in Ligand-Mediated Targeting

✓ Recent advancements in ligand-mediated targeting have focused on enhancing the precision and specificity of drug delivery to diseased tissues or cells. Researchers have identified and characterized new targeting ligands, such as antibodies, peptides, aptamers, and small molecules, that exhibit high affinity and selectivity for molecular targets overexpressed on the surface of cancer cells or diseased tissues [43].

✓ Novel ligands with multifunctional properties have been developed to improve targeting efficiency and therapeutic outcomes. These multifunctional ligands can simultaneously target multiple receptors or molecular pathways implicated in disease progression, enabling synergistic effects and enhanced therapeutic efficacy[44].

✓ Advances in ligand design and engineering have enabled the customization of ligands to optimize their binding affinity, stability, and pharmacokinetic properties. Rational design approaches, computational modeling, and high-throughput screening techniques have facilitated the development of ligands with improved pharmacological properties and reduced off-target effects[45].

✓ Innovative conjugation strategies have been developed to facilitate the conjugation of targeting ligands to drug carriers or nanoparticles with high efficiency and stability. Site-specific conjugation techniques, bio-conjugation chemistry, and click chemistry methodologies enable precise control over

ligand orientation and density on the surface of drug delivery systems, enhancing their targeting specificity and efficacy [46].

- ✓ Recent studies have explored the concept of combination targeting, where multiple targeting ligands are combined to achieve synergistic or complementary effects in drug delivery. By targeting multiple receptors or signalling pathways involved in disease pathogenesis, combination targeting strategies can improve the selectivity and efficiency of drug delivery while minimizing the risk of resistance development [47].
- ✓ Ligand-mediated targeting has been integrated into theranostic platforms for simultaneous imaging and therapy of diseased tissues. By conjugating imaging agents, such as fluorescent dyes or nanoparticles, to targeting ligands, theranostic nanoparticles enable real-time visualization of drug accumulation and distribution within tissues, guiding treatment optimization and monitoring therapeutic responses in vivo [45].
- ✓ Advances in ligand-mediated targeting have led to the development of several targeted drug delivery systems that have progressed into clinical trials for various diseases, including cancer, inflammatory disorders, and infectious diseases. These clinical trials aim to evaluate the safety, efficacy, and pharmacokinetics of targeted therapies in humans, paving the way for the translation of ligand-mediated targeting strategies into clinical practice [43].

9. Smart Drug Delivery Systems

The development of smart drug delivery systems that respond to specific stimuli within the tumor microenvironment represents a promising approach to enhancing the efficacy and specificity of cancer therapy. These systems are designed to exploit the unique physiological characteristics of tumours, such as acidic pH, elevated temperature, and increased enzymatic activity, to trigger drug release selectively within the tumor tissues while minimizing off-target effects on healthy tissues [48].

9.1. pH-Responsive Drug Delivery Systems

Tumours often exhibit acidic extracellular pH due to increased glycolysis and lactate production, which can be exploited for pH-responsive drug delivery. pH-responsive drug carriers, such as liposomes, polymers, and nanoparticles, are designed to undergo structural changes or drug release in response to acidic pH conditions[49]. For example, pH-sensitive liposomes incorporate pH-responsive components, such as acid-labile lipids or polymers, that destabilize the liposomal membrane under acidic conditions, leading to rapid drug release within the acidic tumor microenvironment [50].

Similarly, pH-responsive polymers, such as poly (acrylic acid) (PAA) or poly(histidine), can undergo protonation or deprotonation in response to changes in pH, leading to changes in polymer conformation or solubility and triggering drug release [51].

9.2. Temperature-Sensitive Drug Delivery Systems

Hyperthermia is often used as a therapeutic strategy to selectively heat tumor tissues, which can be exploited for temperature-sensitive drug delivery. Temperature-sensitive drug carriers undergo structural changes or drug release in response to changes in temperature, enabling controlled drug release within hyperthermic tumor tissues [52].

Thermosensitive liposomes and polymers are designed to undergo a phase transition at elevated temperatures, such as the body's normal temperature (37°C) or hyperthermic temperatures (>40°C), leading to drug release from the carrier matrix [53].For example, temperature-sensitive polymers, such as poly (N-isopropyl acrylamide) (PNIPAAm) or poly(N-vinylcaprolactam) (PVCL), exhibit a lower critical solution temperature (LCST) close to body temperature, allowing them to undergo a phase transition from a hydrophilic to a hydrophobic state and trigger drug release at the tumor site during hyperthermia [54].

9.3. Enzyme-Responsive Drug Delivery Systems

Tumors often exhibit elevated enzymatic activity, such as matrix metalloproteinase (MMPs) or proteases, which can be exploited for enzyme-responsive drug delivery. Enzyme-responsive drug carriers are designed to undergo enzymatic cleavage or degradation in response to specific enzymes present in the tumor microenvironment, leading to controlled drug release [51].

Enzyme-responsive peptide linkers or substrates can be incorporated into drug carriers, such as peptides or polymeric nanoparticles, to enable enzyme-triggered drug release[54]. For example, peptide-based drug carriers containing cleavable peptide linkers, such as matrix metalloproteinase-2 (MMP-2) or MMP-9 substrates, can be designed to undergo enzymatic degradation in the presence of overexpressed MMPs within the tumor microenvironment, leading to site-specific drug release [49].

9.4. Dual/Multi-Stimuli-Responsive Drug Delivery Systems

To enhance the specificity and versatility of smart drug delivery systems, researchers have developed dual or multi-stimuli-responsive drug carriers that can respond to multiple stimuli simultaneously or sequentially (Table 3)[49]. For example, dual pH- and temperature-responsive drug carriers can be designed to undergo drug release in response to acidic pH and hyperthermic temperatures within the tumor microenvironment, providing synergistic control over drug release kinetics [53]. Similarly, multi-stimuli-responsive drug carriers can be engineered to respond to combinations of pH, temperature, enzyme activity, or other stimuli present in the tumor microenvironment, allowing for precise spatiotemporal control over drug release [54].

10. Challenges and Future Directions

As with any evolving field, targeted drug delivery systems face several challenges and offer various opportunities for future development [55].

10.1. Overcoming Biological Barriers

10.1.1. Challenge

Drug delivery systems must navigate biological barriers, such as the blood-brain barrier (BBB) or the dense extracellular matrix in solid tumors, to reach their intended target site[56].

Table 3. Potential benefits of smart drug delivery systems [57-59].

Benefits of Smart Drug Delivery Systems	Explanation
Enhanced Tumor Targeting	Targeted delivery to tumours by exploiting specific physiological characteristics (e.g., acidic pH, elevated temperature) for selective drug release within tumor microenvironment.
Minimization of Off-Target Effects	Reduced exposure of healthy tissues to cytotoxic drugs, minimizing systemic toxicity and adverse effects associated with conventional chemotherapy.
Enhanced Therapeutic Efficacy	Precise control over drug release kinetics and spatial distribution within tumor tissues, leading to improved drug uptake by cancer cells and potentiated cytotoxic effects.
Reduction of Drug Resistance	Overcoming mechanisms of drug resistance by bypassing efflux transporters and detoxification pathways, restoring drug sensitivity and improving treatment outcomes.
Personalized Medicine Approach	Tailoring treatment to the specific molecular characteristics of an individual's tumor, optimizing drug delivery strategies for improved therapeutic responses.
Reduced Systemic Toxicity	Lowering systemic toxicity associated with conventional chemotherapy, allowing for higher drug doses, prolonged treatment durations, and improved treatment tolerability.
Synergistic Combination Therapy	Integration with other therapeutic modalities (e.g., immunotherapy, radiotherapy) to achieve synergistic effects and target different aspects of tumor biology, overcoming treatment resistance.

10.1.2. Future Directions

Research efforts focus on developing strategies to enhance drug penetration and retention within target tissues, such as surface modifications, carrier design optimization,

and combination approaches with physical or biochemical interventions [60].

10.2. Precision and Specificity

10.2.1. Challenge:

Achieving precise and specific targeting of diseased tissues while minimizing off-target effects on healthy tissues remains a significant challenge.

10.2.2. Future Directions:

Advancements in targeting ligands, imaging techniques, and computational modeling hold promise for improving the specificity and accuracy of drug delivery systems, enabling personalized medicine approaches tailored to individual patient profiles [61].

10.3. Therapeutic Resistance

10.3.1. Challenge

Cancer cells can develop resistance to targeted therapies and drug delivery systems, limiting treatment efficacy and patient outcomes [62].

10.3.2. Future Directions

Combination therapy approaches, integration with immunotherapy, and novel drug delivery strategies aim to overcome therapeutic resistance by targeting multiple signalling pathways, modulating the tumor microenvironment, and enhancing immune responses [63].

10.4. Clinical Translation

10.4.1. Challenge

The translation of preclinical research findings into clinically viable drug delivery systems faces numerous regulatory, logistical, and financial hurdles [64].

10.4.2. Future Directions

Collaborations between academia, industry, and regulatory agencies, along with investments in clinical trial infrastructure and innovative funding mechanisms, are essential for accelerating the clinical translation of promising drug delivery technologies [65].

10.5. Scalability and Manufacturing

10.5.1. Challenge

Scaling up production and manufacturing of drug delivery systems to meet clinical demand while maintaining product quality and consistency is a complex and resource-intensive process [61].

10.5.2. Future Directions

Advances in manufacturing technologies, process optimization, and quality control methodologies are necessary to streamline production, reduce costs, and ensure the reproducibility and scalability of drug delivery systems[56].

10.6. Biomarker Discovery and Validation

10.6.1. Challenge

The identification and validation of reliable biomarkers for patient stratification, treatment response prediction, and disease monitoring are crucial for the success of targeted drug delivery systems.

10.6.2. Future Directions

Integrating omics technologies, artificial intelligence, and big data analytics to identify and validate biomarkers associated with disease progression, treatment response, and drug delivery system efficacy, enabling personalized and precision medicine approaches [65].

10.7. Regulatory and Reimbursement Landscape

10.7.1. Challenge

Navigating the complex regulatory pathways and securing reimbursement for novel drug delivery systems pose significant barriers to market entry and adoption [66].

10.7.2. Future Directions

Collaboration between regulatory agencies, industry stakeholders, and payers to establish clear guidelines, streamline approval processes, and ensure equitable access to innovative drug delivery technologies, fostering a supportive ecosystem for development and commercialization [65].

11. Conclusion

In conclusion, recent advances in drug delivery systems for targeted cancer therapy mark a pivotal turning point in oncology, offering unprecedented precision and efficacy in treatment strategies. The integration of nanotechnology, smart drug delivery systems, and combination therapies has revolutionized the way we approach cancer treatment, enabling selective delivery of therapeutic agents to tumor sites while minimizing systemic toxicity. These advancements hold immense promise for improving patient outcomes, overcoming treatment resistance, and ushering in a new era of personalized medicine tailored to the specific molecular characteristics of each patient's tumor. However, further research is needed to address challenges such as regulatory approval, manufacturing scalability, and biomarker discovery, ensuring the successful translation of these innovations from bench to bedside. With continued innovation and collaboration, targeted drug delivery systems have the potential to significantly impact cancer therapy and transform the lives of patients worldwide.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publication

All authors read and approved the final manuscript for publication.

Informed Consent

The authors declare not used any patients in this research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Conceptualization: All authors.
Data curation: All authors.
Formal analysis: All authors.
Investigation: All authors.
Methodology: All authors.
Project administration: All authors.
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Advances in Bioresearch

REVIEW ARTICLE

A Review on Bioanalysis of Recently-Approved Kinase Inhibitors

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ABSTRACT

Several steps in the development of cancer are linked to protein kinase dysregulation. There has been a dramatic change in the way cancer is treated since the introduction of protein kinase inhibitors (KIs). The Food and Drug Administration (FDA) has licensed a number of protein kinase inhibitors throughout the past few decades. Recent years (2020-2023) have seen an increase in the number of kinase inhibitors receiving authorization from the FDA. Consequently, there is a growing need for bioanalytical techniques to qualitatively and quantitatively analyze these drugs, and many articles have reported the development, validation, and adoption of such techniques in the context of KIs. The analytical procedures that may quantify KIs in plasma, CSF, urine, tissue, and liver microsomes are described in detail in the majority of published works. Most papers discuss the technological framework that has enabled the assessment of drug concentrations in a range of samples. Plasma, dried blood spots, and tissue analyses all fall under this category. This article provides a comprehensive overview of the several bioanalytical methods now available for determining the concentration of newly licensed kinase inhibitors in biological samples.

Keywords: *Kinase inhibitors, Bioanalysis, Serum, plasma, liquid chromatography-tandem mass spectroscopy (LC-MS/MS), Ultra-high performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS), pharmacokinetics.*

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INTRODUCTION

A critical function for kinases in the tumorigenesis and metastasis of numerous cancers has been disclosed by the latest advances in our knowledge of the basic molecular pathways underpinning tumor cell signaling. The majority of protein kinases are linked to cancer development due to their role in promoting cell proliferation, survival, and migration upon persistent overexpression or activation. Evidence from investigations of kinase mutations throughout the genome suggests that some hereditary variations of certain kinases are directly linked to cancer development, promotion, progression, and recurrence. Mutations and chromosomal rearrangements have been found in various types of cancer

during the last three decades, and these changes have been linked to the regulation and malfunction of protein and lipid kinases and inactive phosphatases. In addition to their roles in metabolic and cell cycle control, protein kinases also play important roles in preserving cells and specialization. Intracellular enzymes called protein kinases govern cell division and proliferation, in addition to the initiation and modulation of immunological responses. Protein kinases are phosphotransferases that attach phosphate group to the serine, threonine, or tyrosine side chain residues in cells. To begin immune cell signaling inside the cell, kinases are required. For example, kinases attach to the internal element found in T and B cell membrane receptors, and when these cells are stimulated by their extrinsic ligands, they initiate intracellular signaling pathways. Protein kinases catalyze the transfer of phosphate (P) from ATP to side chains of serine, threonine, or tyrosine residue in a protein (Figure 1). This phosphorylation serves as a "molecular shift" that may activate or inactivate proteins. In contrast, protein phosphatases are enzymes that remove phosphate groups from proteins, so blocking the activity of kinases and reversing the effects of phosphorylation.

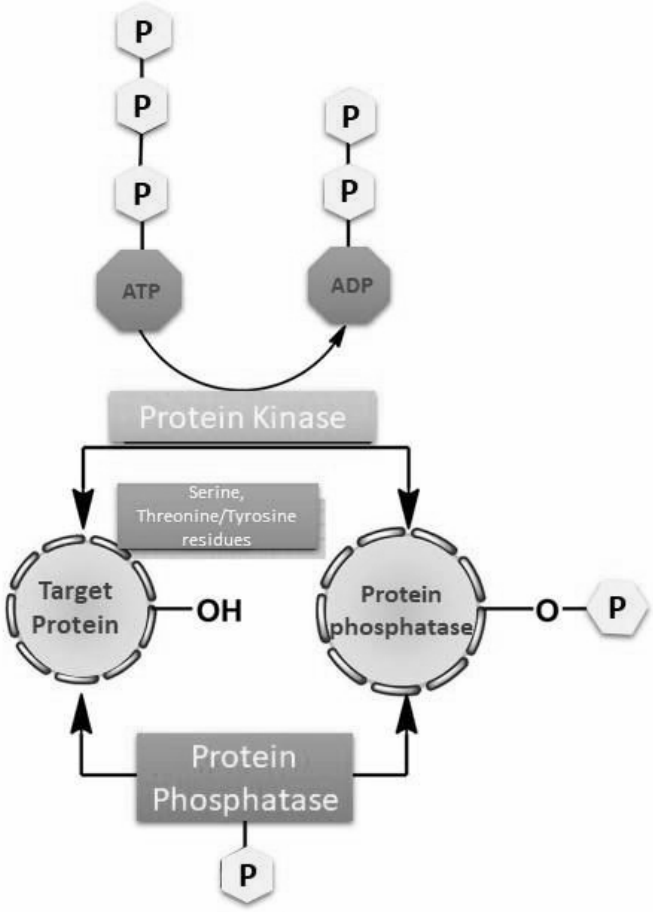

Figure 1: Protein kinase phosphorylation

Several steps in the development of cancer are linked to protein kinase dysregulation. There has been a dramatic change in the way cancer is treated since the introduction of protein kinase inhibitors. The Food and Drug Administration (FDA) has licensed a number of protein kinase inhibitors throughout the past few decades. Recent years (2020-2023) have seen an increase in the number of kinase inhibitors receiving authorization from the FDA, and this study will concentrate on the bioanalytical methods utilized to quantify these compounds.

Avapritinib

Avapritinib (Ayvakit™, Blueprint Medicines Corp.) has been approved by the FDA for the treatment of adults with advanced systemic mastocytosis (AdvSM), which includes patients with aggressive systemic mastocytosis (ASM), systemic mastocytosis with a comorbid hematological neoplasm (SM-AHN), and mast cell leukemia (MCL). Xu et al. looked into a rapid and accurate UPLC-MS/MS technique to confirm and quantify avapritinib content in rat plasma. Avapritinib and IS produced believable improvements in terms of recovery, stability, and matrix impact. A single oral dosage of avapritinib (30 mg/kg) was given

to the rats. Avapritinib concentrations in pharmacokinetic studies were determined using the proposed technique [1]. To develop an LC-MS/MS-based approach that is both straightforward and reliable for measuring avapritinib in plasma from rats. Both the intraday and interday %CV readings were confirmed to be acceptable. Freeze-thaw, Autosampler, benchtop, and long-term stability testing all showed the medication to be stable [2].

Brigatinib

Brigatinib (ALUNBRIG, ARIAD Pharmaceuticals Inc.) was authorized by the FDA for the treatment of adults with anaplastic lymphoma kinase (ALK)-positive, metastatic non-small cell lung cancer (NSCLC). For the purpose of quantifying brigatinib, alectinib, and lorlatinib in human plasma samples, an LC-MS/MS assay was designed and validated. Brain, liver, kidney, and spleen homogenates were also used to partly validate the procedure, along with diluted mouse plasma. Most of the conditions tested did not affect the compounds' stability. The effects of brigatinib on the body and how it's distributed in the body's tissues have been documented in a preliminary investigation [3]. The concentration of brigatinib in human plasma was determined using HPLC-ESI-MS/MS and a brigatinib-D6 internal standard (IS) prepared in accordance with a standardized protein precipitation procedure. The linear regression model yields a standard curve spanning a range of 15.00-120.00 pg/ml and a correlation coefficient (r2) exceeding 0.999 [4].

Cabozantinib

The FDA authorized Cabozantinib (Cabometyx, Exelixis, Inc.) on September 17, 2021, for the treatment of adults and children over the age of 12 with metastatic differentiated thyroid cancer (DTC) that continues to advance even with VEGFR-targeted therapy and in individuals who are unsuitable for or resistant to radioactive iodine. For the determination of Cabozantinib in human plasma, Srikanth Inturi et al. developed a straightforward, sensitive, and specific LC-MS/MS approach. Freeze-thaw, benchtop, and postoperative stability investigations all showed that Cabozantinib was stable [5]. A pharmacokinetic investigation of Cabozantinib in rats using this technique yielded positive results [6]. High throughput is ensured by an efficient and reliable assay, which has been effectively utilized in the monitoring of KI levels in patients [7]. Another analytical technique has been established for the routine measurement of Cabozantinib levels in human plasma. The half-life of the medication in plasma is 48 hours at room temperature or 4 °C [8], while in whole blood it is at least 6 hours at room temperature (after sample).

Capmatinib

Capmatinib (Tabrecta, Novartis Pharmaceuticals Corp.) received full FDA approval on August 10, 2022, for the treatment of adult patients with metastatic NSCLC whose tumors possess a genetic mutation that results in mesenchymal-epithelial transition (MET) exon 14 deletions. Following oral therapy of 5, 10, and 20 mg/kg of Capmatinib in rats, pharmacokinetic research was conducted, and UPLC-MS/MS indicated exceptional results in linearity, precision, reliability, and stability [9]. Capmatinib in plasma was quantified for the first time using two novel HPLC techniques that use Fluorescence detection (FLD) and Photodiode Array Detection (DAD). These techniques may be used to augment pharmacokinetic research, especially in bioanalytical laboratories without LC-MS/MS instruments [10]. Capmatinib concentration in human plasma was determined using an LC-MS/MS approach that was subsequently validated for use in rabbit pharmacokinetic studies. Pharmacokinetics were studied following oral therapy of Capmatinib to healthy rabbits, validating the established approach [11]. Capmatinib (INC280) in rat plasma was quantified using an LC-MS/MS technique. This validated technique is currently being employed for the measurement of Capmatinib in pre-clinical investigations after having been successfully deployed to assess the pharmacokinetics of the drug using plasma samples from rats [12].

Crizotinib

Crizotinib (Xalkori, Pfizer Inc.) was authorized by the FDA on July 14, 2022, for the treatment of adults and children older than 1 year who have unresectable, relapsed, or refractory inflammatory ALK-positive myofibroblastic tumors (IMT). In order to determine alectinib (ALC), ceritinib (CER), and crizotinib (CRZ) in rat plasma simultaneously, a novel UPLC-MS/MS technique was designed and validated. To further investigate the potential PK interaction between bromelain and the selected drugs in Wistar rats, the suggested approach was used. The results showed that the ingestion of bromelain with CER or CRZ induced a considerable drop in plasma levels of these drugs [13]. To assist current clinical and preclinical pharmacokinetic research, an LC-ESI-MS/MS technique for measuring crizotinib in human and mouse plasma was established. Crizotinib levels in plasma samples were effectively analyzed using this approach in phase I pediatric trials [14], suggesting the usage of crizotinib for the therapy of pediatric brain tumors. Rat plasma [15] and human plasma [16] crizotinib concentrations were determined using an LC-MS/MS technique. Crizotinib was effectively quantified and its pharmacokinetics were studied in rats

using this LC-MS/MS test after both intravenous and oral administration of the drug. Oral administration of crizotinib resulted in a 68.6 ± 9.63% absolute bioavailability in rats [17]. Researchers have also used LC-MS/MS to analyze the pharmacokinetics of CRZ and CRZ-lactam in human plasma following a single oral dosage of 250 mg. According to the findings, CRZ was promptly converted into its metabolite, crizotinib-lactam, which had in vivo exposure that was 38.50 percent lower than that of crizotinib [18]. The toxicity caused by crizotinib is a major clinical concern. Investigating which bodily systems this chemical affects would need tissue distribution research. A simple LC-MS/MS technique for the detection of crizotinib in different mouse tissues was devised. The research indicated that the highest concentrations of crizotinib were in the digestive system, with the lungs, liver, and spleen serving as secondary targets. Crizotinib toxicity might be better understood because of this study since it gives a dependable approach to measuring the drug [19]. Therapeutic drug monitoring (TDM) of TKIs has been proven to increase treatment success and decrease adverse events in many trials. This led to the development and validation of an LC-MS/MS technique for the TDM of 12 TKIs, including crizotinib, in individuals with NSCLC, which is now used for regular TDM of these TKIs. NSCLC patients may benefit from individualized dosage modification and better management of side effects by monitoring the plasma levels of TKIs [20].

Dabrafenib

On March 16, 2023, the FDA authorized the combination of dabrafenib (Tafinlar, Novartis) and trametinib (Mekinist, Novartis) for the systemic treatment of children and young adults aged 1 and older who have been diagnosed with low-grade glioma (LGG) with a BRAF V600E mutation. Both medications have had new oral formulations authorized by the FDA that make them easier for individuals with swallowing difficulties to use. Using EMA recommendations, a micellar liquid chromatographic technique was designed and validated for the determination of dabrafenib and other kinase inhibitors in plasma. With little investment of money, time, energy, and potentially less harmful substances, the process was successfully completed. The technique proved helpful for clinical analysis [21] since it allowed for the determination of the medications' target concentrations. Human plasma (22-24] and mouse plasma [25] dabrafenib concentrations were determined using an LC-MS/MS method that was developed and validated. The proposed approach has been shown to be useful for pharmacokinetics and bioequivalence research due to its high sensitivity, higher accuracy, precision, and excellent recovery for the plasma samples [26]. The LC-MS/MS generic test has shown to be a valuable resource in the quest to better characterize the pharmacology of dabrafenib, and it might serve as a foundation for the development of drug-specific analyses with an even greater characterization of their performance [27]. The clinical viability of volumetric absorptive micro sampling (VAMS) was confirmed by its effective implementation in real-world situations. Samples of capillary blood were analyzed for dabrafenib concentration using VAMS by certified medical personnel or by patients themselves at home [28].

Encorafenib

Encorafenib (BRAFTOVI, Array BioPharma Inc.) in combination with cetuximab has been authorized by the FDA for the treatment of adults with BRAF V600E mutation–positive metastatic colorectal cancer (CRC) after previous therapy. The concentration of Encorafenib in rat plasma was determined using a recently designed and completely validated LC-MS/MS bioanalytical technique. When administered orally, Encorafenib (20 mg/kg), pharmacokinetic parameters may be efficiently quantified attributable to the proposed assay's established broad range of calibration curves. The current method stands out due to its efficient extraction recovery and its resilience to matrix influence. Having a total run duration of 2 minutes and a verified sensitivity of 0.2 ng/mL, this assay is suitable for efficient routine tests in pharmacokinetic investigations [29].

Futibatinib

Futibatinib (Lytgobi, Taiho Oncology, Inc.) was given expedited authorization by the FDA on September 30, 2022, for the treatment of adults with recurrent, inoperable, or metastatic intrahepatic cholangiocarcinoma that contains a fusion or reorganization of the fibroblast growth factor receptor (FGFR)-2 gene. For use in the metabolic stability test, a UPLC-MS/MS analytical technique for quantifying futibatinib was developed and validated. This technique provides a precise, sensitive, and time-efficient way of assessing the microsomal stability of futibatinib in HLMs [30] The approach has been effectively used to study the pharmacokinetics of futibatinib in beagle dogs, and it was also an excellent tool for determining its levels in plasma. Drug-drug interaction (DDI) research might also benefit from this methodology [31].

Ibrutinib

Ibrutinib (Imbruvica, Pharmacyclics LLC) was authorized by the FDA on August 24, 2022, for the treatment of children patients above 1 year of age, who have been suffering from cGVHD after having

previously failed on 1 or more lines of systemic medication. It was stated that the drug remained stable for longer periods under different stability settings, and the established LC-MS/MS technology was effectively adaptable to the routine investigation of ibrutinib in biological matrices [32]. Ibrutinib (IBR) and its metabolite dihydrodiol-ibrutinib (DIBR) in human plasma were quantified using LC-MS/MS and shown to be reliable throughout a concentration range of 0.5 to 100 ng/ml. Bile acids were shown to interact with DIBR during the evaluation of plasma samples from a clinical investigation in people with hepatic impairment. Samples from people with hepatic impairment showed considerable interference, but this had no effect on the outcomes of any of the other clinical investigations reviewed [33]. Whether kept in the fridge or the freezer, both IBR and DIBR were shown to be stable [34]. Human CSF and plasma samples [35], beagle dog plasma [36], and rat plasma [37] were used to effectively quantify ibrutinib and PCI-45227 using UHPLC-MS/MS. Lenalidomide, ibrutinib, and the active metabolite PCI-45227 were all simultaneously estimated using an LC-MS/MS to assist pharmacokinetic research in Wistar rats. In a re-analysis study, Veeraraghavan et al. (2015) showed that the assay could be replicated with reliability using data from 18 replicate samples.

Infigratinib

Infigratinib (Truseltiq, QED Therapeutics, Inc.) recently received expedited authorization by the FDA for the treatment of patients with already treated, inoperative locally progressed or metastatic cholangiocarcinoma who also have an FGFR2 fusion or other rearrangements. Using an LC-MS/MS analytical technique, the metabolic stability of INF was evaluated, and it was shown to have a modest extraction ratio, suggesting relatively excellent anticipated oral bioavailability in HLMs in vitro tests [38]. By administering 10 mg/kg INF through gavage to SD rats, the pharmacokinetics of this compound were studied, and the major pharmacokinetic characteristics were derived via the use of an analytical technique devised using UPLC-MS/MS to measure the content in plasma. Xu et al. found that patients using numerous oral medicines (e.g., CYP3A inducers or inhibitors) or individuals with liver or renal impairment may need TDM to obtain customized doses of INF [39].

Lenvatinib

The FDA has authorized the first-line therapy of adults with advanced renal cell carcinoma (RCC) with a combination of lenvatinib (Lenvima, Eisai) and pembrolizumab (Keytruda, Merck). Using LC-MS/MS, Talari et al. (2022) validated the bioanalytical technique and analyzed the pharmacokinetics of Lenvatinib and its metabolites in rat plasma. In a different investigation, an LC-MS/MS technique was established for the determination of lenvatinib concentrations in human plasma [40]. Lenvatinib was shown to be stable in both human serum and phosphate-buffered saline (PBS) throughout a range of stability tests. Results from clinical trials demonstrating lenvatinib's strong protein binding in serum confirmed its effective application for in vivo protein binding investigations [41]. Five different labs developed seven different bioanalytical procedures using LC-MS/MS, and the results imply that lenvatinib levels in human plasma may be analyzed across labs and clinical trials [42]. Qualitative investigation [43] confirmed the viability of this approach for assessing Lenvatinib's kinetic distribution. Lenvatinib in human plasma was quantified by RP-HPLC for therapeutic and pharmacokinetic research [44]. Lenvatinib was shown to reduce the systemic intake of telmisartan, as determined by simultaneous estimate using UPLC-MS/MS. Cui et al. (2022) found evidence of a possible pharmacological link between lenvatinib and telmisartan.

Lorlatinib

Lorlatinib (Lorbrena, Pfizer Inc.) was given authorization by the FDA on March 3, 2021, for the treatment of patients with metastatic NSCLC with ALK-positive tumors, as identified by an FDA-approved test. Preliminary pharmacokinetic investigations in male and female wild-type mice were successful because of the development and validation of a bio-analytical LC-MS/MS assay for lorlatinib in mouse plasma [45] and tissue homogenate [46]. The maximum blood levels of lorlatinib (2,705.683 ± 539.779 μg/L) were reached at 0.625 ± 0.231 h after oral treatment. The kidneys had the lowest concentration (548.83 ng/100 mg), while the liver had the highest (3,153.93 ng/100 mg) and the stomach had the third-highest (2,159.92 ng/100 mg) [47]. The results of a second, completely separate experiment (cross-validation) on lorlatinib homogenate samples confirmed the original results. The therapeutic use of UPLC-MS/MS for quantifying lorlatinib in human plasma was proven by quantifying numerous samples from a pharmacokinetic trial for individuals with lung cancer [48].

Mobocertinib

Mobocertinib (Exkivity, Takeda Pharmaceuticals, Inc.) received an expedited authorization by the FDA for the treatment of adults with NSCLC who experience progression of disease despite receiving platinum-based chemotherapy and whose tumors have been shown to have epidermal growth factor receptor (EGFR) exon 20 insertion modifications, as per an FDA-approved test. Rat plasma Mobocertinib concentrations were determined using an LC-MS/MS technology that was designed and optimized

specifically for this purpose. Mobocertinib pharmacokinetics were investigated in rats given 2, 6, and 18 mg/kg by oral gavage using this methodology. The results showed that mobocertinib was stable in the investigated settings. After being given orally to rats at doses ranging from 2.0 to 18.0 mg/kg, mobocertinib exhibited linear pharmacokinetic properties [49].

Neratinib

On February 25, 2020, neratinib plus capecitabine received authorization from FDA for use in adult patients with advanced or metastatic HER2-positive breast cancer who were given more than 2 previous anti-HER2-based therapies in the metastatic setting. Determination of neratinib was performed using a bioanalytical UPLC-MS/MS approach in rat plasma and tissue homogenates [50], in human plasma [51-52] and in rat plasma [53]. In biological metrics, the approach was successfully used for the pharmacokinetic study of pure formulations. Oral simultaneous treatment with neratinib and apigenin was investigated for potential DDIs by Maher et al. (54]. TDM of cancer sufferers on such regimens will benefit greatly from the findings of this investigation. Results from estimating neratinib and naringenin in rat plasma by UPLC-MS/MS [55] and neratinib and curcumin in human plasma supported the method's sustainability. Quantitation of neratinib in human plasma was also performed using an LC-MS/MS and a stable internal standard [56].

Osimertinib

For use in individuals with NSCLC with tumors that have EGFR exon 19 omissions or exon 21 L858R modifications, as identified by an FDA-approved test, FDA granted authorization to osimertinib (TAGRISSO, AstraZeneca Pharmaceuticals LP) as adjuvant therapy following tumor surgery. A precise bioanalytical UHPLC-MS/MS technique for osimertinib and its metabolites was developed to investigate their metabolic pathway. Osimertinib showed only moderate stability. Samples from patients receiving 80 mg of osimertinib once a day showed that the test was clinically applicable since they contained measurable and quantifiable quantities of all studied chemicals [57]. Osimertinib is among the several kinase inhibitors that have been studied and analyzed using an LC-MS/MS that has been validated for use in normal clinical practice [58-61]. High throughput was achieved using this quick and sensitive technique to track kinase inhibitor levels in patients [62]. An HPLC-UV/DAD was validated for quantifying osimertinib along with other KIs in order to offer a more practical substitute. After comparing results to LC-MS/MS, researchers concluded that the newly developed HPLC-UV/DAD technology is "fit-for-TDM" in clinical practice and provides a viable alternative to LC-MS/MS.

Pemigatinib

Pemigatinib (Pemazyre, Incyte Corporation) was authorized by the FDA on August 26, 2022, to treat individuals with recurrent or refractory myeloid/lymphoid neoplasms (MLNs) that have FGFR1 translocation. In order to quantitatively evaluate the metabolic stability of pemigatinib in human liver microsomes (HLM), an LC-MS/MS analytical approach was devised. The extraction ratio for PMB was modest, indicating high bioavailability [63]. A UPLC-MS/MS technique has been established to quantify pemigatinib concentration in rat plasma. In a pharmacokinetic investigation, the test was also acceptable for detecting the blood levels of pemigatinib following a single oral administration of 1.35 mg/kg to rats [64].

Pralsetinib

Pralsetinib (GAVRETO, Blueprint Medicines Corporation) was granted FDA approval for the treatment of adults and children over the age of 12 with RET-mutant medullary thyroid cancer (MTC) who need chemotherapy, as well as those with RET fusion-positive thyroid cancer who need chemotherapy and are radioactive iodine-refractory. K2-EDTA plasma [65] and mouse plasma [66] were analyzed for Pralsetinib concentrations using LC-MS/MS. Both plasma samples showed stability for a minimum of 7 days when stored at 2-8 degrees Celsius, and for at least 24 hours when stored at 15-25 degrees Celsius.

Ripretinib

In adults with advanced gastrointestinal stromal tumors (GIST), ripretinib (QINLOCK, Deciphera Pharmaceuticals, LLC.) has been authorized by the FDA for use in combination with three or more KIs. Ripretinib was measured in rat plasma using HPLC-FLD, and this approach may be useful for pharmacokinetics and bioequivalence investigations of ripretinib in plasma samples [67]. To determine the ripretinib levels in the plasma of beagle dogs, a novel and robust UPLC-MS/MS method was devised and improved to its utmost potential. Itraconazole and voriconazole were shown in a pharmacokinetic investigation to enhance the plasma clearance of ripretinib in beagle dogs by inhibiting its metabolism [68].

Ruxolitinib

The FDA granted approval to ruxolitinib (Jakafi, Incyte Corp.) on September 22, 2021, for the treatment of chronic graft-versus-host disease (cGVHD) in patients above 12 years who had previously failed on one or

two lines of systemic therapy. Human plasma and serum were analyzed using LC-MS/MS to determine the concentrations of ruxolitinib and other kinase inhibitors [69,70]. Charlier et al. (2019) verified RP-HPLC with FLD in plasma samples, proposing that it should be taken into account alongside other methods. For the purpose of tracking the drug levels of kinase inhibitors, including ruxolitinib, in dried capillary blood, a volumetric absorptive micro sampling (VAMS) approach was developed and validated [71].

Selpercatinib

For adults with locally advanced or metastatic NSCLC with a rearranged during transfection (RET) gene fusion, selpercatinib (Retevmo, Eli Lilly, and Company) was licensed by the FDA on September 21, 2022. LC-MS/MS was used to design and validate a bioanalytical test for selpercatinib and Pralsetinib in mouse plasma [72], in mouse tissue homogenates [73] and human K2-EDTA plasma [74] samples. Drug stability was not affected by matrix effects or extraction inefficiencies under any of the scenarios tested. Additionally, this approach was used in a selpercatinib mouse pilot trial, which was followed by a cost-effective reanalysis of the original sample.

Selumetinib

Selumetinib (KOSELUGO, AstraZeneca) was granted FDA approval for the treatment of incurable plexiform neurofibromas (PN) in children and young adults with neurofibromatosis type 1 (NF1). The clinical study is being supported by the application of robust and sensitive LC-MS/MS techniques for the detection of selumetinib along with its metabolites, which were validated using human biological samples [75,76].

Tivozanib

Tivizanib (Fotivda, AVEO Pharmaceuticals, Inc.), a kinase inhibitor, has been authorized by the FDA for the treatment of adults with relapsed or refractory advanced renal cell carcinoma (RCC) after two or more previous systemic therapies. A preliminary bioanalytical assay for tivozanib has been developed and validated across a linear range of 0.5–5000 ng/mL in human plasma, mouse plasma, and tissue homogenates. In this study, they showed that the linear range could be increased from 2-4 decades by detecting two MRM transitions for tivozanib. Pharmacokinetic experiments in mice and a transport test both used the LC-MS/MS assay with acceptable results [77]. To determine the concentration of tivozanib in rat plasma and liver microsomes, researchers created and employed two novel HPLC techniques combined with FLD or DAD The presented techniques are appropriate for facilitating in vivo and in vitro tivozanib research, notably DDI studies, especially in bioanalytical laboratories without LC-MS/MS capabilities [78] due to their accessibility, speed, and cost-effectiveness.

Tucatinib

Tucatinib (Tukysa, Seagen Inc.) alongside trastuzumab received accelerated approval from the FDA on January 19, 2023, for the treatment of RAS wild-type, HER2-positive colorectal cancer that has advanced after receiving fluoropyrimidine, oxaliplatin, and irinotecan-based chemotherapy and is incurable or has metastasized. To investigate the impact of quercetin on tucatinib metabolism in rats, a UPLC-MS/MS was built and effectively used to measure tucatinib levels in rat plasma. There may be therapeutic relevance to the interaction between quercetin and tucatinib at high doses. Three labs employing five different approaches analyzed tucatinib plasma levels for pharmacokinetic analysis utilizing MS/MS approach. Using a 'base' bioanalysis done by one laboratory and technique, a five-way cross-validation approach was designed to verify the accuracy of the other four procedures. For a more comprehensive population pharmacokinetic study, researchers were able to pool data from various clinical trials of tucatinib using this strategy [79].

Zanubrutinib

On Jan 19, 2023, FDA approval was granted to zanubrutinib (Brukinsa, BeiGene USA, Inc.) for chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). Clinical patient plasma levels of zanubrutinib were quantified using a validated LC-MS/MS technique. Mouse plasma zanubrutinib levels were also quantified using the same technique. After orally administering zanubrutinib to beagle dogs, a UPLC-MS/MS technique was established for its quantification in the dogs' plasma [80].

Table 1: A Summary of the bioanalytical techniques reported in this article

Drug	Analytical technique	Biological matrix
Avapritinib	UPLC-MS/MS	Rat plasma
	LC-MS/MS	Rat plasma
Brigatinib	LC-MS/MS	Human plasma
	HPLC-ESI-MS/MS	Human plasma
Cabozantinib		Human plasma
		Rat plasma

	LC-MS/MS	Human serum and plasma
		Human plasma
Capmatinib	UPLC-MS/MS	Rat plasma
	HPLC	Rat plasma, human liver microsomes
	LC-MS/MS	Human and rabbit plasma
		Rat plasma
Crizotinib	UPLC–MS/MS	Rat plasma
	LC-ESI–MS/MS	Human and mouse plasma
	LC-MS/MS	Human plasma
		Rat plasma
		Human plasma
		Mouse tissues
		Plasma samples from patients with NSCLC
Dabrafenib	Micellar liquid chromatography	Plasma
	LC-MS/MS	Human plasma
		Mouse plasma
	Volumetric absorptive microsampling (VAMS)	Plasma samples
Encorafenib	LC-MS/MS	Rat plasma
Futibatinib	UPLC-MS/MS	Human liver microsomes
		Beagle dog plasma
Ibrutinib	LC-MS/MS	Human plasma
		Wistar rat plasma
	UHPLC-MS/MS	Human CSF and plasma
		Beagle dog plasma
		Rat plasma
Infigratinib	LC-MS/MS	Human liver microsomes
	UPLC-MS/MS	SD rats plasma
Lenvatinib	LC-MS/MS	Rat plasma
		Human plasma
		Human serum
		Human plasma
	RP-HPLC	Human palsma
	UPLC-MS/MS	Rat plasma
Lorlatinib	LC-MS/MS	Mouse plasma and tissue homogenate
		Mouse plasma
		Mouse serum and tissue sample
	UPLC-MS/MS	Human plasma
Mobocertinib	LC-MS/MS	Rat plasma
Neratinib	UPLC–MS/MS	Rat plasma and tissue homogenates
		Human plasma
		Rat plasma
	LC-MS/MS	Human plasma
Osimertinib	UHPLC–MS/MS	Human plasma
	LC-MS/MS	Human serum
		Human plasma
		Serum and plasma
		Plasma and CSF
	HPLC-UV/DAD	Human serum
Pemigatinib	LC-MS/MS	Human liver microsomes
	UPLC-MS/MS	Rat plasma

Pralsetinib	LC-MS/MS	K2-EDTA plasma
		Mouse plasma
Ripretinib	HPLC-FLD	Rat plasma
	UPLC-MS/MS	Beagle dog plasma
Ruxolitinib	LC-MS/MS	Human plasma
		Human serum and plasma
	RP-HPLC	Plasma samples
	LC-MS/MS with volumetric absorptive microsampling (VAMS)	Dried capillary blood
Selpercatinib	LC-MS/MS	Human K2-EDTA plasma
		Mouse plasma and tissue homogenates
		Mouse plasma
Selumetinib	LC-MS/MS	Human biological matrices
Tivozanib	LC-MS/MS	Mouse plasma
	HPLC	Rat plasma and liver microsomes
Tucatinib	UPLC–MS/MS	Rat plasma
	MS/MS	Human plasma
Zanubrutinib	LC-MS/MS	Human plasma
		Mouse plasma
	UPLC–MS/MS	Beagle dog plasma

FUTURE PROSPECTS

Simple, high-throughput procedures that need few or no human interactions are ideal for bioanalysis. The use of chromatographic separation continues to be vital, and the decrease in analysis time shown over the last several decades is a clear indication of this. We expect that MS detection will continue to be the gold standard for bioanalysis, particularly for quantitative purposes. As MS detectors have become more reasonably priced, they may be found at a wider variety of research and academic institutions. Some scientists choose to use Q-TOF or Q-Orbitrap equipment for their high-resolution mass spectrometry. Not mentioned previously in this paper is the ion-mobility interface, which has the potential to improve chromatography-free bioanalysis and might potentially distinguish isobaric chemicals or even chiral molecules.

Using rat/HLMs, various research looked into the drug metabolites and biotransformation routes of different kinase inhibitors. The results highlight the need to include liver microsomes in subsequent bioanalyses of KI candidates to strengthen metabolic studies.

KIs bioanalysis is crucial for the study of cancer and therapeutic development. In recent years, numerous assays have been developed to shed light on topics such as drug metabolism These techniques, in conjunction with research into the desired therapeutic drug level and the clinical dose-effect connection, may be used for TDM to increase clinical effectiveness and decrease the toxicological effects of KIs.

CONCLUSION

The bioanalysis of KIs is a crucial technique for gaining a deeper understanding of the complete efficacy-toxicity ratio of KIs, which can then be utilized to enhance their therapeutic efficacy. While multianalyte and metabolite analysis are on the rise, chromatographic techniques remain the most employed separation method for KI bioanalytical tests. The majority of KI bioanalytical procedures use the US FDA and EMA criteria as their validation framework. To sum up, LC-MS/MS is the gold standard separation method. Future qualitative investigations may make more use of UPLC-MS/MS.

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Bacterial Nanocarriers for Site-Specific Drug Delivery: Harnessing Microorganisms for Precision Medicine

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Abstract:
Bacterial nanocarriers represent a promising approach for site-specific drug delivery, offering precise targeting and controlled release of therapeutic agents. This review provides an overview of the application of bacterial nanocarriers in precision medicine, focusing on their potential to target specific sites within the body for enhanced therapeutic outcomes. The key characteristics of bacterial nanocarriers, including size and shape, surface modifications for targeting and stealth capabilities, payload capacity, loading efficiency, stability, and biocompatibility, are discussed. This review explores various types of bacterial nanocarriers, including bacterial outer membrane vesicles (OMVs), bacterial spores, engineered bacteria, and other bacteria-based nanocarriers, highlighting their unique properties and applications. The mechanisms of site-specific drug delivery, including passive targeting via the enhanced permeability and retention (EPR) effect, active targeting using ligands and receptors, and tumor microenvironment-specific activation, are examined to elucidate the precise delivery mechanisms employed by bacterial nanocarriers. Furthermore, this review discusses the diverse applications of bacterial nanocarriers in precision medicine, including cancer therapy, infectious diseases, and chronic diseases, emphasizing their potential for targeted delivery of chemotherapeutic agents, immunotherapy, antibiotics, vaccines, and sustained release formulations. Challenges such as immunogenicity, manufacturing scalability, regulatory hurdles, and approval processes are addressed, along with future directions and emerging technologies in the field. Overall, bacterial nanocarriers hold immense promise as versatile platforms for site-specific drug delivery, paving the way for advancements in precision medicine and personalized therapeutics.

Keywords: bacterial nanocarriers, precision medicine, drug delivery, targeted therapy, cancer, nanotechnology.

I. Introduction

Precision medicine is a medical approach that takes into account individual variability in genes, environment, and lifestyle for each person. It recognizes that each individual is unique, and thus, treatments should be tailored to specific characteristics rather than adopting a one-size-fits-all approach[1]. Precision medicine aims to improve treatment outcomes, minimize side effects, and optimize healthcare resources by targeting therapies to patients who are most likely to benefit from them.Precision medicine encompasses various fields, including genomics, proteomics, metabolomics, and other omics disciplines, along with clinical and health data analysis[2]. This has led to advancements in disease prevention, diagnosis, and

treatment across various medical specialties, including oncology, cardiology, neurology, and infectious disease[3].

The foundation of precision medicine lies in understanding the molecular mechanisms underlying diseases and identifying biomarkers that can predict disease susceptibility, progression, and response to treatment. With the advent of high-throughput technologies and bioinformatics tools, researchers can analyse vast amounts of biological data to elucidate disease pathways and develop targeted therapies[4].Precision medicine has the potential to revolutionize healthcare by shifting from a reactive approach to a proactive and personalized approach. By identifying individuals at risk of disease and providing targeted interventions, precision medicine aims to improve patient outcomes, reduce healthcare costs, and enhance overall population health[5].Site-specific drug delivery is a crucial aspect of precision medicine that aims to deliver therapeutic agents directly to the site of action within the body while minimizing systemic exposure and off-target effects. Traditional drug delivery systems often result in low drug concentrations at the target site, leading to suboptimal efficacy and increased risk of adverse reactions[6].Site-specific drug delivery offers several advantages over conventional systemic administration. It allows for higher drug concentrations at the desired site, leading to enhanced therapeutic efficacy and reduced dosage requirements. By minimizing exposure to healthy tissues, site-specific drug delivery can also reduce side effects and improve patient compliance with treatment regimens[7].Site-specific drug delivery is particularly important in the treatment of localized diseases, such as cancer, inflammatory disorders, and infections. In cancer therapy, for example, delivering chemotherapy directly to the tumor site can increase the likelihood of tumor regression while minimizing damage to surrounding healthy tissues[8]. Similarly, targeted drug delivery can improve the efficacy of antimicrobial agents in treating infections by delivering high concentrations of drugs to the site of infection.Various approaches have been developed for site-specific drug delivery, including passive and active targeting strategies. Passive targeting relies on the physiological properties of tissues, such as the enhanced permeability and retention (EPR) effect observed in tumors, to deliver drugs selectively to the target site. Active targeting involves the use of ligands, antibodies, or other targeting moieties to facilitate specific interactions with receptors or antigens expressed on target cells, further enhancing drug delivery efficiency[9].

Bacterial nanocarriers represent a promising platform for site-specific drug delivery, harnessing the unique properties of bacteria to deliver therapeutic agents to specific tissues or cells within the body. Bacteria possess several inherent advantages as drug delivery vehicles, including their small size, ability to penetrate biological barriers, and capacity for targeted localization.Bacterial nanocarriers can be engineered to express specific surface molecules or proteins that facilitate targeting to particular tissues or cells[10]. Moreover, bacteria can be genetically modified to produce and release therapeutic agents directly at the site of action, providing sustained drug release and minimizing systemic exposure.Several types of bacterial nanocarriers, including bacterial outer membrane vesicles (OMVs), bacterial spores, and engineered bacteria, have been explored for drug delivery applications[11]. OMVs are naturally produced by bacteria and can be loaded with therapeutic cargo for targeted delivery to host cells. Bacterial spores, such as those produced by Clostridium species, have been investigated as vehicles for delivering drugs or imaging agents specifically to hypoxic regions

within solid tumors. Engineered bacteria can be designed to target specific tissues or cells and deliver therapeutic payloads in response to environmental cues or stimuli[12].

The aim of this review article is to provide a comprehensive overview of bacterial nanocarriers for site-specific drug delivery and their potential applications in precision medicine. This review covers the characteristics of bacterial nanocarriers, including size, shape, surface modifications, payload capacity, and stability. The mechanisms of site-specific drug delivery by bacterial nanocarriers, including passive and active targeting strategies, are also discussed[13].Furthermore, this review highlights the applications of bacterial nanocarriers in precision medicine, with a focus on cancer therapy, infectious diseases, and chronic conditions. The challenges and limitations associated with bacterial nanocarriers, such as immunogenicity, manufacturing scalability, and regulatory considerations, are discussed. Finally, this review provides insights into future directions and emerging technologies in the field of bacterial nanocarriers for site-specific drug delivery.

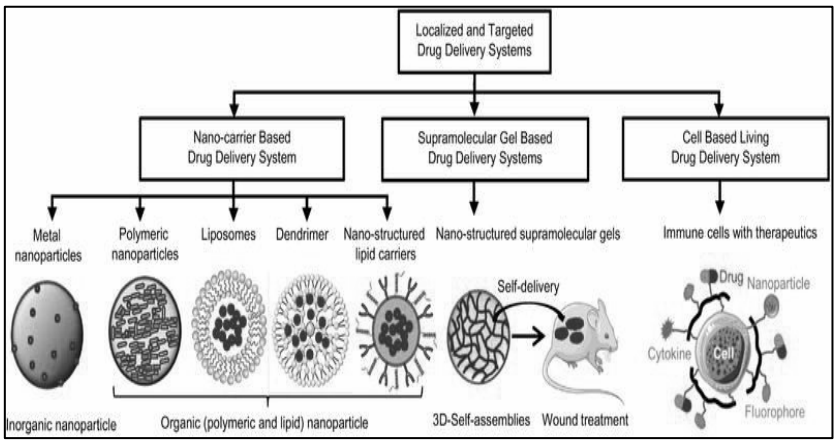

Figure 1: Diverse Carrier-Based Systems for Localized and Targeted Drug Delivery

II. Characteristics of the Bacterial Nanocarriers

A. Size and shape considerations

The size and shape of bacterial nanocarriers play a crucial role in determining their behavior and efficacy as drug delivery vehicles. Bacterial nanocarriers are typically nanosized particles ranging from a few nanometres to several hundred nanometres in diameter[14]. The small size of bacterial nanocarriers enables them to penetrate biological barriers, such as cell membranes and tissue barriers, and reach target sites within the body more efficiently.The shape of bacterial nanocarriers can also influence their biodistribution, cellular uptake, and targeting capabilities[15]. Various shapes, including spherical, rod-shaped, and filamentous, have been explored for use as bacterial nanocarriers. For example, spherical nanoparticles may exhibit prolonged circulation times in the bloodstream and enhanced cellular uptake, while rod-shaped or filamentous particles may improve tumor penetration and retention[16].

Furthermore, the aspect ratio of bacterial nanocarriers, defined as the ratio of their length to width, can impact their interactions with biological systems. High aspect ratio particles, such as nanorods or nanowires, may exhibit enhanced cellular uptake and internalization compared to spherical particles due to their elongated shape. However, excessively high aspect ratios

may also increase the risk of cytotoxicity or immune recognition, highlighting the importance of optimizing the shape and aspect ratio of bacterial nanocarriers for specific applications[17].

B. Surface modifications for targeting and stealth capabilities

Surface modifications of bacterial nanocarriers play a crucial role in enhancing their targeting specificity and stealth capabilities while minimizing immune recognition and clearance[5]. The surface of bacterial nanocarriers can be modified with various functional groups, polymers, peptides, or antibodies to facilitate specific interactions with target cells or tissues[18].Targeting ligands, such as antibodies, peptides, or small molecules, can be conjugated to the surface of bacterial nanocarriers to recognize and bind to specific receptors or antigens expressed on target cells[6]. This enables precise targeting of therapeutic agents to diseased tissues while minimizing off-target effects on healthy cells. For example, antibodies targeting overexpressed receptors on cancer cells can be conjugated to bacterial nanocarriers to achieve tumor-specific drug delivery[2].In addition to targeting ligands, surface modifications can also impart stealth properties to bacterial nanocarriers to evade immune detection and clearance[19]. PEGylation, the conjugation of polyethylene glycol (PEG) chains to the surface of nanoparticles, is a commonly used strategy to increase the circulation half-life of bacterial nanocarriers by reducing opsonization and phagocytosis by the reticuloendothelial system (RES). PEGylation creates a hydrophilic barrier around bacterial nanocarriers, preventing protein adsorption and recognition by immune cells[20].

C. Payload capacity and loading efficiency

The payload capacity and loading efficiency of bacterial nanocarriers are critical factors that determine their therapeutic efficacy and practical utility as drug delivery vehicles[13]. The payload capacity refers to the maximum amount of therapeutic agent that can be loaded onto or encapsulated within bacterial nanocarriers, while the loading efficiency reflects the percentage of drug encapsulated relative to the total capacity of the nanocarrier[21].Bacterial nanocarriers offer several advantages for drug loading and encapsulation, including their large surface area, internal compartmentalization, and potential for genetic engineering. Therapeutic agents can be loaded onto bacterial nanocarriers through physical adsorption, chemical conjugation, encapsulation within vesicles or compartments, or genetic expression and secretion by engineered bacteria[22].The payload capacity of bacterial nanocarriers can vary depending on factors such as the size, shape, and composition of the nanocarrier, as well as the physicochemical properties of the therapeutic agent. For example, small-molecule drugs may be loaded at higher concentrations than larger biologics or nucleic acids due to differences in molecular size and solubility[8].Loading efficiency is influenced by various parameters, including the method of drug loading, the affinity between the drug and nanocarrier, and the stability of the drug-nanocarrier complex. Optimizing loading efficiency is essential to maximize the therapeutic payload delivered to the target site while minimizing waste and ensuring cost-effectiveness[23].

D. Stability and biocompatibility

The stability and biocompatibility of bacterial nanocarriers are critical considerations for their safe and effective use in drug delivery applications[3]. Bacterial nanocarriers must maintain their structural integrity and drug-loading capacity during storage, transportation, and administration to ensure reliable and consistent therapeutic outcomes[24].Stability encompasses various aspects, including physical stability (e.g., aggregation, sedimentation, or degradation), chemical stability (e.g., drug degradation or release), and biological stability (e.g., susceptibility to enzymatic degradation or immune recognition)[2,4]. Strategies to enhance the stability of bacterial nanocarriers include surface modifications, encapsulation within protective matrices or coatings, and formulation optimization[25].Biocompatibility refers to the compatibility of bacterial nanocarriers with biological systems, including cells, tissues, and the immune system. Bacterial nanocarriers should exhibit minimal cytotoxicity, immunogenicity, or inflammatory responses to ensure their safety and tolerability in vivo[11]. Biocompatibility can be influenced by factors such as the composition, surface chemistry, and degradation products of bacterial nanocarriers, as well as their interactions with host cells and tissues[6].Various in vitro and in vivo assays, including cell viability assays, cytokine profiling, histological analysis, and pharmacokinetic studies, are used to assess the stability and biocompatibility of bacterial nanocarriers. Preclinical safety evaluations are essential for identifying potential adverse effects and guiding the design and optimization of bacterial nanocarriers for clinical translation[26].

III. Types of Bacterial Nanocarriers

A. Bacterial outer membrane vesicles (OMVs)

OMVs are nanoscale spherical structures naturally released by gram-negative bacteria as part of their normal growth and metabolism[5]. OMVs are composed of outer membrane lipids, proteins, and various cargo molecules, including toxins, enzymes, nucleic acids, and cell wall components. These vesicles range in size from 20 to 300 nanometers and are enriched in outer membrane proteins and lipopolysaccharides[27].OMVs have garnered significant interest as potential drug delivery vehicles due to their biocompatibility, stability, and ability to encapsulate and protect cargo molecules from degradation. OMVs can be engineered to display specific antigens or ligands on their surface, allowing for targeted delivery to specific cell types or tissues[2,9]. Additionally, OMVs can be loaded with therapeutic agents, such as drugs, vaccines, or nucleic acids, for targeted delivery to diseased tissues or cells.The unique properties of OMVs make them promising candidates for various biomedical applications, including vaccine delivery, cancer therapy, and infectious disease treatment[28]. OMV-based vaccines have been developed against bacterial pathogens, such as *Neisseria meningitidis* and Vibrio cholerae, by loading OMVs with antigenic proteins or polysaccharides derived from target pathogens. These vaccines have shown promising efficacy in preclinical and clinical studies, providing protection against bacterial infections.In addition to their use in vaccine delivery, OMVs have been explored for targeted drug delivery in cancer therapy[3,8]. Engineered OMVs can be loaded with chemotherapeutic agents or nucleic acids and functionalized with targeting ligands to specifically deliver drugs to tumor cells while minimizing off-target effects on healthy tissues. Moreover, OMVs can be used as adjuvants to enhance the immune response and efficacy of cancer immunotherapies[29].

B. Bacterial spores

Bacterial spores are dormant, highly resistant structures formed by certain bacterial species in response to adverse environmental conditions. Spores are characterized by their tough outer coat, which protects the bacterial genome and cellular contents from desiccation, heat, radiation, and chemical damage. Bacterial spores can remain viable for extended periods, making them attractive candidates for drug delivery and biotechnological applications[19].Bacterial spores have been explored as natural carriers for drug delivery due to their unique properties, including their small size (1-2 micrometres), stability, and capacity for payload encapsulation[30]. Spores can be genetically engineered to produce and release therapeutic agents in response to specific stimuli or environmental cues, such as pH, temperature, or the presence of target molecules.One of the most extensively studied bacterial spores for drug delivery is the spore-forming bacterium *Clostridium difficile*[12]. Engineered *C. difficile* spores have been used as targeted delivery vehicles for cancer therapy by exploiting the hypoxic microenvironment of solid tumors. These spores are engineered to germinate selectively within the tumor microenvironment and release therapeutic payloads, such as cytotoxic drugs or imaging agents, specifically within the tumor tissue[31].

C. Engineered bacteria

Engineered bacteria are genetically modified microbial strains designed to deliver therapeutic agents or perform specific functions within the body for biomedical applications. Engineered bacteria offer unique advantages as drug delivery vehicles, including their inherent targeting capabilities, capacity for self-replication, and potential for on-demand drug production[23].

One of the most widely studied engineered bacteria for drug delivery is *Escherichia coli* (*E. coli*), a common gram-negative bacterium[17]. Engineered *E. coli* strains have been designed to express and release therapeutic proteins, peptides, enzymes, or nucleic acids in response to specific environmental cues or stimuli. These bacteria can be administered orally, intravenously, or directly to target tissues to deliver therapeutic payloads to desired sites within the body.Engineered bacteria can be programmed to target specific cell types or tissues by modifying their surface proteins or introducing targeting ligands or peptides[32]. Moreover, bacteria can be engineered to express and release therapeutic agents in a controlled manner, providing sustained drug release and minimizing systemic toxicity[22].Several strategies have been explored to enhance the safety and efficacy of engineered bacteria for drug delivery, including the use of containment mechanisms to prevent bacterial replication in vivo, inducible expression systems for controlled drug release, and genetic circuitry to enable bacterial communication and coordination within microbial consortia.Engineered bacteria hold great promise for a wide range of biomedical applications, including cancer therapy, infectious disease treatment, and metabolic engineering[17]. However, challenges remain in the development of engineered bacteria, including issues related to biosafety, immunogenicity, and regulatory approval. Ongoing research efforts continue to address these challenges and advance the clinical translation of engineered bacteria for precision medicine[33].

D. Other bacterial-based nanocarriers

In addition to OMVs, bacterial spores, and engineered bacteria, other bacteria-based nanocarriers have been explored for drug delivery applications[20]. These include engineered bacterial ghosts, bacterial cell-derived nanoparticles, and bacterial-derived extracellular vesicles.Engineered bacterial ghosts are empty bacterial cell envelopes derived from gram-negative bacteria that have been genetically modified to remove their genetic material. These empty cell envelopes retain the surface structures and membrane components of the original bacteria and can be loaded with therapeutic agents for targeted drug delivery[34]. Bacterial ghosts offer advantages such as biocompatibility, immunogenicity, and potential for surface functionalization.Bacterial cell-derived nanoparticles are nanosized particles derived from intact bacterial cells through various physical or chemical methods, such as sonication, homogenization, or extrusion[18]. These nanoparticles retain the structural and functional properties of the original bacteria and can be loaded with drugs, proteins, or nucleic acids for targeted delivery. Bacterial cell-derived nanoparticles offer advantages such as biocompatibility, stability, and potential for surface modification[35].

IV. Mechanisms of Site-Specific Drug Delivery

A. Passive targeting via the enhanced permeability and retention (EPR) effect

Passive targeting exploits the unique physiological characteristics of diseased tissues, such as tumors, to achieve site-specific drug delivery. The enhanced permeability and retention (EPR) effect is a phenomenon commonly observed in solid tumors, wherein leaky blood vessels and impaired lymphatic drainage lead to the accumulation of macromolecules and nanoparticles within the tumor microenvironment[31].Solid tumors are characterized by abnormal vasculature with discontinuous endothelial cell junctions and fenestrations, allowing for increased vascular permeability[36]. This abnormal vascular architecture, coupled with poor lymphatic drainage, results in the retention of macromolecules and nanoparticles within the tumor interstitium. As a result, systemically administered drugs or nanoparticles can preferentially accumulate in tumor tissues, providing a passive targeting mechanism for site-specific drug delivery[25].The EPR effect is primarily exploited for the delivery of nanoparticle-based therapeutics, such as liposomes, polymeric micelles, and nanoparticles, which are too large to penetrate normal blood vessels but can extravasate and accumulate in tumor tissues through leaky tumor vasculature[36]. Once they accumulate within the tumor interstitium, these nanoparticles can release their payload of therapeutic agents, such as chemotherapy drugs, nucleic acids, or imaging agents, to exert their therapeutic effects.The passive targeting approach offers several advantages for site-specific drug delivery, including simplicity, noninvasiveness, and broad applicability to various types of solid tumors[21]. However, the efficacy of passive targeting via the EPR effect can be highly variable depending on the tumor type, size, and stage, as well as individual patient factors. Moreover, the heterogeneity of the tumor vasculature and the presence of stromal components within the tumor microenvironment can limit the extent of nanoparticle accumulation and hinder drug delivery efficiency[37].

B. Active targeting using ligands and receptors

Active targeting involves specific interactions between targeting ligands, such as antibodies, peptides, aptamers, or small molecules, conjugated to the surface of drug carriers, and receptors or antigens overexpressed on the surface of target cells or tissues[20]. This targeted approach enhances the specificity and efficiency of drug delivery while minimizing off-target effects on healthy tissues.Targeting ligands can be selected or engineered to recognize specific biomarkers associated with diseased tissues, such as cancer cells, inflammatory cells, or pathogenic microorganisms[38]. These ligands bind to their cognate receptors with high affinity and specificity, facilitating the selective uptake of drug carriers into target cells via receptor-mediated endocytosis or other internalization mechanisms[19].Various types of targeting ligands have been employed for active targeting, including monoclonal antibodies, antibody fragments (e.g., Fab or scFv), peptides (e.g., cell-penetrating peptides or tumor-homing peptides), aptamers (e.g., nucleic acid-based ligands), and small molecules (e.g., folate or transferrin)[7]. These ligands can be conjugated to the surface of drug carriers, such as liposomes, nanoparticles, or polymer micelles, using chemical, biological, or physical coupling methods.Active targeting using ligands and receptors offers several advantages over passive targeting, including enhanced specificity, reduced systemic toxicity, and improved therapeutic efficacy[39]. By directing drug carriers specifically to diseased tissues or cells, active targeting can increase the local concentration of therapeutic agents, improve cellular uptake, and overcome barriers to drug delivery, such as the blood–brain barrier or multidrug resistance mechanisms[3].Moreover, active targeting can be combined with other strategies, such as stimuli-responsive drug release or synergistic combination therapy, to further enhance the therapeutic outcomes of targeted drug delivery. For example, stimuli-responsive drug carriers can release their payload selectively in response to specific environmental cues or physiological conditions within the target tissue, further improving drug efficacy and reducing off-target effects[40].

C. Tumor microenvironment-specific activation

Tumor microenvironment-specific activation is a strategy for site-specific drug delivery that exploits the unique biochemical and physiological characteristics of the tumor microenvironment to trigger the release or activation of therapeutic agents within tumor tissues[21]. The tumor microenvironment is a complex and dynamic ecosystem composed of tumor cells, stromal cells, immune cells, blood vessels, and extracellular matrix components[41].

The tumor microenvironment exhibits several distinctive features that can be targeted for site-specific drug delivery, including acidic pH, hypoxia, elevated levels of enzymes and biomarkers, and aberrant expression of receptors or signalling pathways. By harnessing these tumor-specific cues, drug delivery systems can be designed to respond selectively to the tumor microenvironment and release therapeutic agents specifically within tumor tissues while sparing healthy tissues[19].One approach for tumor microenvironment-specific activation involves the use of stimuli-responsive drug carriers that undergo structural changes or release their payload in response to specific stimuli or environmental cues present within the tumor microenvironment[16]. For example, pH-sensitive nanoparticles can be designed to

release their cargo in response to the acidic pH of tumor tissues, leading to localized drug delivery and enhanced therapeutic efficacy[42].

Another approach involves the use of prodrugs or inactive drug precursors that are activated selectively within the tumor microenvironment by tumor-specific enzymes or metabolic pathways[8]. Upon activation, these prodrugs are converted into their active form, exerting their therapeutic effects specifically within tumor tissues while minimizing systemic toxicity.Furthermore, nanotechnology-based platforms, such as liposomes, nanoparticles, or polymer micelles, can be functionalized with targeting ligands or antibodies to achieve tumor-specific accumulation and cellular uptake[43]. These targeted drug delivery systems can exploit tumor-specific biomarkers or receptors for selective binding and internalization into tumor cells, enhancing drug delivery efficiency and therapeutic outcomes.Tumor microenvironment-specific activation strategies offer several advantages for site-specific drug delivery, including enhanced specificity, reduced systemic toxicity, and improved therapeutic efficacy[18]. By targeting tumor-specific cues and mechanisms, these strategies can overcome barriers to drug delivery and enhance the accumulation and retention of therapeutic agents within tumor tissues[44].

Table 1. Antitumour drug-loaded nanocarriers for the treatment of various tumors

Nanocarrier	**Drug(s)**	**Tumor**	**Benefits**	**References**
Liposomes	Doxorubicin, Paclitaxel	Breast cancer	Controlled drug release, reduced systemic toxicity	[2]
Polymeric nanoparticles	Docetaxel, Cisplatin	Lung cancer	Enhanced drug stability, targeted delivery to lung tissue	[5]
Dendrimers	Methotrexate, Paclitaxel	Ovarian cancer	High drug loading capacity, tumor penetration	[10]
Carbon nanotubes	Cisplatin, Gemcitabine	Pancreatic cancer	Ability to penetrate deep into tumor tissue, sustained drug release	[18]
Gold nanoparticles	Cisplatin, 5-Fluorouracil	Colorectal cancer	Enhanced cellular uptake, photothermal therapy enhancement	[13]
Magnetic nanoparticles	Methotrexate, Doxorubicin	Brain tumors	Magnetic targeting, enhanced blood–brain barrier penetration	[22]
Mesoporous silica nanoparticles	Paclitaxel, Docetaxel	Prostate cancer	High surface area for drug loading, pH-responsive drug release	[29]
Nanogels	Methotrexate, Irinotecan	Gastric cancer	Stimuli-responsive drug release, prolonged circulation time	[34]

V. Applications of Bacterial Nanocarriers in Precision Medicine

A. Cancer therapy

Cancer therapy represents one of the most promising applications of bacterial nanocarriers in precision medicine, offering targeted and personalized treatment options for patients with various types of cancer. Bacterial nanocarriers hold great potential for improving the efficacy and safety of cancer therapy through targeted drug delivery, immunomodulation, and combination therapy approaches[45].

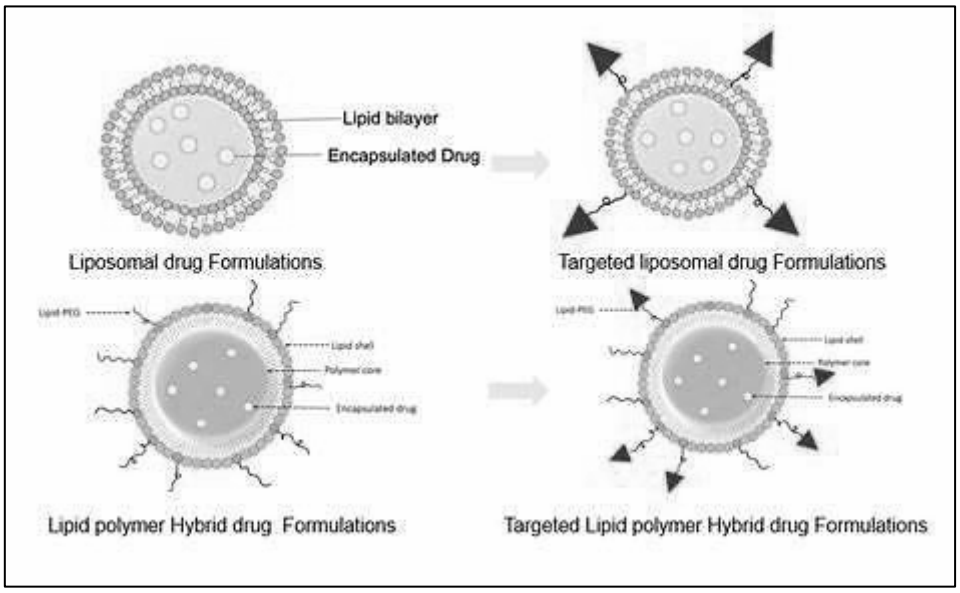

Figure 2: Nanoparticle-Based Systems for Delivering Cancer Therapeutics

Targeted delivery of chemotherapeutic agents

Chemotherapy is a cornerstone of cancer treatment, but its efficacy is often limited by systemic toxicity and off-target effects on healthy tissues. Bacterial nanocarriers can address these limitations by delivering chemotherapeutic agents selectively to tumor tissues while minimizing exposure to healthy organs[6]. By exploiting passive or active targeting mechanisms, bacterial nanocarriers can enhance the accumulation of chemotherapeutic drugs within tumors, leading to improved therapeutic outcomes and reduced side effects.Passive targeting via the enhanced permeability and retention (EPR) effect allows bacterial nanocarriers to accumulate preferentially in tumor tissues due to the leaky vasculature and impaired lymphatic drainage characteristic of solid tumors[46]. This passive targeting mechanism can be further enhanced by optimizing the size, shape, and surface properties of bacterial nanocarriers to improve their tumor penetration and retention[26].Active targeting strategies involve the conjugation of targeting ligands, such as antibodies, peptides, or small molecules, to the surface of bacterial nanocarriers to facilitate specific interactions with receptors or antigens overexpressed on tumor cells. These targeted drug delivery systems can enhance the specificity and efficiency of drug delivery to tumor tissues, resulting in improved therapeutic efficacy and reduced systemic toxicity[29].Moreover, bacterial nanocarriers can be engineered to release chemotherapeutic drugs in response to specific stimuli or environmental cues within the tumor microenvironment, such as acidic pH, hypoxia, or enzymatic activity. Stimuli-responsive drug delivery systems enable controlled and triggered

release of therapeutic agents within tumor tissues, further enhancing drug efficacy and minimizing off-target effects[47].

Immunotherapy using bacterial vectors

In addition to targeted drug delivery, bacterial nanocarriers hold promise for cancer immunotherapy by harnessing the immune system to recognize and eliminate tumor cells[5]. Bacterial vectors can be engineered to express and deliver immunomodulatory agents, such as cytokines, chemokines, or immune checkpoint inhibitors, to the tumor microenvironment, thereby enhancing the antitumour immune response and overcoming immune evasion mechanisms employed by cancer cells[48].Bacterial nanocarriers can serve as potent adjuvants for cancer vaccines, promoting antigen presentation and activation of tumor-specific immune responses. Engineered bacteria can be designed to express tumor-associated antigens or neoantigens and deliver them to antigen-presenting cells, such as dendritic cells, to stimulate T-cell-mediated immune responses against tumor cells[15]. Moreover, bacterial vectors can be modified to express immunostimulatory molecules, such as interleukins or costimulatory ligands, to enhance the activation and proliferation of antitumour immune cells within the tumor microenvironment[20].

Furthermore, bacterial nanocarriers can be engineered to modulate the tumor microenvironment and promote antitumour immune responses while inhibiting the immunosuppressive mechanisms employed by cancer cells[7]. For example, bacteria can be engineered to express enzymes that convert immunosuppressive metabolites, such as adenosine or indoleamine 2,3-dioxygenase (IDO), into immunostimulatory molecules, thereby reversing the immunosuppressive tumor microenvironment and enhancing the efficacy of cancer immunotherapy[49].

B. Infectious diseases

Bacterial nanocarriers hold promise for the treatment of infectious diseases, offering targeted delivery of antibiotics, vaccines, and immunomodulatory agents to combat microbial pathogens while minimizing systemic toxicity and antimicrobial resistance[13].

Targeted delivery of antibiotics

Antibiotic resistance poses a significant threat to global public health, necessitating the development of novel strategies for targeted and personalized antimicrobial therapy. Bacterial nanocarriers can enhance the efficacy and specificity of antibiotic delivery by targeting microbial pathogens directly to infected tissues or cells while sparing the commensal microbiota and minimizing off-target effects[50].

Bacterial nanocarriers can be engineered to encapsulate or conjugate antibiotics and deliver them selectively to sites of infection, such as bacterial biofilms, intracellular pathogens, or localized infections[10]. By exploiting passive or active targeting mechanisms, bacterial nanocarriers can enhance the accumulation of antibiotics within infected tissues, leading to improved antimicrobial efficacy and a reduced risk of resistance development.Passive targeting strategies leverage the enhanced permeability and retention (EPR) effect to deliver antibiotic-loaded bacterial nanocarriers preferentially to sites of infection, such as inflamed tissues or bacterial biofilms[6]. Active targeting approaches involve the conjugation of

targeting ligands or peptides to the surface of bacterial nanocarriers to facilitate specific interactions with microbial surface molecules or host cell receptors, enabling selective binding and internalization of antibiotic-loaded carriers into infected cells or pathogens[51].

Vaccines and immunomodulation

Bacterial nanocarriers offer promising platforms for the development of novel vaccines and immunomodulatory therapies to prevent and treat infectious diseases[13]. Engineered bacteria can be designed to express and deliver antigens or immunomodulatory molecules to the immune system, thereby stimulating protective immune responses against microbial pathogens or modulating host immune responses to enhance pathogen clearance and resolution of infection[4].Bacterial vectors can be engineered to express antigens derived from microbial pathogens and deliver them to antigen-presenting cells, such as dendritic cells, to stimulate the activation and proliferation of pathogen-specific T and B cells[52]. Moreover, bacterial nanocarriers can be modified to express adjuvants or immunostimulatory molecules that enhance the immune response to vaccination, promoting the production of protective antibodies and memory T cells against microbial pathogens.Furthermore, bacterial nanocarriers can be engineered to modulate host immune responses and promote immune-mediated clearance of microbial pathogens[2]. Engineered bacteria can express immunomodulatory molecules, such as cytokines, chemokines, or Toll-like receptor agonists, that enhance innate and adaptive immune responses against infection. Moreover, bacterial nanocarriers can be designed to target specific immune cell populations or tissues involved in host defense mechanisms, such as mucosal surfaces or lymphoid organs, to enhance the efficacy of vaccination or immunotherapy[53].

C. Chronic diseases

Bacterial nanocarriers hold potential for the treatment of chronic diseases, offering targeted delivery to specific organs or tissues affected by pathological processes while minimizing systemic toxicity and off-target effects. Moreover, bacterial nanocarriers can be designed to provide sustained release formulations that deliver therapeutic agents over an extended period, enabling long-term disease management and improved patient compliance[7].

Targeted delivery to specific organs or tissues

Chronic diseases, such as cardiovascular disease, neurodegenerative disorders, and autoimmune diseases, often require targeted drug delivery to specific organs or tissues affected by pathological processes[7]. Bacterial nanocarriers can be engineered to deliver therapeutic agents selectively to diseased tissues while sparing healthy organs, thereby enhancing therapeutic efficacy and minimizing systemic toxicity[54].Passive and active targeting strategies can be employed to achieve site-specific drug delivery in chronic diseases. Passive targeting via the enhanced permeability and retention (EPR) effect enables bacterial nanocarriers to preferentially accumulate in diseased tissues with leaky vasculature or impaired lymphatic drainage, such as inflamed or fibrotic tissues[13]. Active targeting approaches involve the conjugation of targeting ligands or peptides to the surface of bacterial nanocarriers to facilitate specific interactions with receptors or antigens overexpressed on cells or tissues affected by chronic diseases[55].Moreover, bacterial nanocarriers can be

engineered to respond to specific cues or stimuli associated with pathological processes within target tissues, such as inflammation, oxidative stress, or metabolic dysregulation, to trigger the release of therapeutic agents at the site of disease[23]. Stimuli-responsive drug delivery systems enable controlled and triggered release of drugs within diseased tissues, enhancing therapeutic efficacy while minimizing systemic exposure and off-target effects[56].

Sustained release formulations

Chronic diseases often require long-term or continuous administration of therapeutic agents to achieve optimal disease management and symptom control[21]. Bacterial nanocarriers can be designed to provide sustained release formulations that deliver drugs over an extended period, thereby reducing dosing frequency, improving patient compliance, and maintaining therapeutic concentrations of drugs within target tissues.Sustained release formulations can be achieved by encapsulating drugs within bacterial nanocarriers or modifying their surface properties to control drug release kinetics[57]. By modulating factors such as particle size, surface charge, and polymer composition, bacterial nanocarriers can be tailored to release drugs at a controlled rate, ensuring prolonged therapeutic effects while minimizing fluctuations in drug concentrations.Moreover, stimuli-responsive drug delivery systems can be employed to achieve on-demand release of therapeutic agents in response to specific cues or triggers associated with disease progression or symptom exacerbation[55]. For example, bacterial nanocarriers can be engineered to respond to changes in pH, temperature, or enzyme activity within target tissues, enabling the controlled release of drugs in response to disease-specific stimuli[57].

VI. Challenges and Future Perspectives

A. Immunogenicity and safety concerns

Immunogenicity and safety concerns represent significant challenges in the development and clinical translation of bacterial nanocarriers for precision medicine applications. Bacterial nanocarriers, including engineered bacteria and bacterial-derived vesicles, have the potential to elicit immune responses and adverse reactions in patients, which can limit their therapeutic efficacy and safety[33].

Immunogenicity of bacterial nanocarriers

Bacterial nanocarriers, particularly live bacteria or bacterial vectors, can induce immune responses in the host due to their foreign antigenicity and potential for dissemination or persistence within the body[22]. Engineered bacteria may express surface antigens or pathogen-associated molecular patterns (PAMPs) that activate innate immune cells and trigger inflammatory responses, leading to adverse effects such as fever, cytokine release syndrome, or systemic inflammation[58].Moreover, bacterial nanocarriers may elicit adaptive immune responses, including antibody production and T-cell activation, against bacterial antigens or vector components, which can impact their therapeutic efficacy and lead to immune-mediated clearance or neutralization. Preexisting immunity to bacterial vectors, acquired through prior exposure or vaccination, can also influence the immune response to bacterial nanocarriers and affect their clinical performance[59].

Safety considerations of bacterial nanocarriers

Safety concerns associated with bacterial nanocarriers include the risk of infection, systemic toxicity, and unintended off-target effects[33]. Live bacteria or bacterial vectors may pose a risk of infection or dissemination within the host, particularly in immunocompromised or susceptible individuals. Bacterial nanocarriers may also produce toxins or virulence factors that contribute to adverse effects or exacerbate underlying disease conditions[12,1].Furthermore, bacterial nanocarriers must be engineered to ensure the containment and control of their behavior within the body to minimize the risk of unintended off-target effects or environmental release. Strategies to enhance the safety of bacterial nanocarriers include genetic modifications to attenuate virulence, improve containment, or enhance biocontainment, as well as formulation optimization to reduce toxicity and immunogenicity[60].

B. Manufacturing scalability

Manufacturing scalability represents a key challenge in the production of bacterial nanocarriers for precision medicine applications. The scalable and cost-effective production of bacterial nanocarriers is essential for their widespread adoption and commercialization, but it poses technical and logistical challenges that must be overcome to meet the growing demand for precision medicine therapies[19].

Complexity of bacterial nanocarrier production

The production of bacterial nanocarriers involves multiple steps, including bacterial cultivation, genetic engineering, purification, and formulation, each of which presents challenges in terms of scalability and reproducibility[12]. Bacterial cultivation requires the optimization of culture conditions, growth media, and fermentation processes to achieve high cell densities and product yields. Genetic engineering of bacteria or bacterial vectors involves the manipulation of complex biological systems and pathways, requiring expertise in molecular biology, synthetic biology, and genetic engineering techniques[61].

Moreover, the purification of bacterial nanocarriers from culture supernatants or cell lysates can be challenging due to the presence of host cell debris, contaminants, and heterogeneous populations of nanocarriers. Purification processes must be scalable, efficient, and cost-effective to yield high-purity products suitable for clinical use[14]. The formulation of bacterial nanocarriers into stable drug delivery systems, such as liposomes, nanoparticles, or hydrogels, requires the optimization of formulation parameters and quality control measures to ensure product consistency and stability[3,8].

Scalability of production processes

Scaling up production processes for bacterial nanocarriers from the laboratory scale to the industrial scale poses technical and logistical challenges, including the optimization of bioreactor systems, the automation of culture and purification processes, and compliance with regulatory requirements for good manufacturing practices (GMPs). Bioreactor design, operation, and control must be optimized to achieve high cell densities, high product yields, and high reproducibility while minimizing process variability and contamination risks[62].

C. Future directions and emerging technologies

Future directions and emerging technologies hold promise for advancing the field of bacterial nanocarriers and unlocking new opportunities for precision medicine applications. Continued research efforts are needed to address existing challenges, explore novel strategies, and

capitalize on emerging technologies to further enhance the therapeutic potential of bacterial nanocarriers.

Advancements in genetic engineering and synthetic biology

Advances in genetic engineering and synthetic biology are driving innovations in the design and engineering of bacterial nanocarriers for precision medicine applications. Novel gene editing tools, such as CRISPR-Cas9, enable precise manipulation of bacterial genomes to engineer desired traits, functionalities, and properties in bacterial nanocarriers. Synthetic biology approaches allow for the design and construction of synthetic biological systems and pathways for programmable and customizable control of bacterial nanocarrier behavior and function[63].

Development of multifunctional and smart nanocarriers

The development of multifunctional and smart nanocarriers holds promise for enhancing the therapeutic efficacy, specificity, and responsiveness of bacterial nanocarriers in precision medicine applications[64]. Multifunctional nanocarriers can integrate multiple functionalities, such as targeting ligands, stimuli-responsive materials, imaging agents, and therapeutic payloads, into a single platform to enable synergistic effects and personalized treatment strategies. Smart nanocarriers can respond dynamically to environmental cues or stimuli within the body, such as pH, temperature, or enzyme activity, to trigger the controlled release of therapeutic agents and optimize drug delivery kinetics[65].

Integration of nanotechnology and artificial intelligence

The integration of nanotechnology and artificial intelligence (AI) offers opportunities for optimizing the design, characterization, and optimization of bacterial nanocarriers for precision medicine applications[66]. AI-driven approaches, such as machine learning, computational modelling, and data analytics, can accelerate the discovery and development of novel bacterial nanocarriers by predicting structure–function relationships, optimizing formulation parameters, and identifying candidate therapies with enhanced therapeutic properties[67]. Nanotechnology-enabled AI platforms can enable high-throughput screening, rapid prototyping, and personalized optimization of bacterial nanocarriers for specific disease targets or patient populations[68].

Translation of bacterial nanocarriers from bench to bedside

The translation of bacterial nanocarriers from bench to bedside requires collaboration and integration across multiple disciplines, including basic research, translational science, clinical medicine, regulatory affairs, and commercialization. Multidisciplinary teams of researchers, clinicians, engineers, and industry partners must work together to address technical, clinical, regulatory, and commercial challenges in the development and clinical translation of bacterial nanocarrier-based therapies. Strategic partnerships with academic institutions, biopharmaceutical companies, and government agencies can facilitate the translation of bacterial nanocarriers from early-stage research to clinical development and commercialization[66].

Conclusion

In this review, we explored the role of bacterial nanocarriers in site-specific drug delivery for precision medicine applications. We discussed the characteristics, types, mechanisms, and applications of bacterial nanocarriers, highlighting their potential to revolutionize drug delivery and therapy in various disease contexts.Bacterial nanocarriers exhibit unique properties that make them attractive candidates for precision medicine. Their small size, versatile surface modifications, high payload capacity, and inherent targeting capabilities enable precise delivery of therapeutic agents to diseased tissues while minimizing systemic exposure and off-target effects. By leveraging the biological and engineering principles of bacteria, researchers can engineer sophisticated nanocarrier platforms tailored to specific disease targets, patient populations, and clinical needs.Bacterial nanocarriers hold great promise for advancing precision medicine by enabling targeted, personalized, and efficacious drug delivery strategies for a wide range of diseases, including cancer, infectious diseases, and chronic conditions. By harnessing the unique properties of bacteria, researchers can overcome the limitations of conventional drug delivery systems and achieve unprecedented levels of precision and specificity in therapeutic interventions. The ability of bacterial nanocarriers to target specific tissues, cells, or microenvironments within the body offers significant advantages for precision medicine. These nanocarriers can deliver therapeutic agents directly to diseased tissues while sparing healthy organs, minimizing side effects, and maximizing therapeutic efficacy. Moreover, bacterial nanocarriers can be engineered to respond to disease-specific cues or stimuli, enabling on-demand drug release and tailored treatment regimens for individual patients.

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PYRAZOLE SCAFFOLDS: A PROMISING FRONTIER IN DRUG DISCOVERY

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ABSTRACT : Pyrazole scaffolds have emerged as a promising frontier in drug discovery, offering a rich source of chemical diversity and pharmacological activity. This review explores the synthesis, biological significance, medicinal chemistry strategies, computational approaches, recent advances, and future perspectives of pyrazole-based compounds in pharmacotherapy. The chemical structure and properties of pyrazole scaffolds are examined, highlighting their structural characteristics, physicochemical properties and synthetic routes. The biological significance of pyrazole derivatives is elucidated through an exploration of their targeted receptors, pharmacological activities, and therapeutic potential in anticancer, anti-inflammatory, antimicrobial, and other disease contexts. Medicinal chemistry strategies utilizing pyrazole scaffolds, including structure-activity relationship studies, pharmacophore mapping, and molecular docking simulations, are discussed for rational drug design and optimization. Case studies of pyrazole-derived drugs, emerging trends and applications are presented to underscore the diverse pharmacological activities and clinical applications of pyrazole-based compounds. Computational approaches in pyrazole-based drug design, such as molecular modeling techniques, virtual screening methods and quantitative structure-activity relationship studies, are examined for their role in accelerating the discovery and optimization of novel therapeutics. Future perspectives on the potential for future drug development, integration with emerging technologies and concluding remarks highlight the significance of pyrazole scaffolds in addressing unmet medical needs and advancing precision medicine. In conclusion, pyrazole scaffolds represent a promising avenue for the development of novel therapeutics with enhanced efficacy, selectivity and safety profiles, shaping the future of pharmacotherapy in the evolving landscape of modern medicine.

Key words : Pyrazole scaffolds, drug discovery, biological significance, computational approaches, synthetic methodologies, molecular modeling.

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INTRODUCTION

Pyrazole scaffolds represent a class of organic compounds characterized by a five-membered ring containing three carbon atoms and two nitrogen atoms in adjacent positions (Sharma and Singh, 2020). This distinctive structure has garnered significant attention in drug discovery due to its diverse pharmacological activities and potential therapeutic applications (An *et al*, 2020). This review aims to provide an in-depth exploration of pyrazole scaffolds, elucidating their chemical properties, biological significance, medicinal chemistry strategies, recent advances, computational approaches, synthetic methodologies and future prospects (Behbehani and Ibrahim, 2019). Pyrazole scaffolds serve as a versatile platform for the design and synthesis of bioactive molecules. Their unique structural features, including aromaticity and functional group tolerance, contribute to their broad applicability in medicinal

chemistry (Lusardi *et al*, 2023). Pyrazole derivatives can be synthesized via various synthetic routes, allowing for structural modifications and optimization to enhance their pharmacological properties (Sharma and Bhatt, 2021). Additionally, the presence of diverse substituents on the pyrazole ring enables the development of compounds with tailored activities for specific therapeutic targets (Ebenezer *et al*, 2022). Consequently, pyrazole scaffolds have emerged as valuable building blocks in the development of novel drugs across various therapeutic areas, ranging from anticancer agents to antimicrobial compounds. Pyrazole scaffolds hold immense importance in drug discovery due to their wide range of pharmacological activities and therapeutic potential (Aslan *et al,* 2022). Over the years, numerous pyrazole-based drugs have been developed and commercialized for the treatment of various diseases, underscoring the significance of this scaffold in modern medicine (Sharma *et al*, 2022). A fourth Bruton's tyrosine kinase inhibitor for mantle cell lymphoma (2023). Their ability to interact with specific molecular targets, such as enzymes, receptors, and ion channels, makes them attractive candidates for drug development (Goyal *et al,* 2022). Furthermore, the relatively straightforward synthesis of pyrazole derivatives facilitates the exploration of structure-activity relationships (SAR), allowing medicinal chemists to optimize their properties for improved efficacy and safety profiles (Croop *et al,* 2022).

This review comprehensively examines the multifaceted role of pyrazole scaffolds in drug discovery. It encompasses a broad spectrum of topics, including the chemical structure and properties of pyrazole derivatives, their biological significance, medicinal chemistry strategies, recent advances in drug development, computational approaches in rational drug design, synthetic methodologies for scaffold synthesis, and future prospects in the field (Scuteri *et al*, 2022). By providing a holistic overview of pyrazole-based drug discovery, this review aims to facilitate a deeper understanding of the potential applications and challenges associated with this promising class of compounds. Furthermore, it seeks to inspire further research and innovation in the pursuit of novel therapeutics leveraging pyrazole scaffolds (Link *et al*, 2020).

Chemical structure and properties of Pyrazole Scaffolds

Pyrazole scaffolds are organic compounds characterized by a five-membered ring containing three carbon atoms and two nitrogen atoms arranged in adjacent positions. This structural arrangement imparts unique properties to pyrazole derivatives, making them valuable building blocks in drug discovery and medicinal chemistry (Paik, 2022).

Structural characteristics

The core structure of pyrazole scaffolds consists of a planar, aromatic five-membered ring with alternating single and double bonds, reminiscent of other heterocyclic aromatic compounds such as pyridine and pyrimidine (Sahu *et al,* 2024). The nitrogen atoms at positions 1 and 3 confer basicity to the pyrazole ring, making it amenable to protonation and participation in hydrogen bonding interactions (Tuan and Ogbuagu, 2023). Additionally, the ð-electron delocalization within the ring contributes to its aromaticity, resulting in enhanced stability and reactivity. Pyrazole derivatives can undergo various structural modifications by substitution at different positions on the ring. Common substituents include alkyl, aryl, heteroaryl, and functional groups such as halogens, hydroxyl, amino, and carbonyl moieties (Malik *et al*, 2023). These substitutions can significantly influence the physicochemical properties and biological activities of pyrazole compounds by altering their electronic, steric, and lipophilic properties. Moreover, the regioselectivity of substitution reactions plays a crucial role in determining the spatial orientation and interactions of pyrazole derivatives with their molecular targets (De, 2023).

Physicochemical properties

Pyrazole scaffolds exhibit a diverse range of physicochemical properties, which govern their behavior in biological systems and pharmaceutical applications. The lipophilicity of pyrazole derivatives, determined by factors such as the nature and position of substituents, influences their solubility, membrane permeability, and distribution within the body (Goyal *et al,* 2023). Additionally, the presence of functional groups on the pyrazole ring can impart specific chemical reactivity, such as nucleophilicity or electrophilicity, thereby influencing their metabolism and interactions with biological macromolecules (Raghuwanshi *et al,* 2022). The acidity and basicity of pyrazole derivatives depend on the protonation state of the nitrogen atoms within the ring. While pyrazoles are weakly basic due to the lone pair of electrons on the nitrogen atoms, certain substituents can modulate their pKa values and alter their protonation equilibrium in physiological conditions (Kaur and Singh, 2021). Furthermore, the aromaticity of the pyrazole ring contributes to its stability and resistance to chemical degradation, rendering pyrazole scaffolds suitable for drug development applications (Schoepfer *et al,* 2018).

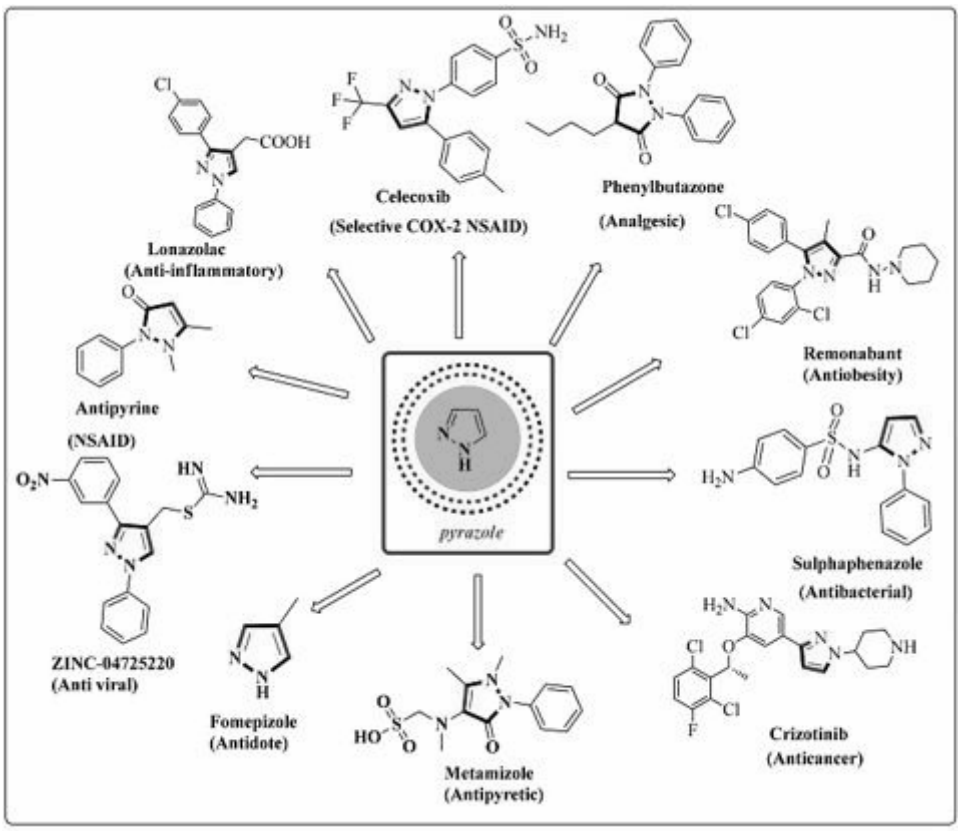

Fig. 1 : Exploring the Diverse Pharmacological activities of the Pyrazole Scaffold sourced from Alam *et al* (2022).

Synthetic routes and modifications

Pyrazole derivatives can be synthesized through various synthetic routes, offering flexibility in structural design and modification (Pankaj, 2021). One of the most common methods for pyrazole synthesis is the condensation reaction between a 1,3-dicarbonyl compound and a hydrazine derivative, known as the Knorr or Bohlmann–Rahtz pyrazole synthesis (De, 2023). This versatile approach allows for the incorporation of diverse substituents on the pyrazole ring, enabling the synthesis of complex molecular architectures with tailored properties. In addition to the Knorr pyrazole synthesis, several other synthetic methodologies have been developed for the efficient preparation of pyrazole derivatives (Siddiqi *et al,* 2023). These include the Hantzsch pyrazole synthesis, the Gewald reaction, the Paal-Knorr pyrazole synthesis and transition metal-catalyzed reactions. Each of these methods offers unique advantages in terms of substrate compatibility, reaction conditions, and regioselectivity, facilitating the synthesis of diverse pyrazole scaffolds for drug discovery applications (Malik *et al,* 2023). Furthermore, synthetic modifications of pyrazole derivatives can be employed to fine-tune their physicochemical and biological properties. These modifications may involve the introduction of specific functional groups, stereochemical manipulation, or scaffold decoration strategies to enhance the potency, selectivity, and pharmacokinetic properties of pyrazole-based drugs. Rational design principles, guided by structure-activity relationship (SAR) studies and computational modeling, play a crucial role in optimizing the structure of pyrazole scaffolds for targeted therapeutic applications (Salhotra *et al*, 2023).

Biological significance of Pyrazole Scaffolds

Pyrazole scaffolds have garnered significant attention in the field of medicinal chemistry and drug discovery due to their diverse pharmacological activities and potential therapeutic applications.

Targeted receptors and pathways

Pyrazole derivatives interact with a variety of molecular targets, including receptors, enzymes, ion channels and signaling pathways, thereby modulating various physiological processes and disease states (Schweitzer *et al*, 2022). The molecular mechanisms underlying the pharmacological activities of pyrazole scaffolds are often specific to the structural features and functional groups present in the compounds. Some of the key receptors and pathways targeted by pyrazole derivatives include:

G protein-coupled receptors (GPCRs) : Pyrazole derivatives exhibit affinity for several subtypes of GPCRs,

including adrenergic, dopaminergic, serotoninergic and cannabinoid receptors (Malik *et al,* 2022). By binding to these receptors, pyrazole compounds can modulate neurotransmitter signaling, leading to diverse physiological effects such as regulation of blood pressure, mood, appetite, and pain perception (Kotian *et al*, 2021).

Protein kinases : Pyrazole scaffolds serve as potent inhibitors of various protein kinases implicated in cancer progression, inflammation and other pathological conditions. These include receptor tyrosine kinases (*e.g.*, EGFR, VEGFR), serine/threonine kinases (*e.g*., MAPK, PKC) and other signaling kinases involved in cell proliferation, survival, and differentiation (Farkas and Balla, 2023).

Enzymes : Pyrazole derivatives can inhibit enzymes involved in key metabolic pathways, such as cyclooxygenases (COX), lipoxygenases (LOX) and phosphodiesterases (PDE). By modulating the activity of these enzymes, pyrazole compounds exert anti-inflammatory, analgesic and antiplatelet effects, making them potential candidates for the treatment of inflammatory disorders and pain (Subbiah *et al*, 2022).

Nuclear receptors : Certain pyrazole derivatives act as ligands for nuclear receptors, including peroxisome proliferator-activated receptors (PPARs) and estrogen receptors (ERs). Through their interaction with these receptors, pyrazole compounds can regulate gene expression and modulate lipid metabolism, glucose homeostasis and hormonal signaling pathways (Gainor *et al*, 2021).

Ion channels : Pyrazole scaffolds exhibit modulatory effects on ion channels, such as voltage-gated sodium channels (NaV), calcium channels (CaV) and potassium channels (KV). By altering ion flux across cell membranes, pyrazole compounds can affect neuronal excitability, muscle contraction and cardiac function, offering potential therapeutic benefits in neurological and cardiovascular disorders (Goyal *et al*, 2022).

Pharmacological activities

Pyrazole derivatives display a wide spectrum of pharmacological activities, ranging from anticancer and anti-inflammatory effects to antimicrobial and other biological activities (Sahu *et al*, 2024). The multifaceted pharmacological profile of pyrazole scaffolds makes them attractive candidates for drug development across different therapeutic areas (Subbiah *et al*, 2021). Some of the key pharmacological activities associated with pyrazole derivatives include:

Anticancer potential

Pyrazole derivatives have emerged as promising candidates for cancer therapy due to their ability to inhibit key signaling pathways involved in tumor growth, metastasis, and drug resistance. These compounds exert cytotoxic effects on cancer cells through various mechanisms, including:

a. Targeting protein kinases : Pyrazole derivatives inhibit receptor tyrosine kinases (e.g., EGFR, HER2), mitogen-activated protein kinases (MAPK), phosphoinositide 3-kinase (PI3K)/Akt/mTOR pathway, and other signaling kinases implicated in cancer progression (Addeo *et al*, 2023).

b. Inducing apoptosis : Pyrazole compounds can trigger programmed cell death in cancer cells by activating intrinsic and extrinsic apoptotic pathways, disrupting mitochondrial function, and modulating apoptotic protein expression.

c. Inhibiting angiogenesis : Pyrazole derivatives inhibit angiogenic signaling pathways, such as vascular endothelial growth factor (VEGF) and angiopoietin/Tie2, thereby suppressing tumor neovascularization and metastatic spread (Wu *et al*, 2019).

d. Overcoming drug resistance : Pyrazole scaffolds sensitize cancer cells to chemotherapy and targeted therapy by overcoming drug resistance mechanisms, such as efflux pump activation, DNA repair pathways and anti-apoptotic protein expression.

Anti-inflammatory effects

Pyrazole derivatives possess potent anti-inflammatory properties, making them effective agents for the treatment of inflammatory disorders, autoimmune diseases, and allergic reactions (Karrouchi *et al*, 2018). These compounds exert anti-inflammatory effects through various mechanisms, including:

a. Inhibition of prostaglandin synthesis : Pyrazole compounds inhibit cyclooxygenase (COX) enzymes, thereby suppressing the production of pro-inflammatory prostaglandins implicated in pain, fever and inflammation (Byon *et al*, 2019).

b. Modulation of cytokine signaling : Pyrazole derivatives regulate the production and release of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) and chemokines involved in the recruitment and activation of immune cells at inflammatory sites.

c. Attenuation of leukocyte migration : Pyrazole scaffolds inhibit leukocyte adhesion molecules (e.g., selectins, integrins) and chemotactic receptors, reducing the recruitment and infiltration of immune cells into inflamed tissues (Fustero *et al,* 2011).

d. Suppression of inflammatory mediators : Pyrazole compounds downregulate the expression of inflammatory mediators, such as nitric oxide (NO), reactive oxygen species (ROS) and matrix metalloproteinases (MMPs), which contribute to tissue damage and remodeling in chronic inflammation (Gomes *et al,* 2020).

Antimicrobial activity

Pyrazole derivatives exhibit broad-spectrum antimicrobial activity against bacterial, fungal, parasitic, and viral pathogens, making them potential candidates for the treatment of infectious diseases. These compounds exert antimicrobial effects through various mechanisms, including:

a. Inhibition of bacterial enzymes : Pyrazole compounds target essential enzymes involved in bacterial cell wall synthesis (e.g., peptidoglycan biosynthesis, cell division) and nucleic acid metabolism (e.g., DNA gyrase, RNA polymerase), leading to bactericidal or bacteriostatic effects (Turkan *et al*, 2018).

b. Disruption of fungal cell membranes: Pyrazole derivatives disrupt fungal cell membrane integrity by altering membrane permeability, inhibiting ergosterol biosynthesis, and disrupting membrane ion gradients, resulting in fungal growth inhibition and cell death (Xu *et al*, 2023).

c. Interference with parasite metabolism : Pyrazole scaffolds interfere with essential metabolic pathways in parasitic organisms, such as protozoa and helminths, by targeting enzymes involved in energy metabolism (e.g., glycolysis, oxidative phosphorylation), nucleotide synthesis and protein biosynthesis (Syed, 2020).

d. Inhibition of viral replication : Pyrazole compounds inhibit viral replication by targeting viral enzymes (e.g., proteases, polymerases) and structural proteins involved in viral assembly, maturation and entry into host cells, thereby suppressing viral propagation and dissemination (Ridley and Condren, 2020).

Other Biological activities

In addition to their anticancer, anti-inflammatory, and antimicrobial activities, pyrazole derivatives exhibit a variety of other biological effects, including:

a. Antioxidant activity : Pyrazole compounds possess antioxidant properties, scavenging free radicals and reactive oxygen species (ROS) implicated in oxidative stress, aging and chronic diseases (Pacenta and Macy, 2018).

b. Neuroprotective effects : Pyrazole scaffolds exert neuroprotective effects by modulating neurotransmitter signaling, enhancing neuronal survival, and attenuating neuroinflammation and excitotoxicity in neurological disorders.

c. Metabolic modulation : Pyrazole derivatives regulate metabolic pathways involved in lipid, carbohydrate, and energy metabolism, offering potential therapeutic benefits in obesity, diabetes and metabolic syndrome (Markham and Duggan, 2019).

d. Cardiovascular effects : Pyrazole compounds exhibit cardioprotective effects by modulating cardiac ion channels, vascular tone, and platelet aggregation, thereby reducing the risk of cardiovascular diseases such as hypertension, ischemic heart disease and thrombosis (Loriot *et al*, 2019).

Medicinal chemistry strategies utilizing pyrazole scaffolds

Pyrazole scaffolds have emerged as valuable building blocks in medicinal chemistry, offering diverse opportunities for the design and optimization of bioactive molecules (Sun *et al*, 2023).

Structure-Activity Relationship (SAR) studies

Structure-activity relationship (SAR) studies are integral in deciphering the correlation between the chemical structure of pyrazole derivatives and their pharmacological activities (Al-Snafi *et al,* 2022). Through systematic manipulation of substituents, functional groups, and molecular scaffolds, medicinal chemists gain insights into the crucial structural determinants driving biological effects (Sampath *et al,* 2023). SAR studies illuminate the landscape of pyrazole scaffolds' structure-activity relationships, informing the rational design and optimization of novel drug candidates with enhanced potency, selectivity and pharmacokinetic profiles (Bhatt, 2018). Key principles underlying SAR studies involve investigating substituent effects by varying substituents such as alkyl, aryl and heteroaryl groups to discern their impact on pharmacological activity. Scaffold modifications, including ring fusion, expansion, or contraction are explored to understand their influence on biological activity (Obeime *et al,* 2023). Functional group optimization is emphasized, with the introduction of electron-donating or -withdrawing groups, polar or lipophilic moieties and bioisosteric replacements to modulate physicochemical and pharmacokinetic properties (Goyal *et al,* 2022). Additionally, SAR studies elucidate regioselectivity and stereochemistry effects, highlighting optimal substituent placement and stereochemical motifs for maximizing binding affinity and biological activity of pyrazole derivatives (Koelblinger *et al,* 2018).

Pharmacophore mapping

Pharmacophore mapping is essential for identifying and characterizing the key molecular features (pharmacophores) necessary for ligand-receptor interactions and biological activity (Kaurav *et al,* 2023). In the realm of pyrazole scaffolds, pharmacophore mapping enables the elucidation of critical structural elements responsible for binding to specific molecular targets and modulating relevant biological pathways (Federman and McDermott, 2019). By amalgamating experimental data, computational modeling techniques, and structural information, pharmacophore mapping facilitates the rational design and virtual screening of novel pyrazole derivatives with desired pharmacological properties (Scott, 2019). Key components of pharmacophore mapping for pyrazole scaffolds encompass the identification of key binding interactions, generation of pharmacophore models, validation and refinement of these models, and subsequent virtual screening and lead identification efforts (Kaur and Singh, 2021). Pharmacophore models are crucial for predicting ligand binding and guiding lead optimization, ultimately enhancing the discovery of novel pyrazole-based therapeutics with improved potency, selectivity and pharmacokinetic properties (Syed, 2019).

Molecular Docking studies

Molecular docking studies are pivotal in understanding the binding modes, interaction kinetics, and structure-activity relationships of pyrazole derivatives with their target receptors or enzymes (Bailly *et al*, 2020). Through computational prediction of energetically favorable binding conformations and affinities within the active site of the target protein, molecular docking elucidates the mechanism of action and binding kinetics of pyrazole-based drug candidates (Van Andel *et al*, 2017). Key aspects include protein structure preparation, ligand conformational sampling, binding affinity estimation, and analysis of docking results (Kaurav *et al*, 2023). By exploring ligand-receptor interactions at the atomic level, these studies provide insights into critical binding residues, hydrogen bonding patterns and structure-activity relationships, guiding the rational design and optimization of novel drug candidates (Lee, 2021). Molecular docking facilitates the selection of lead compounds for further experimental validation and optimization, thus advancing drug discovery efforts targeting pyrazole scaffolds (Dillon *et al*, 2021).

Recent Advances in Pyrazole-Based Drug Discovery

Pyrazole-based drug discovery has made significant strides, propelled by advancements in synthetic chemistry, computational modeling and biological screening technologies. Celecoxib (Celebrex) serves as a notable example, functioning as a selective inhibitor of cyclooxygenase-2 (COX-2), approved for managing osteoarthritis and rheumatoid arthritis (Kasi, 2018). Rimonabant (Acomplia), a selective cannabinoid receptor type 1 (CB1) antagonist, showed promise in obesity treatment before withdrawal due to psychiatric concerns. Regorafenib (Stivarga), a multi-kinase inhibitor has been approved for colorectal cancer treatment, demonstrating the potential of pyrazole-based kinase inhibitors in oncology (Liu *et al*, 2014). Anakinra (Kineret), a recombinant human interleukin-1 receptor antagonist (IL-1Ra), is utilized for rheumatoid arthritis treatment, showcasing the utility of pyrazole-based biologics in inflammatory conditions (Singh *et al*, 2022). These case studies underscore the diverse pharmacological activities and clinical applications of pyrazole-derived drugs, spanning anti-inflammatory, analgesic, kinase inhibitor, and biologic agent categories (Yusuf *et al*, 2021).

Emerging trends and applications

Beyond established pyrazole-derived drugs, emerging trends and applications in pyrazole-based drug discovery are reshaping pharmaceutical research. Notable trends include the rise of targeted therapy, where advancements in molecular biology enable the selective targeting of specific molecular pathways implicated in disease pathogenesis (Murano *et al*, 2008). Pyrazole-based drugs are increasingly tailored to these targets, yielding precision medicines with enhanced efficacy and reduced off-target effects (Singh *et al,* 2021). Combination therapy is also gaining ground, leveraging the synergy of multiple drugs to overcome resistance and improve outcomes, with pyrazole-based drugs explored alongside chemotherapy, immunotherapy and supportive care medications (Ran *et al,* 2022). Furthermore, the development of pyrazole-based biologics, such as monoclonal antibodies and antibody-drug conjugates (ADCs), expands treatment options, offering targeted solutions for cancer, autoimmune, and infectious diseases. Additionally, drug repurposing and repositioning strategies capitalize on existing pyrazole-based compounds, accelerating development timelines and reducing costs by exploring new therapeutic indications based on emerging evidence (Singh *et al,* 2024). These trends underscore the dynamic landscape of pyrazole-based drug discovery, promising novel therapies and innovative solutions for complex diseases (Frost *et al,* 2013).

Challenges and opportunities

Despite significant progress in pyrazole-based drug

discovery, several challenges and opportunities persist (Granger *et al*, 2011). Firstly, concerns about off-target effects arise due to the promiscuous nature of pyrazole scaffolds, necessitating the design of selective drugs through rational drug design, SAR studies and computational modeling. Secondly, optimizing pharmacokinetic properties, including ADME parameters, is crucial for clinical success, prompting research into improving bioavailability, metabolic stability and tissue penetration through structural modifications and formulations (An *et al*, 2020). Thirdly, the challenge of drug resistance in cancer and infectious diseases requires the development of novel therapeutic strategies and combination therapies to overcome resistance mechanisms such as target mutations and efflux pump overexpression (Singh *et al,* 2022). Lastly, translating preclinical findings into clinical practice faces hurdles like safety concerns and regulatory requirements, emphasizing the need for interdisciplinary collaboration and robust preclinical validation to bridge the gap between preclinical and clinical efficacy of pyrazole-derived drugs (Behbehani and Ibrahim, 2019).

Computational approaches in Pyrazole-based drug design

Computational approaches play a pivotal role in accelerating the drug discovery process and optimizing the therapeutic potential of pyrazole-based compounds.

Molecular Modeling techniques

Molecular modeling techniques encompass a diverse array of computational methods used to predict and analyze the three-dimensional structure, energetics and interactions of molecules at the atomic level (Sharma and Singh, 2020). In the context of pyrazole-based drug design, molecular modeling techniques provide valuable insights into the conformational flexibility, binding affinity, and pharmacological properties of pyrazole derivatives (Lusardi *et al,* 2023).

Molecular dynamics (MD) simulations : Molecular dynamics simulations involve the computational modeling of molecular systems over time to study their dynamic behavior and conformational changes (Sharma and Bhatt, 2021). MD simulations can elucidate the flexibility and stability of pyrazole-based ligands in complex with target proteins, providing insights into ligand-receptor interactions, binding kinetics and structural rearrangements that occur during the binding process (Ebenezer *et al*, 2022).

Quantum mechanical (QM) calculations : Quantum mechanical calculations employ quantum mechanical principles to compute the electronic structure and properties of molecules with high precision (Sharma *et al*, 2022). QM methods can be used to investigate the electronic properties, charge distribution, and chemical reactivity of pyrazole derivatives, facilitating the understanding of their binding affinity, reactivity and metabolism in biological systems(Pirtobrutinib (Jaypirca): A fourth Bruton's tyrosine kinase inhibitor for mantle cell lymphoma (2023).

Molecular docking : Molecular docking is a computational technique used to predict the binding mode and binding affinity of small molecules (ligands) within the active site of a target protein (receptor) (Sahu *et al,* 2024). In pyrazole-based drug design, molecular docking simulations enable the identification of favorable binding poses and key molecular interactions between pyrazole derivatives and target proteins, guiding the rational design and optimization of ligands with enhanced potency and selectivity (Aslan *et al,* 2022).

Homology modeling : Homology modeling, also known as comparative modeling, is a computational technique used to generate three-dimensional models of protein structures based on their sequence similarity to known protein structures (Raghuwanshi *et al,* 2022). Homology modeling can be employed to predict the three-dimensional structure of target proteins for which experimental structures are not available, facilitating structure-based drug design efforts targeting pyrazole-based ligands to specific molecular targets (Croop *et al,* 2022).

Virtual Screening methods

Virtual screening methods involve the computational screening of chemical libraries or compound databases to identify candidate molecules with the potential to interact with a target protein or biological target of interest. Virtual screening methods are widely used in pyrazole-based drug discovery to prioritize lead compounds for experimental validation and optimization (Scuteri *et al,* 2022).

Ligand-based virtual screening : Ligand-based virtual screening involves the comparison of the chemical features and structural properties of pyrazole derivatives with known ligands or bioactive compounds using similarity searching, pharmacophore modeling, and quantitative structure-activity relationship (QSAR) analysis (Pankaj, 2021). Ligand-based virtual screening enables the identification of structurally diverse pyrazole compounds with similar pharmacological profiles to known active compounds, facilitating the exploration of chemical space and the discovery of novel drug candidates (Link *et al*, 2020).

Structure-based virtual screening : Structure-based virtual screening relies on computational docking simulations to predict the binding affinity and binding mode of small molecule ligands within the active site of a target protein (Malik *et al*, 2023). In pyrazole-based drug design, structure-based virtual screening enables the rapid screening of large compound libraries to identify pyrazole derivatives with high binding affinity and favorable interaction patterns with target proteins, accelerating the lead identification and optimization process (Paik, 2022).

Fragment-based virtual screening : Fragment-based virtual screening involves the screening of small molecule fragments or building blocks against a target protein to identify potential binding motifs or pharmacophoric features that can be further optimized to develop lead compounds (Malik *et al,* 2022). Fragment-based virtual screening is particularly useful in the early stages of pyrazole-based drug discovery for exploring chemical space, identifying novel scaffolds and designing focused libraries of pyrazole derivatives with enhanced binding affinity and specificity (Tuan and Ogbuagu, 2023).

QSAR and QSPR studies

Quantitative structure-activity relationship (QSAR) and quantitative structure-property relationship (QSPR) studies involve the computational analysis of chemical structure-property relationships to predict the biological activity or physicochemical properties of pyrazole derivatives based on their molecular descriptors and structural features (De, 2023). QSAR and QSPR studies provide valuable insights into the structure-activity relationships governing the pharmacological activity, ADME (absorption, distribution, metabolism and excretion) properties and toxicological profiles of pyrazole-based compounds (Goyal *et al*, 2023).

QSAR modeling : QSAR modeling aims to establish quantitative relationships between the chemical structure of pyrazole derivatives and their biological activity against specific molecular targets or biological assays (Kumar *et al,* 2019). QSAR models are constructed using statistical methods, machine learning algorithms, or computational regression techniques to correlate molecular descriptors (e.g., physicochemical properties, molecular fingerprints) with experimental activity data, enabling the prediction of the potency, efficacy, and selectivity of pyrazole compounds (Schoepfer *et al,* 2018).

QSPR modeling : QSPR modeling focuses on predicting the physicochemical properties and ADME properties of pyrazole derivatives based on their molecular descriptors and structural features (Kaurav *et al*, 2023). QSPR models are developed using regression analysis, machine learning algorithms, or molecular dynamics simulations to correlate molecular descriptors with experimental property data, such as solubility, lipophilicity, permeability and metabolic stability, facilitating the optimization of pyrazole-based compounds with desirable drug-like properties (De, 2023).

Application in drug design : QSAR and QSPR studies are widely used in pyrazole-based drug design to prioritize lead compounds, guide structural modifications, and optimize pharmacological properties (Kaur and Singh, 2021). By integrating QSAR and QSPR predictions with molecular modeling techniques and experimental validation, researchers can identify structurally diverse pyrazole derivatives with optimized activity profiles, improved drug-likeness, and enhanced therapeutic potential (Siddiqi *et al,* 2023).

Synthetic methodologies for Pyrazole scaffold synthesis

The synthesis of pyrazole scaffolds has been a subject of intense research in organic chemistry due to the diverse pharmacological activities and therapeutic potential of pyrazole-based compounds (Goyal *et al,* 2022).

Classical Synthetic routes

Classical synthetic routes for pyrazole scaffold synthesis typically involve the condensation of hydrazine derivatives with α,β-unsaturated carbonyl compounds or diketones, followed by cyclization and subsequent functionalization (Salhotra *et al,* 2023). The most common classical methods for pyrazole synthesis include:

Knorr pyrazole synthesis : The Knorr pyrazole synthesis is one of the oldest and most widely used methods for the preparation of pyrazole derivatives (Bhatt, 2018). It involves the condensation of hydrazine or hydrazine derivatives with á,â-unsaturated ketones or diketones under acidic conditions, followed by cyclization to form the pyrazole ring. The reaction proceeds via an intermediate enamine or hydrazone intermediate, which undergoes intramolecular cyclization to yield the desired pyrazole product (Schweitzer *et al*, 2022).

Paal-Knorr pyrazole synthesis : The Paal-Knorr pyrazole synthesis is a variation of the Knorr pyrazole synthesis that involves the condensation of 1,3-diketones with hydrazine or hydrazine derivatives under acidic conditions (Bhatt *et al,* 2023). The reaction proceeds via the formation of an α,β-unsaturated intermediate, which undergoes cyclization and dehydration to yield the pyrazole product. The Paal-Knorr pyrazole synthesis is

particularly useful for the synthesis of polysubstituted pyrazoles from readily available starting materials (Kotian *et al,* 2021).

Bohlmann-Rahtz pyrazole synthesis : The Bohlmann-Rahtz pyrazole synthesis involves the condensation of α,β-unsaturated esters or nitriles with hydrazine or hydrazine derivatives under basic conditions (Bhatt *et al*, 2021). The reaction proceeds via the formation of an intermediate vinylhydrazine, which undergoes intramolecular cyclization and subsequent tautomerization to yield the pyrazole product. The Bohlmann-Rahtz pyrazole synthesis offers regioselectivity and functional group compatibility, making it suitable for the synthesis of diverse pyrazole derivatives (Farkas and Balla, 2023).

Hantzsch pyrazole synthesis : The Hantzsch pyrazole synthesis involves the condensation of β-ketoesters or β-diketones with hydrazine or hydrazine derivatives under acidic conditions, followed by cyclization and reduction (Bhatt *et al*, 2018). The reaction proceeds via the formation of an intermediate dihydropyrazole, which undergoes intramolecular cyclization and subsequent reduction to yield the pyrazole product. The Hantzsch pyrazole synthesis offers mild reaction conditions and functional group tolerance, making it applicable to the synthesis of complex pyrazole scaffolds (Subbiah *et al*, 2022).

Modern Synthetic strategies

Modern synthetic strategies for pyrazole scaffold synthesis aim to improve reaction efficiency, selectivity, and diversity through the development of novel synthetic methodologies and reaction protocols (Gainor *et al,* 2021). These strategies often involve transition-metal catalysis, heterocycle synthesis and multicomponent reactions, enabling the rapid assembly of complex pyrazole structures from simple starting materials. Some of the key modern synthetic strategies for pyrazole synthesis include:

Transition-metal catalyzed methods: Transition-metal catalysis has emerged as a powerful tool for the synthesis of pyrazole derivatives via C–H activation, cross-coupling reactions and cycloaddition reactions (Bhatt *et al,* 2023). Palladium, ruthenium, copper, and gold catalysts have been employed in various transformations, including C–H arylation, alkenylation, alkynylation, and heteroatom incorporation, enabling the synthesis of diverse pyrazole scaffolds with high efficiency and selectivity (Subbiah *et al*, 2021).

Click chemistry approaches : Click chemistry refers to a set of highly efficient and selective reactions for the rapid synthesis of complex molecules from readily available starting materials (Bhatt *et al*, 2024). Copper-catalyzed azide-alkyne cycloaddition (CuAAC) and [3+2] cycloaddition reactions have been utilized in the synthesis of pyrazole derivatives from azides and alkynes or nitriles, enabling the formation of triazole-pyrazole hybrids and functionalized pyrazole scaffolds with diverse substitution patterns (Addeo *et al*, 2023).

Multicomponent reactions (MCRs) : Multicomponent reactions involve the simultaneous reaction of three or more reactants to generate complex molecular architectures in a single synthetic step (Bhatt *et al,* 2019). MCRs have been employed in the synthesis of pyrazole derivatives via condensation reactions, cycloaddition reactions and cascade reactions. Examples include the Gewald reaction, the Gewald-amine reaction, and the domino Knoevenagel-hetero Diels-Alder reaction, which enable the efficient assembly of pyrazole scaffolds from simple starting materials under mild reaction conditions (Wu *et al*, 2019).

Heterocycle synthesis strategies : Heterocycle synthesis strategies involve the functionalization of heterocyclic precursors or the construction of heterocyclic rings through ring-closing reactions, ring-expansion reactions, and ring-opening reactions (Baskar *et al*, 2022). Heterocycle synthesis methods have been applied to the synthesis of pyrazole derivatives via intramolecular cyclization of enaminones, ring-opening reactions of epoxides, and ring-expansion reactions of azoles, providing diverse access to substituted pyrazole scaffolds with tunable properties (Karrouchi *et al,* 2018).

Green Chemistry approaches

Green chemistry approaches aim to minimize environmental impact, reduce waste generation, and conserve resources in chemical synthesis processes (Byon *et al,* 2019). In the context of pyrazole scaffold synthesis, green chemistry principles can be applied to develop sustainable and environmentally friendly synthetic methodologies. Some key green chemistry approaches for pyrazole synthesis include:

Solvent-free synthesis: Solvent-free synthesis techniques eliminate the use of organic solvents, reducing waste generation and environmental pollution (Al-Snafi *et al*, 2022). Solid-phase synthesis, mechanochemical synthesis and microwave-assisted synthesis have been utilized in the solvent-free synthesis of pyrazole derivatives, offering advantages in reaction efficiency, product purity, and environmental sustainability (Fustero *et al*, 2011).

Catalysis and reagent recycling : Catalysis plays a crucial role in green chemistry approaches by facilitating

reactions under mild conditions, reducing reaction times, and minimizing waste generation (Ahmed *et al,* 2020). Transition-metal catalysts, organocatalysts and biocatalysts have been employed in pyrazole synthesis to improve reaction efficiency, selectivity, and atom economy (Gomes *et al,* 2020). Additionally, the development of recyclable catalysts and reagent recycling strategies enables the sustainable use of catalytic systems and reduces environmental impact.

Renewable feedstocks: Green chemistry approaches promote the use of renewable feedstocks and bio-based starting materials in chemical synthesis processes (Ahamed *et al*, 2022). Biomass-derived precursors, bio-based solvents, and renewable reagents can be utilized in pyrazole synthesis to reduce reliance on fossil fuels, decrease carbon footprint and promote sustainability in chemical manufacturing (Turkan *et al*, 2018).

Microwave-assisted and flow chemistry : Microwave-assisted synthesis and flow chemistry techniques enable rapid and efficient synthesis of pyrazole derivatives under controlled reaction conditions (Sun *et al*, 2023). Microwave irradiation promotes faster reaction rates, higher yields and improved selectivity, while continuous flow reactors offer advantages in scalability, automation, and reaction optimization, reducing energy consumption and waste generation in pyrazole synthesis processes (Xu *et al*, 2023).

Future perspectives and conclusion

The future of pyrazole-based drug discovery holds immense promise, driven by advancements in synthetic chemistry, computational modeling and biomedical research.

Potential for Future Drug development

Pyrazole scaffolds represent a rich source of chemical diversity and pharmacological activity, offering vast potential for future drug development across a wide range of therapeutic areas (Syed, 2020). The following factors contribute to the continued relevance and importance of pyrazole-based compounds in drug discovery:

Targeted therapy: With increasing understanding of disease mechanisms and molecular pathways, there is growing interest in targeted therapies that selectively modulate specific molecular targets implicated in disease pathogenesis (Koelblinger *et al,* 2018). Pyrazole-based drugs can be designed to target key enzymes, receptors, and signaling pathways involved in cancer, inflammation, metabolic disorders, neurodegenerative diseases, and infectious diseases, offering personalized treatment options with improved efficacy and reduced side effects (Ridley and Condren, 2020).

Multimodal pharmacology: Pyrazole derivatives exhibit diverse pharmacological activities, including anti-inflammatory, anticancer, antidiabetic, antiviral, antibacterial, and antifungal properties (Syed, 2019). Future drug development efforts may focus on harnessing the multimodal pharmacology of pyrazole scaffolds to develop multitargeted therapeutics with synergistic effects, enhanced therapeutic outcomes and reduced risk of drug resistance (Pacenta and Macy, 2018).

Drug repurposing and combination therapy: The repurposing of existing drugs and the development of combination therapies represent promising strategies for expanding the therapeutic applications of pyrazole-based compounds (Bailly *et al,* 2020). By repurposing known pyrazole derivatives for new indications or combining them with other pharmacological agents, researchers can leverage synergistic interactions, overcome drug resistance, and maximize therapeutic efficacy in complex diseases (Markham and Duggan, 2019).

Biologics and nanomedicine: The integration of pyrazole scaffolds with biologics, such as monoclonal antibodies, antibody-drug conjugates (ADCs), and nucleic acid-based therapeutics, offers opportunities for targeted drug delivery, enhanced tissue penetration and improved therapeutic outcomes (Van Andel *et al*, 2017). Additionally, the development of nanomedicine platforms, such as nanoparticle-based drug delivery systems and liposomal formulations, enables the targeted delivery of pyrazole-based drugs to specific tissues or cell types, minimizing off-target effects and maximizing therapeutic efficacy (Loriot *et al*, 2019).

Integration with Emerging Technologies

The integration of pyrazole-based drug discovery with emerging technologies is poised to revolutionize the field of pharmacotherapy and accelerate the development of novel therapeutics (Sun *et al,* 2023). Key emerging technologies that are likely to impact pyrazole-based drug discovery include:

Artificial intelligence (AI) and machine learning: AI and machine learning algorithms offer powerful tools for data analysis, predictive modeling and virtual screening in drug discovery (Granger *et al,* 2011). By leveraging large datasets, computational models, and predictive analytics, AI-driven approaches can accelerate the identification of novel pyrazole derivatives with desired pharmacological properties, guiding lead optimization and candidate selection in drug development pipelines (Sampath *et al,* 2023).

High-throughput screening (HTS) and automation : HTS platforms and laboratory automation technologies enable the rapid screening of large compound libraries for potential drug candidates (Frost *et al,* 2013). Integrated with robotics, liquid handling systems, and data analytics software, HTS platforms facilitate the identification of lead compounds, structure-activity relationships and hit-to-lead optimization for pyrazole-based drug discovery programs (Obeime *et al*, 2023).

CRISPR-based genome editing: CRISPR-based genome editing technologies enable precise manipulation of genetic targets and functional genomics studies in disease models (Ran *et al,* 2022). By integrating CRISPR-based screening techniques with pyrazole-based drug discovery efforts, researchers can elucidate disease mechanisms, validate drug targets and identify genetic modifiers that influence drug response and resistance, informing the development of personalized therapies (Koelblinger *et al*, 2018).

Organs-on-chips and 3D tissue models : Organs-on-chips and 3D tissue models recapitulate the physiological complexity and microenvironmental cues of human tissues and organs, providing more predictive preclinical models for drug testing and toxicity screening (Dillon *et al*, 2021). By incorporating pyrazole-based compounds into organoid cultures, microfluidic devices, and tissue engineering platforms, researchers can evaluate drug efficacy, pharmacokinetics and safety profiles in more clinically relevant settings, accelerating translation from bench to bedside (Scott, 2019).

CONCLUSION

Pyrazole scaffolds offer a diverse array of opportunities in drug discovery, driven by their structural flexibility, pharmacological potency and therapeutic versatility. Through classical synthetic routes, modern synthetic strategies and green chemistry approaches, researchers have unlocked the potential of pyrazole derivatives in targeting various diseases, from cancer and inflammation to infectious diseases and metabolic disorders. The future of pyrazole-based drug development holds promise, as integration with emerging technologies such as artificial intelligence, high-throughput screening, CRISPR-based genome editing and organ-on-chip platforms revolutionizes the field of pharmacotherapy. By embracing innovation, sustainability and interdisciplinary collaboration, the continued exploration of pyrazole scaffolds is poised to yield novel therapeutics with improved efficacy, selectivity and safety profiles, ultimately enhancing patient care and addressing unmet medical needs in the evolving landscape of modern medicine.

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Bacterial Nanocarriers for Site-Specific Drug Delivery: Harnessing Microorganisms for Precision Medicine

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Abstract:
Bacterial nanocarriers represent a promising approach for site-specific drug delivery, offering precise targeting and controlled release of therapeutic agents. This review provides an overview of the application of bacterial nanocarriers in precision medicine, focusing on their potential to target specific sites within the body for enhanced therapeutic outcomes. The key characteristics of bacterial nanocarriers, including size and shape, surface modifications for targeting and stealth capabilities, payload capacity, loading efficiency, stability, and biocompatibility, are discussed. This review explores various types of bacterial nanocarriers, including bacterial outer membrane vesicles (OMVs), bacterial spores, engineered bacteria, and other bacteria-based nanocarriers, highlighting their unique properties and applications. The mechanisms of site-specific drug delivery, including passive targeting via the enhanced permeability and retention (EPR) effect, active targeting using ligands and receptors, and tumor microenvironment-specific activation, are examined to elucidate the precise delivery mechanisms employed by bacterial nanocarriers. Furthermore, this review discusses the diverse applications of bacterial nanocarriers in precision medicine, including cancer therapy, infectious diseases, and chronic diseases, emphasizing their potential for targeted delivery of chemotherapeutic agents, immunotherapy, antibiotics, vaccines, and sustained release formulations. Challenges such as immunogenicity, manufacturing scalability, regulatory hurdles, and approval processes are addressed, along with future directions and emerging technologies in the field. Overall, bacterial nanocarriers hold immense promise as versatile platforms for site-specific drug delivery, paving the way for advancements in precision medicine and personalized therapeutics.

Keywords: bacterial nanocarriers, precision medicine, drug delivery, targeted therapy, cancer, nanotechnology.

I. Introduction

Precision medicine is a medical approach that takes into account individual variability in genes, environment, and lifestyle for each person. It recognizes that each individual is unique, and thus, treatments should be tailored to specific characteristics rather than adopting a one-size-fits-all approach[1]. Precision medicine aims to improve treatment outcomes, minimize side effects, and optimize healthcare resources by targeting therapies to patients who are most likely to benefit from them.Precision medicine encompasses various fields, including genomics, proteomics, metabolomics, and other omics disciplines, along with clinical and health data analysis[2]. This has led to advancements in disease prevention, diagnosis, and

treatment across various medical specialties, including oncology, cardiology, neurology, and infectious disease[3].

The foundation of precision medicine lies in understanding the molecular mechanisms underlying diseases and identifying biomarkers that can predict disease susceptibility, progression, and response to treatment. With the advent of high-throughput technologies and bioinformatics tools, researchers can analyse vast amounts of biological data to elucidate disease pathways and develop targeted therapies[4].Precision medicine has the potential to revolutionize healthcare by shifting from a reactive approach to a proactive and personalized approach. By identifying individuals at risk of disease and providing targeted interventions, precision medicine aims to improve patient outcomes, reduce healthcare costs, and enhance overall population health[5].Site-specific drug delivery is a crucial aspect of precision medicine that aims to deliver therapeutic agents directly to the site of action within the body while minimizing systemic exposure and off-target effects. Traditional drug delivery systems often result in low drug concentrations at the target site, leading to suboptimal efficacy and increased risk of adverse reactions[6].Site-specific drug delivery offers several advantages over conventional systemic administration. It allows for higher drug concentrations at the desired site, leading to enhanced therapeutic efficacy and reduced dosage requirements. By minimizing exposure to healthy tissues, site-specific drug delivery can also reduce side effects and improve patient compliance with treatment regimens[7].Site-specific drug delivery is particularly important in the treatment of localized diseases, such as cancer, inflammatory disorders, and infections. In cancer therapy, for example, delivering chemotherapy directly to the tumor site can increase the likelihood of tumor regression while minimizing damage to surrounding healthy tissues[8]. Similarly, targeted drug delivery can improve the efficacy of antimicrobial agents in treating infections by delivering high concentrations of drugs to the site of infection.Various approaches have been developed for site-specific drug delivery, including passive and active targeting strategies. Passive targeting relies on the physiological properties of tissues, such as the enhanced permeability and retention (EPR) effect observed in tumors, to deliver drugs selectively to the target site. Active targeting involves the use of ligands, antibodies, or other targeting moieties to facilitate specific interactions with receptors or antigens expressed on target cells, further enhancing drug delivery efficiency[9].

Bacterial nanocarriers represent a promising platform for site-specific drug delivery, harnessing the unique properties of bacteria to deliver therapeutic agents to specific tissues or cells within the body. Bacteria possess several inherent advantages as drug delivery vehicles, including their small size, ability to penetrate biological barriers, and capacity for targeted localization.Bacterial nanocarriers can be engineered to express specific surface molecules or proteins that facilitate targeting to particular tissues or cells[10]. Moreover, bacteria can be genetically modified to produce and release therapeutic agents directly at the site of action, providing sustained drug release and minimizing systemic exposure.Several types of bacterial nanocarriers, including bacterial outer membrane vesicles (OMVs), bacterial spores, and engineered bacteria, have been explored for drug delivery applications[11]. OMVs are naturally produced by bacteria and can be loaded with therapeutic cargo for targeted delivery to host cells. Bacterial spores, such as those produced by Clostridium species, have been investigated as vehicles for delivering drugs or imaging agents specifically to hypoxic regions

within solid tumors. Engineered bacteria can be designed to target specific tissues or cells and deliver therapeutic payloads in response to environmental cues or stimuli[12].

The aim of this review article is to provide a comprehensive overview of bacterial nanocarriers for site-specific drug delivery and their potential applications in precision medicine. This review covers the characteristics of bacterial nanocarriers, including size, shape, surface modifications, payload capacity, and stability. The mechanisms of site-specific drug delivery by bacterial nanocarriers, including passive and active targeting strategies, are also discussed[13].Furthermore, this review highlights the applications of bacterial nanocarriers in precision medicine, with a focus on cancer therapy, infectious diseases, and chronic conditions. The challenges and limitations associated with bacterial nanocarriers, such as immunogenicity, manufacturing scalability, and regulatory considerations, are discussed. Finally, this review provides insights into future directions and emerging technologies in the field of bacterial nanocarriers for site-specific drug delivery.

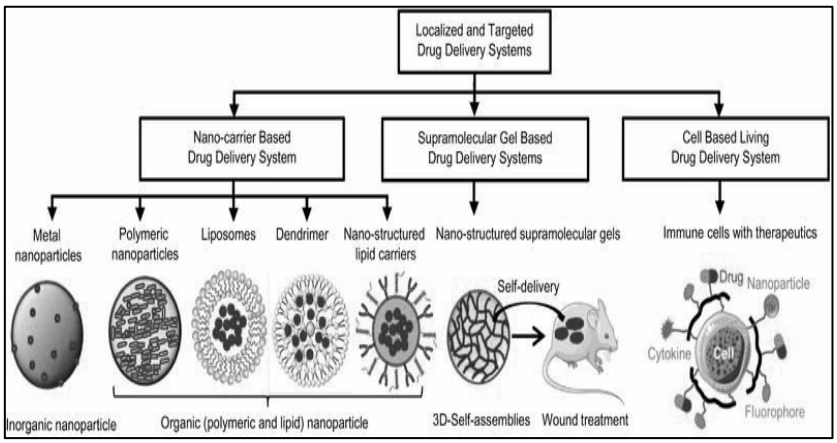

Figure 1: Diverse Carrier-Based Systems for Localized and Targeted Drug Delivery

II. Characteristics of the Bacterial Nanocarriers

A. Size and shape considerations

The size and shape of bacterial nanocarriers play a crucial role in determining their behavior and efficacy as drug delivery vehicles. Bacterial nanocarriers are typically nanosized particles ranging from a few nanometres to several hundred nanometres in diameter[14]. The small size of bacterial nanocarriers enables them to penetrate biological barriers, such as cell membranes and tissue barriers, and reach target sites within the body more efficiently.The shape of bacterial nanocarriers can also influence their biodistribution, cellular uptake, and targeting capabilities[15]. Various shapes, including spherical, rod-shaped, and filamentous, have been explored for use as bacterial nanocarriers. For example, spherical nanoparticles may exhibit prolonged circulation times in the bloodstream and enhanced cellular uptake, while rod-shaped or filamentous particles may improve tumor penetration and retention[16].

Furthermore, the aspect ratio of bacterial nanocarriers, defined as the ratio of their length to width, can impact their interactions with biological systems. High aspect ratio particles, such as nanorods or nanowires, may exhibit enhanced cellular uptake and internalization compared to spherical particles due to their elongated shape. However, excessively high aspect ratios

may also increase the risk of cytotoxicity or immune recognition, highlighting the importance of optimizing the shape and aspect ratio of bacterial nanocarriers for specific applications[17].

B. Surface modifications for targeting and stealth capabilities

Surface modifications of bacterial nanocarriers play a crucial role in enhancing their targeting specificity and stealth capabilities while minimizing immune recognition and clearance[5]. The surface of bacterial nanocarriers can be modified with various functional groups, polymers, peptides, or antibodies to facilitate specific interactions with target cells or tissues[18].Targeting ligands, such as antibodies, peptides, or small molecules, can be conjugated to the surface of bacterial nanocarriers to recognize and bind to specific receptors or antigens expressed on target cells[6]. This enables precise targeting of therapeutic agents to diseased tissues while minimizing off-target effects on healthy cells. For example, antibodies targeting overexpressed receptors on cancer cells can be conjugated to bacterial nanocarriers to achieve tumor-specific drug delivery[2].In addition to targeting ligands, surface modifications can also impart stealth properties to bacterial nanocarriers to evade immune detection and clearance[19]. PEGylation, the conjugation of polyethylene glycol (PEG) chains to the surface of nanoparticles, is a commonly used strategy to increase the circulation half-life of bacterial nanocarriers by reducing opsonization and phagocytosis by the reticuloendothelial system (RES). PEGylation creates a hydrophilic barrier around bacterial nanocarriers, preventing protein adsorption and recognition by immune cells[20].

C. Payload capacity and loading efficiency

The payload capacity and loading efficiency of bacterial nanocarriers are critical factors that determine their therapeutic efficacy and practical utility as drug delivery vehicles[13]. The payload capacity refers to the maximum amount of therapeutic agent that can be loaded onto or encapsulated within bacterial nanocarriers, while the loading efficiency reflects the percentage of drug encapsulated relative to the total capacity of the nanocarrier[21].Bacterial nanocarriers offer several advantages for drug loading and encapsulation, including their large surface area, internal compartmentalization, and potential for genetic engineering. Therapeutic agents can be loaded onto bacterial nanocarriers through physical adsorption, chemical conjugation, encapsulation within vesicles or compartments, or genetic expression and secretion by engineered bacteria[22].The payload capacity of bacterial nanocarriers can vary depending on factors such as the size, shape, and composition of the nanocarrier, as well as the physicochemical properties of the therapeutic agent. For example, small-molecule drugs may be loaded at higher concentrations than larger biologics or nucleic acids due to differences in molecular size and solubility[8].Loading efficiency is influenced by various parameters, including the method of drug loading, the affinity between the drug and nanocarrier, and the stability of the drug-nanocarrier complex. Optimizing loading efficiency is essential to maximize the therapeutic payload delivered to the target site while minimizing waste and ensuring cost-effectiveness[23].

D. Stability and biocompatibility

The stability and biocompatibility of bacterial nanocarriers are critical considerations for their safe and effective use in drug delivery applications[3]. Bacterial nanocarriers must maintain their structural integrity and drug-loading capacity during storage, transportation, and administration to ensure reliable and consistent therapeutic outcomes[24].Stability encompasses various aspects, including physical stability (e.g., aggregation, sedimentation, or degradation), chemical stability (e.g., drug degradation or release), and biological stability (e.g., susceptibility to enzymatic degradation or immune recognition)[2,4]. Strategies to enhance the stability of bacterial nanocarriers include surface modifications, encapsulation within protective matrices or coatings, and formulation optimization[25].Biocompatibility refers to the compatibility of bacterial nanocarriers with biological systems, including cells, tissues, and the immune system. Bacterial nanocarriers should exhibit minimal cytotoxicity, immunogenicity, or inflammatory responses to ensure their safety and tolerability in vivo[11]. Biocompatibility can be influenced by factors such as the composition, surface chemistry, and degradation products of bacterial nanocarriers, as well as their interactions with host cells and tissues[6].Various in vitro and in vivo assays, including cell viability assays, cytokine profiling, histological analysis, and pharmacokinetic studies, are used to assess the stability and biocompatibility of bacterial nanocarriers. Preclinical safety evaluations are essential for identifying potential adverse effects and guiding the design and optimization of bacterial nanocarriers for clinical translation[26].

III. Types of Bacterial Nanocarriers

A. Bacterial outer membrane vesicles (OMVs)

OMVs are nanoscale spherical structures naturally released by gram-negative bacteria as part of their normal growth and metabolism[5]. OMVs are composed of outer membrane lipids, proteins, and various cargo molecules, including toxins, enzymes, nucleic acids, and cell wall components. These vesicles range in size from 20 to 300 nanometers and are enriched in outer membrane proteins and lipopolysaccharides[27].OMVs have garnered significant interest as potential drug delivery vehicles due to their biocompatibility, stability, and ability to encapsulate and protect cargo molecules from degradation. OMVs can be engineered to display specific antigens or ligands on their surface, allowing for targeted delivery to specific cell types or tissues[2,9]. Additionally, OMVs can be loaded with therapeutic agents, such as drugs, vaccines, or nucleic acids, for targeted delivery to diseased tissues or cells.The unique properties of OMVs make them promising candidates for various biomedical applications, including vaccine delivery, cancer therapy, and infectious disease treatment[28]. OMV-based vaccines have been developed against bacterial pathogens, such as *Neisseria meningitidis* and Vibrio cholerae, by loading OMVs with antigenic proteins or polysaccharides derived from target pathogens. These vaccines have shown promising efficacy in preclinical and clinical studies, providing protection against bacterial infections.In addition to their use in vaccine delivery, OMVs have been explored for targeted drug delivery in cancer therapy[3,8]. Engineered OMVs can be loaded with chemotherapeutic agents or nucleic acids and functionalized with targeting ligands to specifically deliver drugs to tumor cells while minimizing off-target effects on healthy tissues. Moreover, OMVs can be used as adjuvants to enhance the immune response and efficacy of cancer immunotherapies[29].

B. Bacterial spores

Bacterial spores are dormant, highly resistant structures formed by certain bacterial species in response to adverse environmental conditions. Spores are characterized by their tough outer coat, which protects the bacterial genome and cellular contents from desiccation, heat, radiation, and chemical damage. Bacterial spores can remain viable for extended periods, making them attractive candidates for drug delivery and biotechnological applications[19].Bacterial spores have been explored as natural carriers for drug delivery due to their unique properties, including their small size (1-2 micrometres), stability, and capacity for payload encapsulation[30]. Spores can be genetically engineered to produce and release therapeutic agents in response to specific stimuli or environmental cues, such as pH, temperature, or the presence of target molecules.One of the most extensively studied bacterial spores for drug delivery is the spore-forming bacterium *Clostridium difficile*[12]. Engineered *C. difficile* spores have been used as targeted delivery vehicles for cancer therapy by exploiting the hypoxic microenvironment of solid tumors. These spores are engineered to germinate selectively within the tumor microenvironment and release therapeutic payloads, such as cytotoxic drugs or imaging agents, specifically within the tumor tissue[31].

C. Engineered bacteria

Engineered bacteria are genetically modified microbial strains designed to deliver therapeutic agents or perform specific functions within the body for biomedical applications. Engineered bacteria offer unique advantages as drug delivery vehicles, including their inherent targeting capabilities, capacity for self-replication, and potential for on-demand drug production[23].

One of the most widely studied engineered bacteria for drug delivery is *Escherichia coli* (*E. coli*), a common gram-negative bacterium[17]. Engineered *E. coli* strains have been designed to express and release therapeutic proteins, peptides, enzymes, or nucleic acids in response to specific environmental cues or stimuli. These bacteria can be administered orally, intravenously, or directly to target tissues to deliver therapeutic payloads to desired sites within the body.Engineered bacteria can be programmed to target specific cell types or tissues by modifying their surface proteins or introducing targeting ligands or peptides[32]. Moreover, bacteria can be engineered to express and release therapeutic agents in a controlled manner, providing sustained drug release and minimizing systemic toxicity[22].Several strategies have been explored to enhance the safety and efficacy of engineered bacteria for drug delivery, including the use of containment mechanisms to prevent bacterial replication in vivo, inducible expression systems for controlled drug release, and genetic circuitry to enable bacterial communication and coordination within microbial consortia.Engineered bacteria hold great promise for a wide range of biomedical applications, including cancer therapy, infectious disease treatment, and metabolic engineering[17]. However, challenges remain in the development of engineered bacteria, including issues related to biosafety, immunogenicity, and regulatory approval. Ongoing research efforts continue to address these challenges and advance the clinical translation of engineered bacteria for precision medicine[33].

D. Other bacterial-based nanocarriers

In addition to OMVs, bacterial spores, and engineered bacteria, other bacteria-based nanocarriers have been explored for drug delivery applications[20]. These include engineered bacterial ghosts, bacterial cell-derived nanoparticles, and bacterial-derived extracellular vesicles.Engineered bacterial ghosts are empty bacterial cell envelopes derived from gram-negative bacteria that have been genetically modified to remove their genetic material. These empty cell envelopes retain the surface structures and membrane components of the original bacteria and can be loaded with therapeutic agents for targeted drug delivery[34]. Bacterial ghosts offer advantages such as biocompatibility, immunogenicity, and potential for surface functionalization.Bacterial cell-derived nanoparticles are nanosized particles derived from intact bacterial cells through various physical or chemical methods, such as sonication, homogenization, or extrusion[18]. These nanoparticles retain the structural and functional properties of the original bacteria and can be loaded with drugs, proteins, or nucleic acids for targeted delivery. Bacterial cell-derived nanoparticles offer advantages such as biocompatibility, stability, and potential for surface modification[35].

IV. Mechanisms of Site-Specific Drug Delivery

A. Passive targeting via the enhanced permeability and retention (EPR) effect

Passive targeting exploits the unique physiological characteristics of diseased tissues, such as tumors, to achieve site-specific drug delivery. The enhanced permeability and retention (EPR) effect is a phenomenon commonly observed in solid tumors, wherein leaky blood vessels and impaired lymphatic drainage lead to the accumulation of macromolecules and nanoparticles within the tumor microenvironment[31].Solid tumors are characterized by abnormal vasculature with discontinuous endothelial cell junctions and fenestrations, allowing for increased vascular permeability[36]. This abnormal vascular architecture, coupled with poor lymphatic drainage, results in the retention of macromolecules and nanoparticles within the tumor interstitium. As a result, systemically administered drugs or nanoparticles can preferentially accumulate in tumor tissues, providing a passive targeting mechanism for site-specific drug delivery[25].The EPR effect is primarily exploited for the delivery of nanoparticle-based therapeutics, such as liposomes, polymeric micelles, and nanoparticles, which are too large to penetrate normal blood vessels but can extravasate and accumulate in tumor tissues through leaky tumor vasculature[36]. Once they accumulate within the tumor interstitium, these nanoparticles can release their payload of therapeutic agents, such as chemotherapy drugs, nucleic acids, or imaging agents, to exert their therapeutic effects.The passive targeting approach offers several advantages for site-specific drug delivery, including simplicity, noninvasiveness, and broad applicability to various types of solid tumors[21]. However, the efficacy of passive targeting via the EPR effect can be highly variable depending on the tumor type, size, and stage, as well as individual patient factors. Moreover, the heterogeneity of the tumor vasculature and the presence of stromal components within the tumor microenvironment can limit the extent of nanoparticle accumulation and hinder drug delivery efficiency[37].

B. Active targeting using ligands and receptors

Active targeting involves specific interactions between targeting ligands, such as antibodies, peptides, aptamers, or small molecules, conjugated to the surface of drug carriers, and receptors or antigens overexpressed on the surface of target cells or tissues[20]. This targeted approach enhances the specificity and efficiency of drug delivery while minimizing off-target effects on healthy tissues.Targeting ligands can be selected or engineered to recognize specific biomarkers associated with diseased tissues, such as cancer cells, inflammatory cells, or pathogenic microorganisms[38]. These ligands bind to their cognate receptors with high affinity and specificity, facilitating the selective uptake of drug carriers into target cells via receptor-mediated endocytosis or other internalization mechanisms[19].Various types of targeting ligands have been employed for active targeting, including monoclonal antibodies, antibody fragments (e.g., Fab or scFv), peptides (e.g., cell-penetrating peptides or tumor-homing peptides), aptamers (e.g., nucleic acid-based ligands), and small molecules (e.g., folate or transferrin)[7]. These ligands can be conjugated to the surface of drug carriers, such as liposomes, nanoparticles, or polymer micelles, using chemical, biological, or physical coupling methods.Active targeting using ligands and receptors offers several advantages over passive targeting, including enhanced specificity, reduced systemic toxicity, and improved therapeutic efficacy[39]. By directing drug carriers specifically to diseased tissues or cells, active targeting can increase the local concentration of therapeutic agents, improve cellular uptake, and overcome barriers to drug delivery, such as the blood–brain barrier or multidrug resistance mechanisms[3].Moreover, active targeting can be combined with other strategies, such as stimuli-responsive drug release or synergistic combination therapy, to further enhance the therapeutic outcomes of targeted drug delivery. For example, stimuli-responsive drug carriers can release their payload selectively in response to specific environmental cues or physiological conditions within the target tissue, further improving drug efficacy and reducing off-target effects[40].

C. Tumor microenvironment-specific activation

Tumor microenvironment-specific activation is a strategy for site-specific drug delivery that exploits the unique biochemical and physiological characteristics of the tumor microenvironment to trigger the release or activation of therapeutic agents within tumor tissues[21]. The tumor microenvironment is a complex and dynamic ecosystem composed of tumor cells, stromal cells, immune cells, blood vessels, and extracellular matrix components[41].

The tumor microenvironment exhibits several distinctive features that can be targeted for site-specific drug delivery, including acidic pH, hypoxia, elevated levels of enzymes and biomarkers, and aberrant expression of receptors or signalling pathways. By harnessing these tumor-specific cues, drug delivery systems can be designed to respond selectively to the tumor microenvironment and release therapeutic agents specifically within tumor tissues while sparing healthy tissues[19].One approach for tumor microenvironment-specific activation involves the use of stimuli-responsive drug carriers that undergo structural changes or release their payload in response to specific stimuli or environmental cues present within the tumor microenvironment[16]. For example, pH-sensitive nanoparticles can be designed to

release their cargo in response to the acidic pH of tumor tissues, leading to localized drug delivery and enhanced therapeutic efficacy[42].

Another approach involves the use of prodrugs or inactive drug precursors that are activated selectively within the tumor microenvironment by tumor-specific enzymes or metabolic pathways[8]. Upon activation, these prodrugs are converted into their active form, exerting their therapeutic effects specifically within tumor tissues while minimizing systemic toxicity.Furthermore, nanotechnology-based platforms, such as liposomes, nanoparticles, or polymer micelles, can be functionalized with targeting ligands or antibodies to achieve tumor-specific accumulation and cellular uptake[43]. These targeted drug delivery systems can exploit tumor-specific biomarkers or receptors for selective binding and internalization into tumor cells, enhancing drug delivery efficiency and therapeutic outcomes.Tumor microenvironment-specific activation strategies offer several advantages for site-specific drug delivery, including enhanced specificity, reduced systemic toxicity, and improved therapeutic efficacy[18]. By targeting tumor-specific cues and mechanisms, these strategies can overcome barriers to drug delivery and enhance the accumulation and retention of therapeutic agents within tumor tissues[44].

Table 1. Antitumour drug-loaded nanocarriers for the treatment of various tumors

Nanocarrier	**Drug(s)**	**Tumor**	**Benefits**	**References**
Liposomes	Doxorubicin, Paclitaxel	Breast cancer	Controlled drug release, reduced systemic toxicity	[2]
Polymeric nanoparticles	Docetaxel, Cisplatin	Lung cancer	Enhanced drug stability, targeted delivery to lung tissue	[5]
Dendrimers	Methotrexate, Paclitaxel	Ovarian cancer	High drug loading capacity, tumor penetration	[10]
Carbon nanotubes	Cisplatin, Gemcitabine	Pancreatic cancer	Ability to penetrate deep into tumor tissue, sustained drug release	[18]
Gold nanoparticles	Cisplatin, 5-Fluorouracil	Colorectal cancer	Enhanced cellular uptake, photothermal therapy enhancement	[13]
Magnetic nanoparticles	Methotrexate, Doxorubicin	Brain tumors	Magnetic targeting, enhanced blood–brain barrier penetration	[22]
Mesoporous silica nanoparticles	Paclitaxel, Docetaxel	Prostate cancer	High surface area for drug loading, pH-responsive drug release	[29]
Nanogels	Methotrexate, Irinotecan	Gastric cancer	Stimuli-responsive drug release, prolonged circulation time	[34]

V. Applications of Bacterial Nanocarriers in Precision Medicine

A. Cancer therapy

Cancer therapy represents one of the most promising applications of bacterial nanocarriers in precision medicine, offering targeted and personalized treatment options for patients with various types of cancer. Bacterial nanocarriers hold great potential for improving the efficacy and safety of cancer therapy through targeted drug delivery, immunomodulation, and combination therapy approaches[45].

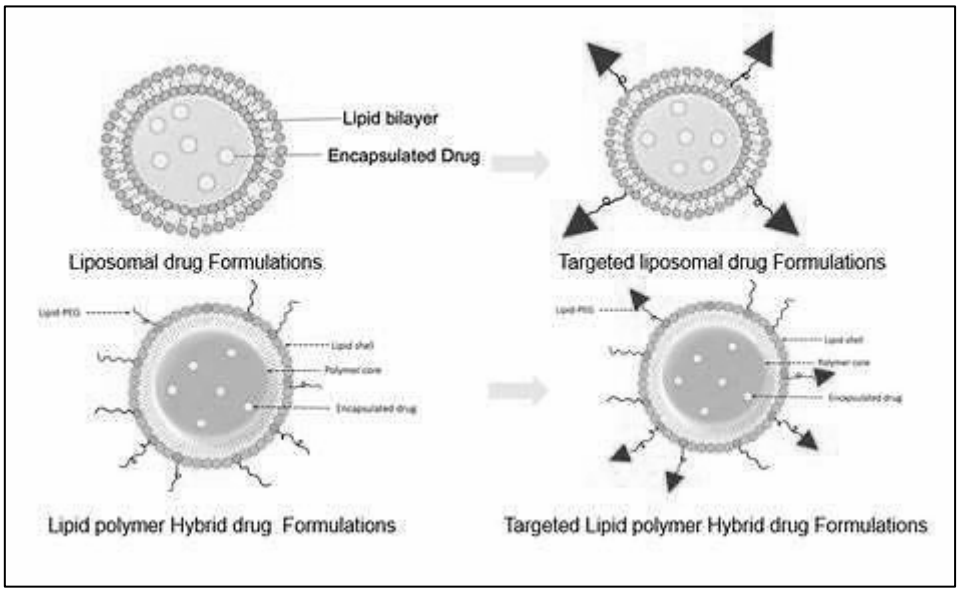

Figure 2: Nanoparticle-Based Systems for Delivering Cancer Therapeutics

Targeted delivery of chemotherapeutic agents

Chemotherapy is a cornerstone of cancer treatment, but its efficacy is often limited by systemic toxicity and off-target effects on healthy tissues. Bacterial nanocarriers can address these limitations by delivering chemotherapeutic agents selectively to tumor tissues while minimizing exposure to healthy organs[6]. By exploiting passive or active targeting mechanisms, bacterial nanocarriers can enhance the accumulation of chemotherapeutic drugs within tumors, leading to improved therapeutic outcomes and reduced side effects.Passive targeting via the enhanced permeability and retention (EPR) effect allows bacterial nanocarriers to accumulate preferentially in tumor tissues due to the leaky vasculature and impaired lymphatic drainage characteristic of solid tumors[46]. This passive targeting mechanism can be further enhanced by optimizing the size, shape, and surface properties of bacterial nanocarriers to improve their tumor penetration and retention[26].Active targeting strategies involve the conjugation of targeting ligands, such as antibodies, peptides, or small molecules, to the surface of bacterial nanocarriers to facilitate specific interactions with receptors or antigens overexpressed on tumor cells. These targeted drug delivery systems can enhance the specificity and efficiency of drug delivery to tumor tissues, resulting in improved therapeutic efficacy and reduced systemic toxicity[29].Moreover, bacterial nanocarriers can be engineered to release chemotherapeutic drugs in response to specific stimuli or environmental cues within the tumor microenvironment, such as acidic pH, hypoxia, or enzymatic activity. Stimuli-responsive drug delivery systems enable controlled and triggered

release of therapeutic agents within tumor tissues, further enhancing drug efficacy and minimizing off-target effects[47].

Immunotherapy using bacterial vectors

In addition to targeted drug delivery, bacterial nanocarriers hold promise for cancer immunotherapy by harnessing the immune system to recognize and eliminate tumor cells[5]. Bacterial vectors can be engineered to express and deliver immunomodulatory agents, such as cytokines, chemokines, or immune checkpoint inhibitors, to the tumor microenvironment, thereby enhancing the antitumour immune response and overcoming immune evasion mechanisms employed by cancer cells[48].Bacterial nanocarriers can serve as potent adjuvants for cancer vaccines, promoting antigen presentation and activation of tumor-specific immune responses. Engineered bacteria can be designed to express tumor-associated antigens or neoantigens and deliver them to antigen-presenting cells, such as dendritic cells, to stimulate T-cell-mediated immune responses against tumor cells[15]. Moreover, bacterial vectors can be modified to express immunostimulatory molecules, such as interleukins or costimulatory ligands, to enhance the activation and proliferation of antitumour immune cells within the tumor microenvironment[20].

Furthermore, bacterial nanocarriers can be engineered to modulate the tumor microenvironment and promote antitumour immune responses while inhibiting the immunosuppressive mechanisms employed by cancer cells[7]. For example, bacteria can be engineered to express enzymes that convert immunosuppressive metabolites, such as adenosine or indoleamine 2,3-dioxygenase (IDO), into immunostimulatory molecules, thereby reversing the immunosuppressive tumor microenvironment and enhancing the efficacy of cancer immunotherapy[49].

B. Infectious diseases

Bacterial nanocarriers hold promise for the treatment of infectious diseases, offering targeted delivery of antibiotics, vaccines, and immunomodulatory agents to combat microbial pathogens while minimizing systemic toxicity and antimicrobial resistance[13].

Targeted delivery of antibiotics

Antibiotic resistance poses a significant threat to global public health, necessitating the development of novel strategies for targeted and personalized antimicrobial therapy. Bacterial nanocarriers can enhance the efficacy and specificity of antibiotic delivery by targeting microbial pathogens directly to infected tissues or cells while sparing the commensal microbiota and minimizing off-target effects[50].

Bacterial nanocarriers can be engineered to encapsulate or conjugate antibiotics and deliver them selectively to sites of infection, such as bacterial biofilms, intracellular pathogens, or localized infections[10]. By exploiting passive or active targeting mechanisms, bacterial nanocarriers can enhance the accumulation of antibiotics within infected tissues, leading to improved antimicrobial efficacy and a reduced risk of resistance development.Passive targeting strategies leverage the enhanced permeability and retention (EPR) effect to deliver antibiotic-loaded bacterial nanocarriers preferentially to sites of infection, such as inflamed tissues or bacterial biofilms[6]. Active targeting approaches involve the conjugation of

targeting ligands or peptides to the surface of bacterial nanocarriers to facilitate specific interactions with microbial surface molecules or host cell receptors, enabling selective binding and internalization of antibiotic-loaded carriers into infected cells or pathogens[51].

Vaccines and immunomodulation

Bacterial nanocarriers offer promising platforms for the development of novel vaccines and immunomodulatory therapies to prevent and treat infectious diseases[13]. Engineered bacteria can be designed to express and deliver antigens or immunomodulatory molecules to the immune system, thereby stimulating protective immune responses against microbial pathogens or modulating host immune responses to enhance pathogen clearance and resolution of infection[4].Bacterial vectors can be engineered to express antigens derived from microbial pathogens and deliver them to antigen-presenting cells, such as dendritic cells, to stimulate the activation and proliferation of pathogen-specific T and B cells[52]. Moreover, bacterial nanocarriers can be modified to express adjuvants or immunostimulatory molecules that enhance the immune response to vaccination, promoting the production of protective antibodies and memory T cells against microbial pathogens.Furthermore, bacterial nanocarriers can be engineered to modulate host immune responses and promote immune-mediated clearance of microbial pathogens[2]. Engineered bacteria can express immunomodulatory molecules, such as cytokines, chemokines, or Toll-like receptor agonists, that enhance innate and adaptive immune responses against infection. Moreover, bacterial nanocarriers can be designed to target specific immune cell populations or tissues involved in host defense mechanisms, such as mucosal surfaces or lymphoid organs, to enhance the efficacy of vaccination or immunotherapy[53].

C. Chronic diseases

Bacterial nanocarriers hold potential for the treatment of chronic diseases, offering targeted delivery to specific organs or tissues affected by pathological processes while minimizing systemic toxicity and off-target effects. Moreover, bacterial nanocarriers can be designed to provide sustained release formulations that deliver therapeutic agents over an extended period, enabling long-term disease management and improved patient compliance[7].

Targeted delivery to specific organs or tissues

Chronic diseases, such as cardiovascular disease, neurodegenerative disorders, and autoimmune diseases, often require targeted drug delivery to specific organs or tissues affected by pathological processes[7]. Bacterial nanocarriers can be engineered to deliver therapeutic agents selectively to diseased tissues while sparing healthy organs, thereby enhancing therapeutic efficacy and minimizing systemic toxicity[54].Passive and active targeting strategies can be employed to achieve site-specific drug delivery in chronic diseases. Passive targeting via the enhanced permeability and retention (EPR) effect enables bacterial nanocarriers to preferentially accumulate in diseased tissues with leaky vasculature or impaired lymphatic drainage, such as inflamed or fibrotic tissues[13]. Active targeting approaches involve the conjugation of targeting ligands or peptides to the surface of bacterial nanocarriers to facilitate specific interactions with receptors or antigens overexpressed on cells or tissues affected by chronic diseases[55].Moreover, bacterial nanocarriers can be

engineered to respond to specific cues or stimuli associated with pathological processes within target tissues, such as inflammation, oxidative stress, or metabolic dysregulation, to trigger the release of therapeutic agents at the site of disease[23]. Stimuli-responsive drug delivery systems enable controlled and triggered release of drugs within diseased tissues, enhancing therapeutic efficacy while minimizing systemic exposure and off-target effects[56].

Sustained release formulations

Chronic diseases often require long-term or continuous administration of therapeutic agents to achieve optimal disease management and symptom control[21]. Bacterial nanocarriers can be designed to provide sustained release formulations that deliver drugs over an extended period, thereby reducing dosing frequency, improving patient compliance, and maintaining therapeutic concentrations of drugs within target tissues.Sustained release formulations can be achieved by encapsulating drugs within bacterial nanocarriers or modifying their surface properties to control drug release kinetics[57]. By modulating factors such as particle size, surface charge, and polymer composition, bacterial nanocarriers can be tailored to release drugs at a controlled rate, ensuring prolonged therapeutic effects while minimizing fluctuations in drug concentrations.Moreover, stimuli-responsive drug delivery systems can be employed to achieve on-demand release of therapeutic agents in response to specific cues or triggers associated with disease progression or symptom exacerbation[55]. For example, bacterial nanocarriers can be engineered to respond to changes in pH, temperature, or enzyme activity within target tissues, enabling the controlled release of drugs in response to disease-specific stimuli[57].

VI. Challenges and Future Perspectives

A. Immunogenicity and safety concerns

Immunogenicity and safety concerns represent significant challenges in the development and clinical translation of bacterial nanocarriers for precision medicine applications. Bacterial nanocarriers, including engineered bacteria and bacterial-derived vesicles, have the potential to elicit immune responses and adverse reactions in patients, which can limit their therapeutic efficacy and safety[33].

Immunogenicity of bacterial nanocarriers

Bacterial nanocarriers, particularly live bacteria or bacterial vectors, can induce immune responses in the host due to their foreign antigenicity and potential for dissemination or persistence within the body[22]. Engineered bacteria may express surface antigens or pathogen-associated molecular patterns (PAMPs) that activate innate immune cells and trigger inflammatory responses, leading to adverse effects such as fever, cytokine release syndrome, or systemic inflammation[58].Moreover, bacterial nanocarriers may elicit adaptive immune responses, including antibody production and T-cell activation, against bacterial antigens or vector components, which can impact their therapeutic efficacy and lead to immune-mediated clearance or neutralization. Preexisting immunity to bacterial vectors, acquired through prior exposure or vaccination, can also influence the immune response to bacterial nanocarriers and affect their clinical performance[59].

Safety considerations of bacterial nanocarriers

Safety concerns associated with bacterial nanocarriers include the risk of infection, systemic toxicity, and unintended off-target effects[33]. Live bacteria or bacterial vectors may pose a risk of infection or dissemination within the host, particularly in immunocompromised or susceptible individuals. Bacterial nanocarriers may also produce toxins or virulence factors that contribute to adverse effects or exacerbate underlying disease conditions[12,1].Furthermore, bacterial nanocarriers must be engineered to ensure the containment and control of their behavior within the body to minimize the risk of unintended off-target effects or environmental release. Strategies to enhance the safety of bacterial nanocarriers include genetic modifications to attenuate virulence, improve containment, or enhance biocontainment, as well as formulation optimization to reduce toxicity and immunogenicity[60].

B. Manufacturing scalability

Manufacturing scalability represents a key challenge in the production of bacterial nanocarriers for precision medicine applications. The scalable and cost-effective production of bacterial nanocarriers is essential for their widespread adoption and commercialization, but it poses technical and logistical challenges that must be overcome to meet the growing demand for precision medicine therapies[19].

Complexity of bacterial nanocarrier production

The production of bacterial nanocarriers involves multiple steps, including bacterial cultivation, genetic engineering, purification, and formulation, each of which presents challenges in terms of scalability and reproducibility[12]. Bacterial cultivation requires the optimization of culture conditions, growth media, and fermentation processes to achieve high cell densities and product yields. Genetic engineering of bacteria or bacterial vectors involves the manipulation of complex biological systems and pathways, requiring expertise in molecular biology, synthetic biology, and genetic engineering techniques[61].

Moreover, the purification of bacterial nanocarriers from culture supernatants or cell lysates can be challenging due to the presence of host cell debris, contaminants, and heterogeneous populations of nanocarriers. Purification processes must be scalable, efficient, and cost-effective to yield high-purity products suitable for clinical use[14]. The formulation of bacterial nanocarriers into stable drug delivery systems, such as liposomes, nanoparticles, or hydrogels, requires the optimization of formulation parameters and quality control measures to ensure product consistency and stability[3,8].

Scalability of production processes

Scaling up production processes for bacterial nanocarriers from the laboratory scale to the industrial scale poses technical and logistical challenges, including the optimization of bioreactor systems, the automation of culture and purification processes, and compliance with regulatory requirements for good manufacturing practices (GMPs). Bioreactor design, operation, and control must be optimized to achieve high cell densities, high product yields, and high reproducibility while minimizing process variability and contamination risks[62].

C. Future directions and emerging technologies

Future directions and emerging technologies hold promise for advancing the field of bacterial nanocarriers and unlocking new opportunities for precision medicine applications. Continued research efforts are needed to address existing challenges, explore novel strategies, and

capitalize on emerging technologies to further enhance the therapeutic potential of bacterial nanocarriers.

Advancements in genetic engineering and synthetic biology

Advances in genetic engineering and synthetic biology are driving innovations in the design and engineering of bacterial nanocarriers for precision medicine applications. Novel gene editing tools, such as CRISPR-Cas9, enable precise manipulation of bacterial genomes to engineer desired traits, functionalities, and properties in bacterial nanocarriers. Synthetic biology approaches allow for the design and construction of synthetic biological systems and pathways for programmable and customizable control of bacterial nanocarrier behavior and function[63].

Development of multifunctional and smart nanocarriers

The development of multifunctional and smart nanocarriers holds promise for enhancing the therapeutic efficacy, specificity, and responsiveness of bacterial nanocarriers in precision medicine applications[64]. Multifunctional nanocarriers can integrate multiple functionalities, such as targeting ligands, stimuli-responsive materials, imaging agents, and therapeutic payloads, into a single platform to enable synergistic effects and personalized treatment strategies. Smart nanocarriers can respond dynamically to environmental cues or stimuli within the body, such as pH, temperature, or enzyme activity, to trigger the controlled release of therapeutic agents and optimize drug delivery kinetics[65].

Integration of nanotechnology and artificial intelligence

The integration of nanotechnology and artificial intelligence (AI) offers opportunities for optimizing the design, characterization, and optimization of bacterial nanocarriers for precision medicine applications[66]. AI-driven approaches, such as machine learning, computational modelling, and data analytics, can accelerate the discovery and development of novel bacterial nanocarriers by predicting structure–function relationships, optimizing formulation parameters, and identifying candidate therapies with enhanced therapeutic properties[67]. Nanotechnology-enabled AI platforms can enable high-throughput screening, rapid prototyping, and personalized optimization of bacterial nanocarriers for specific disease targets or patient populations[68].

Translation of bacterial nanocarriers from bench to bedside

The translation of bacterial nanocarriers from bench to bedside requires collaboration and integration across multiple disciplines, including basic research, translational science, clinical medicine, regulatory affairs, and commercialization. Multidisciplinary teams of researchers, clinicians, engineers, and industry partners must work together to address technical, clinical, regulatory, and commercial challenges in the development and clinical translation of bacterial nanocarrier-based therapies. Strategic partnerships with academic institutions, biopharmaceutical companies, and government agencies can facilitate the translation of bacterial nanocarriers from early-stage research to clinical development and commercialization[66].

Conclusion

In this review, we explored the role of bacterial nanocarriers in site-specific drug delivery for precision medicine applications. We discussed the characteristics, types, mechanisms, and applications of bacterial nanocarriers, highlighting their potential to revolutionize drug delivery and therapy in various disease contexts.Bacterial nanocarriers exhibit unique properties that make them attractive candidates for precision medicine. Their small size, versatile surface modifications, high payload capacity, and inherent targeting capabilities enable precise delivery of therapeutic agents to diseased tissues while minimizing systemic exposure and off-target effects. By leveraging the biological and engineering principles of bacteria, researchers can engineer sophisticated nanocarrier platforms tailored to specific disease targets, patient populations, and clinical needs.Bacterial nanocarriers hold great promise for advancing precision medicine by enabling targeted, personalized, and efficacious drug delivery strategies for a wide range of diseases, including cancer, infectious diseases, and chronic conditions. By harnessing the unique properties of bacteria, researchers can overcome the limitations of conventional drug delivery systems and achieve unprecedented levels of precision and specificity in therapeutic interventions. The ability of bacterial nanocarriers to target specific tissues, cells, or microenvironments within the body offers significant advantages for precision medicine. These nanocarriers can deliver therapeutic agents directly to diseased tissues while sparing healthy organs, minimizing side effects, and maximizing therapeutic efficacy. Moreover, bacterial nanocarriers can be engineered to respond to disease-specific cues or stimuli, enabling on-demand drug release and tailored treatment regimens for individual patients.

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Original Article

FORMULATION AND ASSESSMENT OF HERBAL EMULGELS IN THE MANAGEMENT OF ACNE: *IN VITRO* AND *IN VIVO* INVESTIGATIONS

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ABSTRACT

Objective: The main objective of the current research was to prepare herbal emulgel and analyze the effect of herbal formulation in the treatment of acne.

Methods: The plants *Tabernaemontana coronaria* and *Thunbergia alata* were selected for the study because of folklore for their medicinal values. The *T. coronaria* and *T. alata* test extracts were prepared by soxhlet extraction procedure and subjected to physico-chemical evaluation. The formulated herbal emulgels prepared by dispersion technique were investigated for anti-acne properties by *in vitro* and *in vivo* methods. The prepared emulgel formulations were assessed for parameters like viscosity, spreadability, pH, content uniformity, stickiness, zeta potential, particle size, surface morphology, and *in vitro* diffusion studies.

Results: The physico-chemical evaluation of herbal gel revealed that emulgel appeared light green in colour, opaque, and odourless with smooth texture. The emulgels of both the test extracts showed no stickiness, and revealed pH ranging from 5.467±0.13 to 5.889±0.1. When the shear rate was increased, there was a decrease in the viscosity of the test emulgels, with good extrudability. The content uniformity of F5 emulgel for *T. coronaria* and *T. alata*was 99%, and spreadibilty was more with F7 formulation of *T. coronaria* and F6 formulation of *T. alata,* respectively. In the stability testing studies, amongst all the formulations prepared, F5 was found to be stable upon storage for six months. *In vitro* studies, F5 formulation of both the test extracts had a remarkable zone of inhibition; whereas F5 formulation treated histopathological sections in *in vivo* investigation displayed a decline in the overall damage induced by *Propionibacterium acnes*. The results showed no statistical significant difference for measurement of zone of inhibition and histopthological studies between the test formulations and standard drug.

Conclusion: The study concludes that both herbal formulations were promising agents for the treatment of acne vulgaris.

Keywords: *T. coronaria*, *T. alata*, Anti-acne emulgels, *In vivo* studies, Anti-bacterial effect

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INTRODUCTION

According to estimates, 80–95 percent of all teenagers will experience acne at some time in their lifetime, and in some cases, the condition will persist till they become adults and might continue [1]. Male and female are both equally susceptible to the genetic factors that contribute to the development of acne, albeit males often have more severe instances. Acne is more common in teenagers than in any other age group. This is due to the fact that throughout puberty, hormone release increases the sebaceous glands output and the pace of skin-cell turnover in the follicles [2]. Dead cells in the skin and hair follicles in conjunction with oil lead to the formation of acne, that are visualized in the prime areas such as the face, chest region, forehead, backside, and shoulders [3]. Additionally, there exists a chronic inflammatory lesion, seborrhoea generation, and nodules. Research studies revealed that the presence of bacteria is responsible for the pathogenesis of acne formation, the species are *Staphylococcus aureus*, *Propionibacterium acnes* and *Staphylococcus epidermidis*, out of which *P. acnes* is a gm+ve bacteria that grows within and also cause for the inflammatory acne. *P. acnes* have the capacity to activate the complements and let sebaceous triglycerides metabolize into fatty acids, thereby captivating the neutrophils. Furthermore, the aerobic bacteria *Staphylococcus* species remained to blame for the infections which are superficial [4].

As a part of treatment for acne, currently, topical applications like benzoyl peroxide, retinoids, antibiotics such as clindamycin and oral medications like retinoids are available. Antibiotics of tetracycline and macrolides classes are also preferred, in case of severity; drugs are combined and used [5]. Although antibiotics can stop acne-related inflammation and target *P. acnes*, the discovery of innovative treatment drugs is a mandate due to *P. acnes* and other acne-forming bacterial species developing antibiotic resistance. Overuse of antibiotics for extended periods of time has created resistance in the bacteria that cause acne, such as *P. acnes, S. epidermidis,* and *S. aureus*. The unique nature of the association between bacteria and antibiotics, the method of use, host features, and environmental variables are some of the several elements that contribute to the development of antibiotic resistance [6]. In this regard, natural components that have been employed in conventional medical practices, such as various plant parts, spices and condiments, and minerals, could be investigated as potent sources for new anti-acne treatments. Anti-acne formulations come in a variety of forms, including microspheres, patches, gels, and tablets [7]. Emulgels have also been used as anti-acne systems. Anti-acne gels provide a number of benefits, including the ease with which they may be administered across wider surface areas, removed, and improved for absorption. Pure biological polymer and pure synthetic polymer properties alone are frequently inadequate for the production of materials with a good combination of biological, thermal, mechanical, and chemical characteristics. Natural gelling agents like gum tragacanth, gum acacia, and gellan gum have been shown to be rather less effective than synthetic gelling agents like polyvinyl pyrrolidone, carboxymethyl cellulose, and carbopol. Smaller doses of synthetic gelling agents may be combined with natural agents to enhance their gelling properties, resulting in formulations that have prolonged and better action [8].

A perennial bush with a 3-meter named *Tabernaemontana coronaria*, belonged to the Family Apocynaceae. The elongated, wavy green leaves that emerge are dull and glossy. The plant is decorative as well as having a colossal number of alkaloids [9]. Plant parts are detailed to have different phenolic compounds, glycosides, steroids

and terpenoids [10]. In conventional medicine, it is utilized to treat wind and scorpion biting, sore eyes, gastric issues, inflammations, skin diseases, cancer, and hypertension [11, 12].

The evergreen vine *Thunbergia alata*, belonging to the Acanthaceae family, may grow to a height of 1 to 5 meters and is cultivated from seeds. The flowers bloom all year and range in hue from yellow to orange. The fruits are compressed globular capsules with four seeds that ripen throughout the calendar year. The plant was found in both the Western and Eastern Ghats. *T. alata* was identified as a crucial traditional medicine for inflammations, fevers, dysentery, coughs, pains and skin infections. *T. alata's* phytochemical analysis indicated an abundance of polyphenolic substances and glycosides [13, 14].

In the current global scenario acne has been a popular skin disease associated with all ages and genders. The herbal formulations were known as the best suitable components which would improve the disease condition without showing any side effects. The *T. coronaria* and *T. alata* were mentioned in the literature as a good herbal source to treat acne. Hence, the goal of the current study was to formulate and assess an anti-acne emulgel from an ethanolic extract of *T. coronaria* and *T. alata*.

MATERIALS AND METHODS

Procurement of chemicals clove oil, ethyl paraben, propylene glycol, Span 20, Tween 20, liquid paraffin, and Carbapol 940 were obtained from SD Fine Chemicals, Mumbai, India.

Collection of plant material

Leaves of *T. coronaria* and *T. alata* were gathered from rural Tirupati and Chittoor. Dr. K. Venkata Ratnam, Department of Botany, Rayalaseema University, Kurnool, authenticated leaves and voucher specimens were provided (RU/BD/VSN-142 and 163) for future use.

Preliminary phytochemical screening

The two plants extract of *T. coronaria* and *T. alata* were screened for the presence of phytochemical constituents. *T. coronaria* was detected with amino acids, proteins, phenols, alkaloids, flavonoids, terpenoids, tannins, saponins, and glycosides whilst *T. alata* had alkaloids, flavonoids, phenolics and glucosides [15].

Preparation of extracts

T. alata and *T. coronaria* leaves were gathered and shade-dried. The powdered dried leaves were then effectively extracted with ethanol using the Soxhlet. To acquire the solid extract, the solvent was evaporated to dryness, and the % yield was determined [13, 16].

Method of preparation of emulgel

As indicated in table 1, formulations with varying ingredient quantities were prepared; dispersion technique was used to prepare emugels. The cold water was used to dissolve carbopol-940 by continuous agitation at a modest speed to obtain a homogenous mixture, which was used to produce the gel part of the emulgel. Triethanolamine was then used to bring the pH to 6-6.5. To make the emulsion's aqueous phase, tween 20 was solubilized in distilled water, whereas span 20 was solubilized in liquid paraffin to prepare the emulsion's oil phase. Methyl and ethyl paraben were dissolved in propylene glycol to preserve the emulsion, and the extracts were dissolved in ethanol before being combined with the aqueous phase. Mentha oil and clove oil were combined in the oil phase. The oil phase and the aqueous phase were heated separately at 70 °C in a water bath. The aqueous phase was then continuously stirred with a homogenizer (Remi Motors, RQ127 A), for 10 min at a speed of 3000 rpm before being cooled to room temperature. To produce emulgel, the gel and emulsion components were ultimately blended in a 1:1 ratio while being stirred gently [17, 18].

Table 1: Composition of various emulgel formulation batches (%w/w)

Ingredients	F1	F2	F3	F4	F5	F6	F7
EETA/EETC	0.25	0.5	0.75	1.0	1.25	1.5	1.75
Carbapol 940	1	1	1	1	1	1	1
Methyl Paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Ethanol	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Propylene glycol	5	5	5	5	5	5	5
Span 20	1	1	1	1	1	1	1
Tween 20	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Liquid paraffin	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Ethyl Paraben	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Clove oil	----	2	---	4	6	8	10
Mentha oil	10	8	---	-----	6	4	2
Water	q. s	q. s	q. s	q. s	q. s	q. s	q. s

EETA: Ethanolic extract of *Thunbergia alata*; EETC: Ethanolic extract of *Tabernaemontana coronaria*

Characterization and evaluation of topical emulgel

Physicochemical evaluation

Loss on drying

About 1 g of the formulation was weighed and dried for 3 h at a temperature between 100 °C and 105 °C. Test materials were well combined and weighed. The sample was placed in a bottle, with a cap, and the container and contents were precisely weighed by moderate and sideways shake. The sample was distributed to a depth of about 5 mm. In the drying chamber, with the bottle loaded, the sample was dried at the designated temperature. After the chamber is opened, immediately the bottle was sealed and waited to attain room temperature in desiccators before weighing. A weight difference of no more than 0.5 mg was observed between consecutive weights. The formula was used to determine loss on drying [19].

$$\% \text{ LOD} = \frac{(W2 - W3)}{(W2 - W1)} \text{ X } 100$$

Where, W_1 = Weight of empty weighing bottle, W_2 = Weight of weighing bottle+sample, W_3 = Weight of weighing bottle+dried sample.

Stickiness

Little amount of emulgel was applied and observed for the presence or lack of stickiness, the stickiness was thus tested [20].

pH determination

Evaluation of pH is a crucial factor, particularly for topical formulations. To mimic the skin condition, the pH of the emulgel should be between 5 and 7. It may irritate the patient if the prepared emulgel has an acidic or basic pH. By using a digital pH meter (ELICO LI 613), emulgel's pH was measured. 1 g of emulgel was dissolved in 100 ml of distilled water, and then was applied to the glass electrode. Each formulation's pH was measured three times, with the average readings being computed [21].

Viscosity

By utilizing Brookfield viscometer, emulgel's viscosity was determined [22].

Extrudability test

The herbal emulgel formulations were filled in standard caped collapsible lami-tube and sealed. The tube was weight was recorded.

The tube was clamped after being positioned between two glass slides. The cap was opened after a 500 g weight was placed over the glass slide. The amount of emulgel was collected and weighed. The % of emulgel extruded was calculated; and grades were allotted (+++excellent, ++Very good,+Good).

Spreadability

Spreadability was assessed using the "drag" and "sleep" approach. On this block, a ground glass slide was affixed. Two grams of test emulgel were applied to this slide. After that, an additional glass slide with a hook and the same fixed ground side dimension was placed between these two slides, containing the emulgel. The top of this slide was then loaded with weight (40 g). The top slide's time (in seconds) to travel a distance of 6 cm was recorded [23].

The spreadability was then determined using the formula:

$$S = \frac{M\ L}{T}$$

Where, T-Time (sec); L-The glass's length (6 cm); M-Weight tied to the upper slide (40g); S–Spreadability [24].

Extract uniformity

A USP standard for the emulgel formulation was extract uniformity. The upper, middle, and end portions of the sample were taken from the filled tube to estimate the content's uniformity using the UV analysis method. In this instance, 2 g of anti-acne emulgel was mixed with 100 ml of propylene glycol. To this, 2 ml of the sample was added from the previously prepared solution. Propylene glycol was utilized as a blank solution, and the aforementioned concentration solutions were scanned by means of a UV spectrophotometer across 280 and 360 nm [25, 26].

$$\text{Percentage Purity} = \frac{\text{Test content}}{\text{Label claim}} X\ 100$$

***In vitro* drug release**

An *in vitro* drug permeation study was done by means of franz diffusion cell which consisted of two compartments (cells). Upper cell was donor with two open ends, while the lower cell had only one open end (15 ml). Himedia dialysis membrane, which was soaked previously in warm water, was used to cover one end of the donor compartment. A magnetic bead was noticed in receptor cell; temperature was maintained in both the compartments at 37 °C using thermostat. Receptor cell had a phosphate buffer (7.4). A quantity of 5 ml of each formulation was placed on the diffusion cell, out of which 3 ml was withdrawn at each time interval. During such transfer, fresh media was used each time to maintain sink condition. The samples were analysed for drug content using a UV-Visible spectrophotometer at 212 nm at different time intervals–0, 30, 60, 90, 120,150, 240 and 360 min [27].

Skin irritation test (Patch test)

This particular test was carried out on 2-3 mo healthy male Wistar rats (150-170 g), that were procured from National Institute of Nutrition (NIN), Hyderabad, Telangana State bearing Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) registration number. Animals were allowed to acclimatize for about a week before start of the experiment in controlled environment (centrally air-conditioned) at ambient temperature of 22±3 °C with relative humidity of 50±10%, and 12 h light/dark cycle. For the investigation, a group of 12 rats were used. On the appropriately shaved skin of the rat, the emulgel was applied. For a period of 24 h, undesirable skin alterations, such as colour and morphological changes, were monitored [27]. The present study was approved by institutional animal ethical committee with Ref No: 1447/PO/Re/S/11/CPCSEA-64/A.

Particle size and zeta potential

A Zetasizer Nano ZS90 dynamic light scattering particle size analyzer (Malvern Instruments, Malvern, Worcestershire, UK) was used to measure the zeta potential and globule size of the formulation at a temperature of 25 °C, a scattering angle of 90°, at a wavelength of 365 nm [28, 29].

Surface morphology

The formulations were examined using scanning electron microscopy (JEOL JEM 2100 F, USA) after being diluted 1000 times with distilled water and then sprayed over a carbon grid stained with a 2% uranyl acetate solution [30, 31].

Toxicity studies

Wistar albino rats of either sex (200–250 g) were chosen and allocated to eight groups of six rats each. The rats were treated with a single oral dose of ethanolic extract of *T. coronaria* (EETC) and ethanolic extract of *T. alata* (EETA) ranging from a low dose of 50 mg/kg to a high dose of 2000 mg/kg. Rats receiving extract treatment were closely monitored for indications of fatality and toxicity. For the current study, $1/5^{th}$ and $1/10^{th}$ of the maximal dose were selected.

***In vitro* anti-bacterial activity (Anti-acne activity)**

The bacterial strains *S. epidermidis* (MTCC 931), *S. aureus* (MTCC 3160), and *P. acnes* (MTCC 1951) were procured from the Department of Microbiology, National Institute of Nutrition (NIN), Hyderabad and used to assess the anti-bacterial activity. The solutions of the EETA and EETC and F5 of both extracts were prepared by dissolving them in DMSO. Ciprofloxacin (0.1 mg/ml) was used as a standard drug. Both the extract formulation F5's anti-bacterial properties were assessed using a modified agar-well diffusion technique. This is considered as most reliable employed for testing the anti-microbial properties of a test drug. In this technique, 0.2 ml of a *P. acnes* (48 h) broth culture was placed onto each brain-heart infusion media, 0.2 ml of a 24 h *S. aureus* broth culture was placed onto each nutrient agar plate, and 0.2 ml of a 24 h *S. epidermidis* broth culture was placed onto each plate of soybean casein digest media. For, the plates were dried. A sterile 8-mm borer was created on all plates. Extract solutions, formulation F5 of both extracts, and Ciprofloxacin were added to each plate. Plates containing *S. epidermidis*, *S. aureus*, and *P. acnes* were incubated for 24 h and 48 h at 37 °C. To assess the anti-bacterial activity, the diameter of the zones of inhibition (measured in mm) was taken into consideration.

***In vivo* anti-acne activity of EETC and EETA**

Animal grouping and ethical approval

Male Wistar rats (150-180 g) were obtained from the animal breeding unit at National Institute of Nutrition (NIN), Hyderabad. Animals were kept in cleansed, clear polypropylene cages in groups of four in each cage maintained at 25±2 °C with 12 h of light and dark cycle with free access to food pellets and water ad libitum. The present study was approved by institutional animal ethical committee with Ref No: 1447/PO/Re/S/11/CPCSEA-64/A. All the animals were divided into five groups of 6 animals in each group (n=6). Group 1 was considered as control, group 2 has *P. acnes* induced rats (0.14 mg in 50 μL saline; positive control), group 3 has EETC (0.05 mg/ml), group 4 has EETA (0.1 mg/ml), and group 5 has Clindamycin (0.1 mg/ml).

The rats were shaved in the interscapular region, and then applied with the EETC, EETA, and standard for a period of 21 d. After 24 h of application of the final dosage, animals were sacrificed. From the interscapular region, the skin specimens were removed and prepared for electron microscope analysis. *P. acnes* was injected intradermally into the rat's ear for inflammation that was similar to chronic acne and was characterized by edema, cell infiltration, and the development of comedons. The histological parameters were monitored.

Measurement of ear thickness

As a file with a provocative quality and skin breakout, ear thickness was assessed. The thickness was assessed using a Vernier callipers. For ten days, the thickness was measured once regularly.

$$\%\ \text{Inhibition} = \frac{1 - \text{Test}}{\text{Control}}$$

Statistical analysis

All the data was expressed in mean±SD. Statistical analysis was done using Graph Pad Prism Version 6. Analysis of variance (ANOVA) was

applied and the values of test groups were comparable with control and standard groups respectively.

Stability studies

According to the ICH guidelines, stability studies were performed to assess the stability of *T. coronaria* and *T. alata* emulgels. The selected formulations were taken in triplicate and was packed with polyethylene coating and sealed with aluminium, stored in a chamber that was maintained at 40±2 ℃ and 70±5% relative humidity for a period of 6 mo [31]. After storage for a period of 6 mo, the optimized formulation was evaluated physically and was reported.

RESULTS

Physicochemical evaluation and characterization of emulgels

The final formulation prepared appeared light green in colour, opaque, odourless with smooth texture.

Loss on drying

The loss on drying was below the predetermined limits (not more than 0.5%). The LOD of EETC emulgel formulation (F5) was found to be 0.35±0.24%, while LOD of EETA emulgel formulation (F5) was observed to be 0.4±0.22% (table 2).

Table 2: Average loss on drying of formulations of the test drugs

Formulations	Emulgel of EETC	Emulgel of EETA
	Average loss on drying (%)	Average loss on drying (%)
F1	0.28±0.03	0.34±0.3
F2	0.19±0.21	0.32±0.43
F3	0.31±0.16	0.31±0.15
F4	0.29±0.13	0.27±0.27
F5	0.35±0.24	0.40±0.22
F6	0.31±0.3	0.39±0.16
F7	0.32±0.17	0.37±0.28

EETC–Ethanolic extract of *Tabernaemontana coronaria,* EETA–Ethanolic extract of *Thunbergia alata,* All the values were expressed in (n=3) mean±SD

Stickiness

The findings certainly showed that the formulated emulgel was free of stickiness after application, and it was also compared with the commercial formulations. It was also dispersed freely on the skin.

pH of the emulgel

For the formulations F1 to F7, the pH of EETC emulgel varied from 5.473±0.21 to 5.699±0.26. Amongst the formulations F1 to F7, the pH was optimum in F7 formulation. The pH of EETA emulgel varied from 5.467±0.13 to 5.889±0.1. The pH values were suitable to avoid any chance of irritation when applied. The pH was optimum in the F6 formulations (table 3).

Viscosity measurement

A spindle number three was used to obtain viscosity at 10 rpm. Non-Newtonian flow and shear-thinning behaviour were exhibited by all emulgels. After prolonged shearing, the emulgels had a shear-thinning behaviour, indicating that the observed viscosity declined as the shear rate increased (table 3).

Table 3: pH and viscosity of formulations of the test drugs

Formulations	Observations pH at 25 ℃		Viscosity (cps)	
	EETC	EETA	EETC	EETA
F1	5.673±0.14	5.666±0.11	2523±0.21	2433±0.11
F2	5.473±0.21	5.587±0.15	2514±0.16	2256±0.81
F3	5.667±0.24	5.667±0.09	2467±0.3	2542±0.13
F4	5.599±0.12	5.467±0.13	2422±0.18	2314±0.18
F5	5.611±0.18	5.690±0.13	2318±0.11	2289±0.19
F6	5.678±0.22	5.889±0.17	2249±0.18	2397±0.01
F7	5.699±0.26	5.679±0.21	2220±0.31	2215±0.09

EETC–Ethanolic extract of *Tabernaemontana coronaria,* EETA–Ethanolic extract of *Thunbergia alata,* All the values were expressed in (n=3) mean±SD

Spreadability

The spreadability of EETC emulgel varied between 26.9±0.2 cm/sec and 38.1±0.14 cm/sec for formulations F1 to F7. The best spreadability was found with the F7 formulation. While, the spreadability of EETA emulgel varied between 27.4±0.7 cm/sec and 35.8±0.51 cm/sec for formulations F1 to F7. The best spreadability was found with F6 formulations. Table 4 showed the average value of spreadability for all formulations.

Extrudability

The extrudability of EETC and EETA emulgels was found to be very good (table 4).

Table 4: Average spreadability and extrudability of Emulgel formulations

Formulations	Average spreadability (cm/sec)		Extrudability	
	EETC	EETA	EETC	EETA
F1	29.9±0.01	29.3±0.91	Very good	Very good
F2	30.2±0.19	31.2±0.3	Very good	Very good
F3	36.3±0.21	35.3±0.09	Very good	Very good
F4	26.9±0.2	28.9±0.33	Very good	Very good
F5	37.4±0.42	27.4±0.9	Very good	Very good
F6	37.9±0.81	35.8±0.51	Very good	Very good
F7	38.1±0.1	35.2±0.52	Very good	Very good

EETC–Ethanolic extract of *Tabernaemontana coronaria,* EETA–Ethanolic extract of *Thunbergia alata,* All the values were expressed in (n=3) mean±SD

Extract content uniformity

Amongst the different formulations developed, the EETC and EETA extracts of the emulgel formulations were found to be consistent and ranged between 84.7±0.09% and 99.2±0.18% and 83.6±0.31% and 99.5±0.51%, respectively. It was evident from both extracts that the F5 had the highest extract content, which was greater than 98%. Table 5 displays gel formulation's content uniformity.

Table 5: Content uniformity of the Emulgel formulations of test extracts

Formulations	Percentage (%) of Emulgel of EETC	Percentage (1%) of Emulgel of EETA
F1	89.2±0.27	85.5±0.54
F2	88.2±0.16	97.1±0.13
F3	97.2±0.17	94.2±0.65
F4	89.6±0.07	83.6±0.31
F5	99.2±0.18	99.5±0.51
F6	84.7±0.09	89.7±0.32
F7	86.2±0.01	88.2±0.09

EETC–Ethanolic extract of *Tabernaemontana coronaria,* EETA–Ethanolic extract of *Thunbergia alata,* All the values were expressed in (n=3) mean±SD

Drug permeation data for different formulations by *in vitro* method

In this method, the different formulations of both the test extracts were estimated for drug release. The formulation F5 of both the extracts of EETC and EETA showed approximately 90% of drug release in 6 h. Hence, F5 was chosen for preparation of formulation of emulgel (fig. 1 and fig. 2).

Skin irritation test

Up to 24 h, rats did not exhibit any allergy signs including inflammation, redness, or irritation.

Particle size and zeta potential

The particle size for the F5-prepared emulgel was found to be 126.59±1.17 to 154±2.31 nm and zeta potential values ranged from-20.13±4.69mV to-29.04±3.05 for the F5 formulation of both the test drugs. The particle size and zeta potential images optimized formulations of both plant extract emulsions of EETA and EETC were displayed in fig. 3 and 4, respectively.

Surface morphology (SEM)

SEM was used to analyse the surface morphology of emulgels. The images of emulsions of EETA and EETC were depicted in the fig. 5. Analysis of the emulsions using SEM showed that both the EETA and EETC emulsions were nearly spherical in shape, and there was no evidence of droplet coalescence, which indicated that emulsions were stable.

Toxicity studies of EETC and EETA

The maximal therapeutic doses of 250 and 500 mg/kg were selected for the present study.

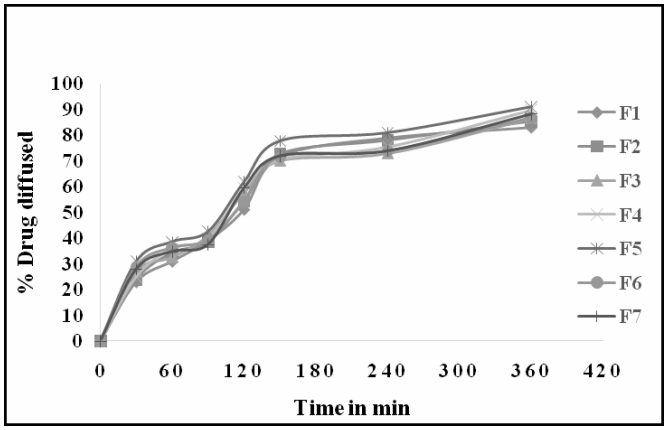

Fig. 1: The % drug release of different formulations at different time intervals of EETC emulgel

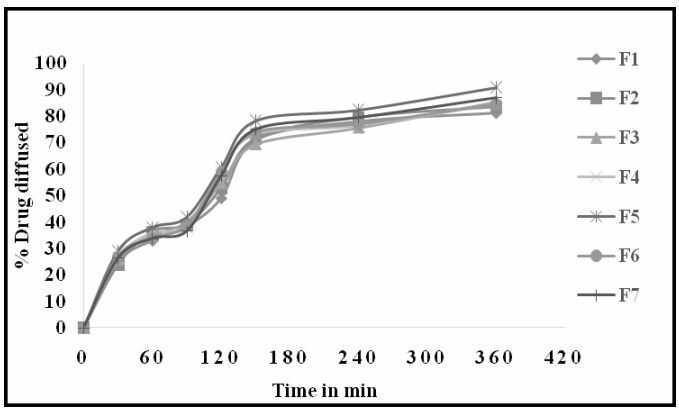

Fig. 2: The % drug release of different formulations at different time intervals of EETA emulgel

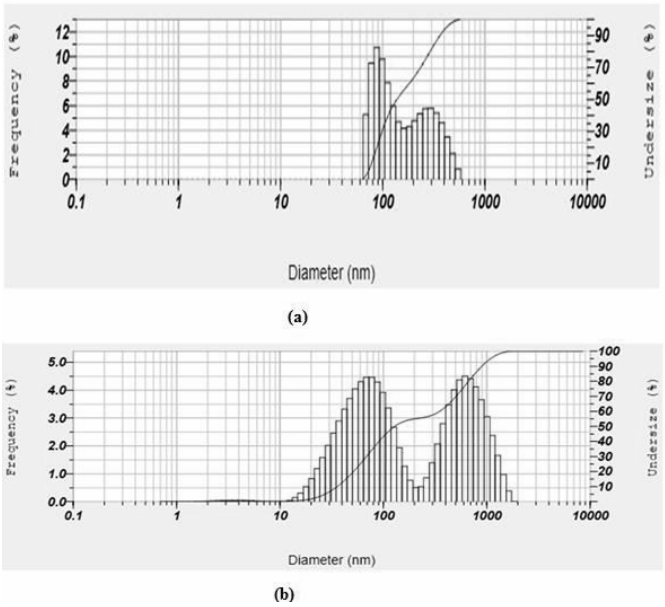

Fig. 3: Particle size of Emulgel of (a) EETA and (b) EETC

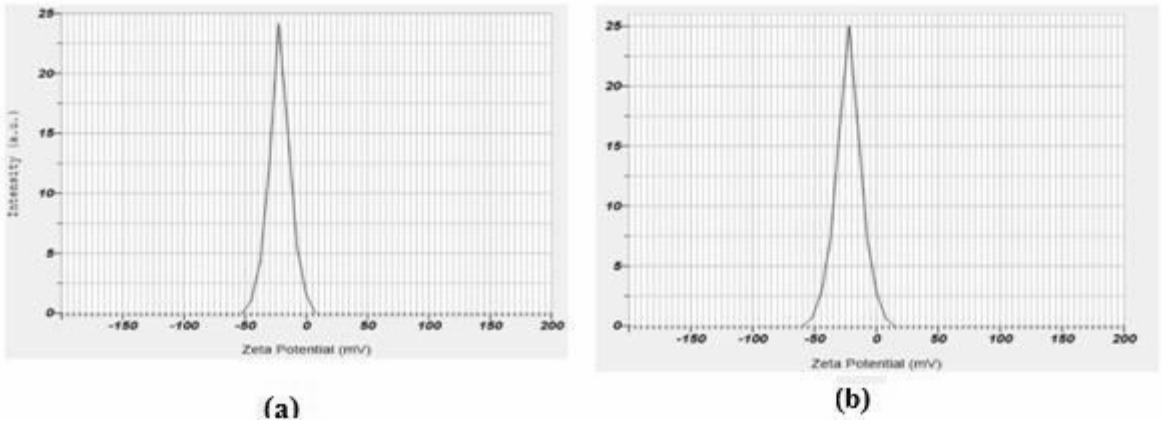

Fig. 4: Zeta potential of emulgel of (a) EETA and (b) EETC

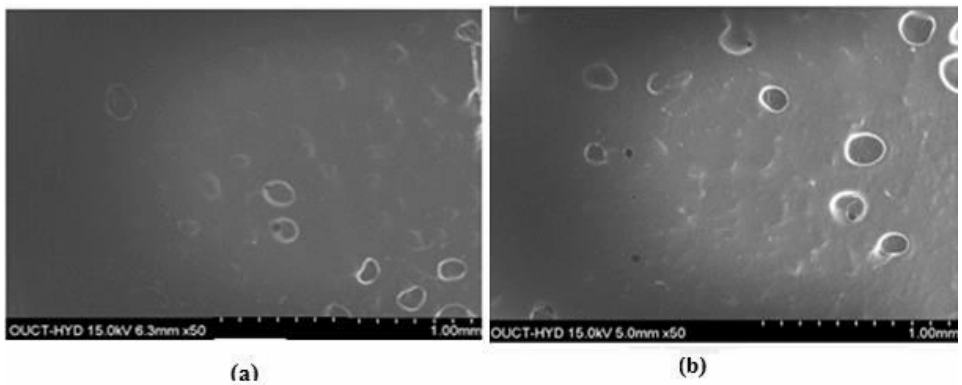

Fig. 5: SEM images of emulgels (a) EETA (b) EETC (bearing measurements of 5 mm magnified into 50 times in 1.00 mm scale)

Evaluation of anti-bacterial activity

Anti-bacterial effect of test extracts

It was evident from Tables 6 and 7 that sample solution concentration was a crucial parameter for controlling the zone of inhibition. For effective suppression of microorganisms, extract concentration must be raised. For *T. alata*, T1 produced 14.33±0.58 mm, 10.67±1.03 mm, and 15±1.72 mm (P<0.05) zones of inhibition, T2 produced 19±2.00 mm, 15.67±1.0 mm, and 20±1.0 mm (P<0.05) of a clear zone of inhibition with *P. acnes*, *S. aureus*, and *S. epidermidis,* respectively. Similarly, for *T. coronaria* T1 produced 17.67 mm, 12.67 mm, and 23.33 mm (P<0.05) of the zone of

inhibition, T2 produced 22 mm, 17.33 mm, and 33 mm (P<0.05) of a clear zone of inhibition with *P. acnes*, *S. aureus*, and *S. epidermidis,* respectively. Ciprofloxacin (0.1 mg/ml) inhibited *S. aureus* with a maximum clear zone of inhibition (30.33 mm). The results showed no statistical significant difference between suppression of microorganisms when compared to standard as depicted by zone of inhibition. This indicates that the prepared extracts showed anti-microbial activity similar to the compared standard.

Table 6: Anti-bacterial effect of Ethanolic extract of *Tabernaemontana coronaria*

Treatment and dose	Zone of inhibition (mm)		
	Propionibacterium acnes	***Staphylococcus aureus***	***Staphylococcus epidermidis***
Control	11.00±1.00	6.67±0.42	11.67±0.58
T1 (0.05 mg/ml)	14.33±0.58*	10.67±1.03*	15±1.72*
T2 (0.1 mg/ml)	19.00±2.00*	15.67±1.0*	20±1.0*
Ciprofloxacin (0.1 mg/ml)	29.00±1.73**	29.02±1.39**	30.31±2.08**

All the values were expressed in (n=3) mean±SD, *P<0.05, **P<0.01 as comparable to the control

Table 7: Anti-bacterial effect of Ethanolic extract of *Thunbergia alata*

Treatment and dose	Zone of Inhibition (mm)		
	Propionibacterium acnes	***Staphylococcus aureus***	***Staphylococcus epidermidis***
Control	7.02±2.0	8.33±1.15	7.64±1.0
T1 (0.05 mg/ml)	17.67±1.17*	12.67±1.29*	16.74±0.65*
T2 (0.1 mg/ml)	22.00±1.0*	17.33±1.15*	23.14±1.21*
Ciprofloxacin (0.1 mg/ml)	27.33±2.08**	28.33±1.61**	25.77±1.23**

All the values were expressed in mean±SD (n=3); *P<0.05, **P<0.01 as comparable to the control

The anti-bacterial effect of emulgel formulations

It was evident from Tables 8 and 9 that a key parameter in controlling the zone of inhibition is the concentration of the test sample. The reported bacteria's zone of inhibition increased when the F5 concentration was raised from 0.5% to 1%. The zone inhibition of the EETC F5 (0.5%) formulation against *P. acnes, S. aureus,* and *S. epidermidis* was 14.33±0.58 mm, 19.00±2.0 mm, and 10.67±1.51 mm (P<0.05) and zone inhibition of the EETC F5 (1%) was 15.67±1.49 mm, 13.62±0.72 mm, and 17.09±0.98 mm (P<0.05), respectively. The zone inhibition of the EETA F5 (0.5%) formulation against *P. acnes*, *S. aureus*, and *S. epidermidis* was 17.67±1.53 mm, 14.79±2.09, and 14.84±1.41 mm (P<0.05) and zone inhibition of the EETA F5 (1%) was 20.07±1.0 mm, 19.63±1.07 mm, and 20.14±1.03 mm (P<0.05), respectively. Although both the extracts F5 showed a good zone of inhibition, the EETA F5 displayed the maximum zone of inhibition. The results showed no statistical significant difference between suppression of microorganisms when compared to standard as depicted by zone of inhibition. This indicates that the prepared optimized formulations showed anti-microbial activity similar to the compared standard.

Table 8:Anti-bacterial activity of F5 of EETC

Treatment and dose	Zone of inhibition (mm)		
	Propionibacterium acnes	***Staphylococcus aureus***	***Staphylococcus epidermidis***
Control	6.25±1.00	6.71±0.53	6.04±0.95
F5 0.5%	14.33±0.58*	10.67±1.51*	13.62±0.72*
F5 1%	19.00±2.0*	15.67±1.49*	17.09±0.98*
Standard ciprofloxacin (0.1 mg/ml)	24.00±1.69**	19.33±1.53**	22.31±1.92**

All the values were expressed in mean±SD (n=3); *P<0.05, **P<0.01 as comparable to the control; EETA: Ethanolic extract of *Tabernaemontana coronaria*

Table 9: Anti-bacterial effect of F5 of EETA

Treatment and dose	Zone of inhibition (mm)		
	Propionibacterium acnes	***Staphylococcus aureus***	***Staphylococcus epidermidis***
Control	6.00±2.00	6.50±1.00	5.87±1.00
F5 0.5%	17.67±1.53*	14.79±2.09*	14.84±1.41*
F5 1%	20.07±1.0*	19.63±1.07*	20.14±1.03*
Standard ciprofloxacin (0.1 mg/ml)	25.94±1.08**	22.43±1.15**	22.33±1.15**

All the values were expressed in mean±SD (n=3), *p<0.05, **p<0.01 as comparable to the control; EETC: Ethanolic extract of *Thunbergia alata*

***In vivo* anti-acne activity of EETC and EETA**

Histopathological studies

Treatment was given for a period of 21 d for acne induced by *P. acnes*, and a histopathological evaluation was done. It was observed that there was lymphocyte transmigration into the follicle wall with neutrophil accumulation on the site of inflammatory lesions thatled to follicle rupture and pustule creation in the dermis (fig. 6). After 24 h, there was a follicle swelling and eventual rupture due to the overabundance of neutrophils. In the vicinity of the final breach, there was a localized loss of the granular layer. Formation of comedones, inflammatory lesions and scars were also noted as components of acne. The negative group (A) was expressed with inflammation, edema and the presence of a sebaceous gland. A standard drug-treated group (B) was manifested with small discolouration, reduced infiltrates of leukocytes and reduced inflammation. Group (C) treated with EETC (500 mg/kg) emulgel showed decreased inflammation and edema whilst group (D) treated

with EETA (sections exhibited a reduction in acne, papules and inflammation. With regard to the ear thickness, there was a significant decline in the overall damage produced by *P. acne* (table 10). The results showed no statistical significant difference between histopathological studies of EETC (250 mg/kg and 500 mg/kg) and EETA (250 mg/kg and 500 mg/kg) when compared to standard (Clindamycin 200 mg/kg) as depicted by ear thickness measurement. This indicates that the prepared optimized formulations showed similar activity when compared to the standard.

Table 10: Effect of EETC and EETA on ear thickness

Treatment and dose	Day 1	Day 3	Day 5	Day 7	Day 10
Control	1.473±0.021	1.343±0.021	1.273±0.021	1.243±0.021	1.223±0.021
Clindamycin 200 mg/kg	0.215±0.004***	0.205±0.004***	0.135±0.004***	0.125±0.004***	0.105±0.004***
EETC 250 mg/kg	0.225±0.003*	0.213±0.001*	0.201±0.003*	0.196±0.004*	0.156±0.003*
EETC 500 mg/kg	0.196±0.003**	0.176±0.001**	0.156±0.003**	0.113±0.002**	0.110±0.001**
EETA 250 mg/kg	0.211±0.003*	0.202±0.001*	0.195±0.003*	0.189±0.004*	0.148±0.003*
EETA 500 mg/kg	0.204±0.003**	0.184±0.001**	0.152±0.003**	0.109±0.002**	0.104±0.001**

All the values were expressed in mean±SD (n=3); *p<0.05, **p<0.01, ***p<0.01 as comparable to the control; EETC: Ethanolic extract of *Thunbergia alata;* EETA: Ethanolic extract of *Tabernaemontana coronaria*

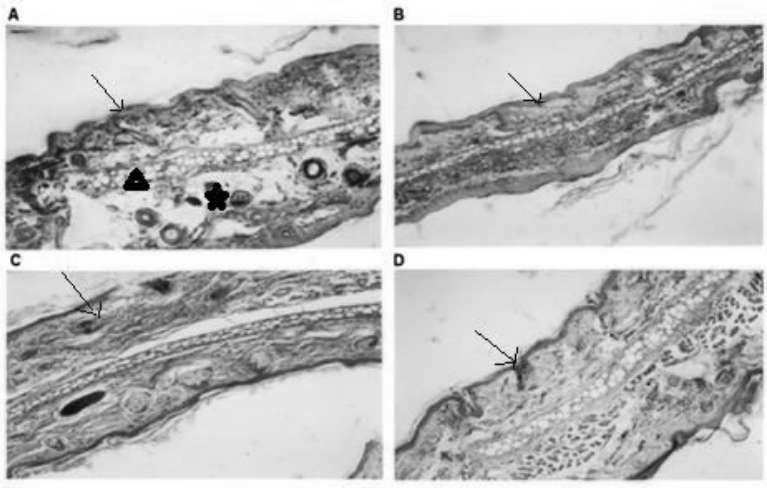

Fig. 6: Histological changes observed in the ear skin of rats, a) Positive group (acne-induced) with inflammation (black arrow), comedones (marked with star) edema and sebaceous gland dilatation (marked with black triangle) b) Clindamycin treated group, with reduced inflammation (black arrow), decreased leukocyte infiltration with almost no acne c) EETC formulation (500 mg/kg) treated group with reduced inflammation (black arrow), and edema d) EETA formulation (500 mg/kg) treated group with decline in inflammation (black arrow), and edema

Stability studies

In the stability testing studies, all the formulations which were prepared were observed and F5 was found to be stable upon storage for six months. There was almost no change in their physical appearance, pH, viscosity, spreadability and drug content. After a period of 6 mo, the formulation was investigated for *in vitro* drug release studies (table 11).

Table 11: Stability studies of F5 emulgel formulations–physical evaluation

Formulations	Viscosity (cps)		Spreadability cm/seconds		% drug content	
	EETC	EETA	EETC	EETA	EETC	EETA
F5	2312	2284	37.8	26.8	99.5	99.7

EETC–Ethanolic extract of *Tabernaemontana coronaria,* EETA–Ethanolic extract of *Thunbergia alata*

DISCUSSION

A chronic disease named acne is considered as a most perturbing and worrisome which is affecting nearly 80 % of adolescents and young individuals. In terms of prevalence, the disease was found to be more prominent in the adolescent stage, more in women>40 y of age different studies reported an average age of prevalence of acne between 24-25 y [32]. Acne is always accompanied by swelling, inflammation and edema in the skin region. Also, the most affected areas belong to the face, chest and back, distinguished by comedones of open and closed type, nodules and papules. Acne requires long-term therapy for an adequate outcome, treatment adherence, but patient compliance and devoid of side effects become crucial and need to be analysed [33]. Acne may also form scars that affect an individual's self-esteem and predispose to depression. As the treatment takes longer for better results, extreme dissatisfaction, insufficient adherence and costly medication may also predominate leading to failure of treatment and other consequences [34]. Acne is contemplated as a disease that affects the quality of life. There are different types of treatments like lifestyle remedies, topical or oral medications, the use of antibiotics or steroids and a few medical strategies [35]. Compared to modern therapies, herbal medicines typically have fewer adverse effects. Antibacterial, anti-

inflammatory, and antiseptic qualities can be found in several herbs. These qualities may aid in the healing of blemishes and the decrease of germs and inflammation that cause acne [36]. Keeping in view of the above considerations, many people rely on traditional cosmetics both in rural and urban areas; this practice has been followed since ancient times.

The present study was designed to evaluate the effect of herbal formulations using *in vitro* and *in vivo* methods. The formulations were prepared from the ethanolic extracts of two herbal plants–*T. alata* and *T. coronaria*, tested for anti-acne (anti-bacterial) effects. Phytochemical screening for both plants was done and was found that *T. alata* contained phenols (caffeoylmalic, feruloylmalic, and p-coumaroylmalic acids), glucosides such as alatoside and thunaloside, stilbericoside respectively. The phytochemical constituents found in *T. coronaria* were sterols, phenols, diterpenes, alkaloids, fatty acids, triterpenoids and tocopherols [36, 37]. From the test drugs, all the formulations were prepared with a homogenous nature. The pH varied showing in the range of 5.4-5.9. Optimum spreadability was maintained with F6 formulation, with preservation of consistency using of viscosity. Similarly, content uniformity was also analyzed, observed as 95 %, and both the formulations of extracts were approximately spherical from the SEM images, which indicated the stability of the preparations. *In vitro* drug release at 6 h was 90 % in F5 formulations of both the test drugs.

The test formulations were tested for *in vitro* anti-bacterial activity against *P. acnes*, *S. aureus, S. epidermidis*. The test extracts and their respective formulations showed almost the same zone of inhibitions in a significant manner. All the zone of inhibition was compared with the marketed product and standard drug Clindamycin. After the treatment for a certain period, the test formulations showed a tremendous effect in combating the invasion of bacteria, subsequently causing the reduction of the genesis of acne. As there is involvement of bacterium in the formation of acne, also it contributes to the development of inflammation by conversion of sebaceous triglycerides to fatty acids, which ultimately grabs neutrophils [38]. After the treatment for 21 d in Wistar rats, the ear sections were examined for histopathological studies. The anti-acne property exhibited by both the test extracts was evident from the images of the ear that were examined microscopically, along with the measurement of ear's thickness. It appeared that the inflammation brought on by different kinins, histamine, and 5-HT has greatly diminished in test drug formulations, with a significant reduction in the thickness of the ears. According to the literature, inflammation is brought on by reactive oxygen species [39]. Anti-oxidant agents overcome these reactive oxygen species and minimize the generation of free radicals caused by oxidative stress [40, 41]. In medicinal plants, phenolic substances and flavonoids were attributed to the same mechanism of exerting anti-oxidant properties and thereby reducing inflammatory response. Whenever there is an infection, the immune system gets activated, accumulates neutrophils, and causes swelling, edema and pain, all of which fall under the symptoms of acne. In the present investigation, both the test drugs showed the presence of these above phytochemical constituents which were responsible for the anti-inflammatory effect, and stood as remarkable anti-acne agents. Furthermore, *T. coronaria* due to the presence of alkaloidal content was found to possess anti-inflammatory, anti-bacterial and analgesic effects respectively.

CONCLUSION

The Ancient system of medicine showed extensive use of herbal remedies, considering the safety and devoid of side effects as compared to conventional medicines. Also, there can be every chance to avoid the resistance induced by the use of antibiotics. This was the primary study conducted to formulate emulgels of plant extracts *T. coronaria* and *T. alata*. In the current study, the topical emulgels of plant extracts *T. coronaria* and *T. alata* were formulated and evaluated for different parameters and were investigated for anti-bacterial activity. The results showed a significant anti-bacterial effect against acne-producing bacteria and the effect was distinguished. As inflammation forms a crucial part in the genesis of acne, the test formulations exhibited an anti-inflammatory effect, a promising action as a topical anti-acne medicine. Thus, phytochemical constituents responsible for the anti-acne property need a thorough investigation at a molecular level. Emulgels are a fantastic option because they have better stability than other topical preparations in the market; hence development of a commercial product for the treatment of acne would be of great choice. In this regard, clinical studies might be planned accordingly for diligent evaluation of prepared herbal emulgel for acne vulgaris.

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AUTHORS CONTRIBUTIONS

SN completed the research work, execution, and writing. AM and VK did the work plan, review, and corrections. All authors agree with the submission and publication. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTERESTS

Declared none

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Sustainable synthesis of Ag–NPs from Cassia auriculata flower extract: synthesis, spectral characterization, its biomedical and environmental applications

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ORIGINAL PAPER

Sustainable synthesis of Ag-NPs from *Cassia auriculata* flower extract: synthesis, spectral characterization, its biomedical and environmental applications

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Abstract

The present study describes the environmentally friendly biosynthesis and characterization of silver nanoparticles derived from a medicinal plant in India. The main objective of this investigation was to use green chemistry for producing silver nanoparticles from *Cassia auriculata* flower extract and their antimicrobial, larvicidal and photocatalytic properties. The synthesized nanoparticles of silver have been evaluated by ultraviolet visible spectroscopy (UV), X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDAX), and Fourier transform infrared spectroscope (FTIR). The presence of therapeutic functional molecules was shown by FT-IR, whereas the Ag-NPs UV-Vis spectrum displayed a significant absorption peak at 295 nm. The XRD results demonstrated that NPs are crystalline. The spherical form of Ag NPs and the presence of silver at 3.5 keV using EDAX are proven by the FESEM data. The size and shape of the silver nanoparticles, which ranged in size from 10.3 to 83.1 nm, were determined by HR-TEM analysis. Furthermore, the antibacterial properties of *C. auriculata* flower produced Ag-NPs (18 nm) for *Staphylococcus aureus* and *Escherichia coli* (11.5 mm) towards both gram-positive and gram-negative bacteria was obtained at 100 µg/mL. In further research, investigations on the larvicidal effects of *C. auriculata* flowers produced Ag-NPs that were effective against *Aedes albopictus* and *Anopheles stephensi* larvae during their fourth instar. The mortality rate of the larvae was found following a 24-hour exposure. The efficacy of synthesized Ag-NPs was evaluated concentrations (50, 100, 150, 200, and 250 mg/L) displayed more effective activity against the 4th instar larvae of *An. stephensi* (LC_{50} = 24.14; LC_{90} = 62.58, mg/L) and *Ae. albopictus* (LC_{50} = 35.53; LC_{90} = 65.27 mg/L). Histological patterns were changed after treatments with Ag-NPs at a concentration of 250 mg/L. Based on a pseudo-first order kinetic demonstrate, it was found that 92% of the (methylene blue) dye degraded in 120 min. The work was remarkable because the silver nanoparticles that were bio-synthesized using *C. auriculata* flower extract had potential larvicidal, photocatalytic, and antimicrobial properties. The potential of biosynthesized Ag-NPs for enhanced medicinal and catalytic applications is proven in the present research.

Keywords *Cassia auriculata* flower · Ag-NPs · TEM · Antibacterial activity · Dengue vector · Dye degradation

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Introduction

The field of nanotechnology is a multidisciplinary discipline of study that has influenced all sectors of research including physics, technology, the study of materials, chemical engineering, the environment, the field of biotechnology bioengineering, pharmaceuticals, and biological sciences [1]. Biologically, chemically, and physically, nanoparticles vary significantly from their parent atoms, molecules, and bulk materials at the nanoscale level [2]. Nanomaterials are special because of their small dimensions and increased surface to volume ratio, which influences their electrical, mechanical, chemical, physical, and magnetic properties [3]. With their variable dimensions, geometries, and surface chemistries, nanomaterials may interact with a wide range of ligands and targeting components, including peptides, proteins, nucleic acids, and antibodies [4]. This property makes them highly useful in the biomedical area.

The commonly used general techniques used to produce nanoparticles are the physical [5] and chemical [6] processes. However, these techniques are not environmentally friendly [7], and the presence of certain toxic metals during the synthesis process could have negative impacts in biomedical applications [8]. Plant- and microbe-mediated biological processes avoid these difficulties in the manufacturing of nanoparticles, and this bio-route has attracted a lot of attention due to its environmentally friendly and biocompatibility [9, 10]. Many metals, including silver, gold, copper, zinc, titanium, and palladium, may be used for producing nanoparticles; but, because of their numerous applications, gold and silver nanoparticles have drawn a lot of interest [11]. Numerous studies have been published on the production of silver nanoparticles employing microorganisms, including bacteria [12], fungi [13], and both external and intracellular forms of [14] and algae [15].

Toxic organic dyes may be used in a number of sectors, such as textiles, leather, paper, plastic, paint, beauty products, and pharmaceuticals [16, 17]. Direct contact between an organism and the dye can cause severe medical concerns, including troubles with the eyes, central nervous system, and mental health [18]. The storage of synthetic organic pollutants from effluent is a significant environmental problem [19, 20]. The removal of synthetic colors from wastewater is unable to be managed solely through physical and chemical treatments, because they're costly and costly to operate. Therefore, more sophisticated approaches are needed [21]. A new approach that effectively replaces conventional physical and chemical procedures for the removal of harmful organic pollutants from wastewater involves the use of metallic nanoparticles [22, 23]. Because Ag-NPs are very affordable compared to other metallic NPs, they have attracted a lot of interest from investigators [24, 25].

The development of innovative antibacterial agents based on inorganic constituents is gaining prominence as a potential replacement for traditional organic agents, as microorganisms are developing resistant to a wide range of antimicrobial drugs [26]. Organic agents have limited uses because of their weak heat resistance, high decomposability, and short life. The field of current nanoscience and technology has drawn considerable attention to plant-assisted nanotechnology manufacturing, primarily due to its ecological sustainability and flexibility. The method utilized to create Ag-NPs from live plants, plant parts, flowers, leaves, bark, or roots is known as "green synthesis." [27]. It is eco-friendly, biocompatible, requires minimal effort and time for synthesis, and doesn't need any hazardous substances or solvents, and doesn't call for extra agents that reduce and cap because different phytochemicals and biomolecules found in various plant extracts, such as polyphenols, flavonoids, alkaloids, tannins, terpenoids, saponins, proteins, enzymes, vitamins, and polysaccharides, are in charge of turning metal ions into metal nanoparticles [28, 29]. They also serve as a capping and stabilizing agent during the synthesis of nanoparticles [30].

Cassia auriculata was an annual herb in the Caesalpiniaceae family that is commonly utilized to treat diabetes, conjunctivitis, rheumatism, and as a tonic [31]. Additionally, previous research has documented the biological effects of *C. auriculata*, including fever, ulcers, skin problems, and urinary abnormalities [32]. The plant *C. auriculata* has previously been noted to be an excellent producer of metallic nanoparticles [33, 34]. Here, we present a simple, economical, and environmentally friendly synthesis of silver nanoparticles (Ag NPs) for biological uses using the cassia plant flower. Following that, examination was conducted using Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and field emission scanning electron microscopy with energy dispersive x-ray spectroscopy (FESEM-EDX). Phenolic and flavonoid groups, which serve as reducing, stabilizing, and capping agents, are abundant in this extract. Furthermore, we discussed about antibacterial properties towards *E. coli* and *S. aureus*. Additionally, the potential of nanoparticles to degradation dyes by photocatalysis against methylene blue (MB) organic hazardous colorants was demonstrated. The bio-route synthesized silver nanoparticles might be a good option for biological uses.

Materials and methods

Preparation and green synthesis of Ag-NPs

The *Cassia auriculata* flower was collected in Guttoor Village, Denkanikottai Taluk, Krishnagiri District (Latitude:

12° 36' 10.58" N Longitude: 77° 51' 7.70" E) and the taxonomy was done by Dr. D. S. Murugesan, Assistant Professor, Department of Botany at Periyar University. 10 g of finely grinded *Cassia auriculata* flower powder was thoroughly mixed with 100 mL of purified water in a conical flask. The resultant mixture was then boiled to 60ºC for 40 min while being continuously stirred with a hot magnetic stirrer at 700 rpm. The combination was then filtered and stored in the refrigerator. The produced aqueous extract was used for fabricating silver nanoparticles and was kept at 4ºC. 10 mL of flower extract was added to an Erlenmeyer flask containing 90 mL of $AgNO_3$ (1 mM) solution (1:9 ratio). For *C. auriculata* flower, the mixture was constantly agitated at 40ºC for 4 h. The change in color of the product of reaction from pale yellow to a deep reddish brown, which indicates the production of silver nanoparticles in the solution, was used to investigate the reaction development for the production of silver nanoparticles. Using a UV–vis spectrophotometer, the solution was analysed range 200–700 nm for wavelength of the absorption signal was recorded [35] (Fig. 1).

Morphological characterizations of NPs

Several spectroscopic approaches were used to characterize the produced Ag NPs. Synthesized Ag NPs UV-Visible spectra were captured using a Shimadzu UV-1601 spectrophotometer, which was programmed to record at a resolution of 20 nm in the 300–700 nm range. Fourier Transform Infrared spectroscopy (FTIR) studies were performed to investigate molecules connected with nanoparticles. Potassium bromide (KBr) was blended with nanoparticles and then squeezed into a pellet. The pellet was pushed into the sample holder, and the 400–4000 cm-1 Alpha II (Brucker, Germany) spectrum was detected in the wavelengths. X-ray diffraction (XRD) was used to examine the nature and structure of the produced nanoparticles. X-ray powder diffraction is a quick analytical method for finding out a crystalline material phase. With Cu Ka radiation in 0–20 setups, the X-Ray diffractometer (PAN analytical BV) performed at a voltage of 40 kV and a current of 30 mA. Applying the Scherrer method and providing that the XRD peaks are devoid of nonuniform stresses, the crystallite region diameter was determined from their breadth. D¼0.94 λ/ β cos θ, where θ is the diffraction position, μ is the X-ray wavelength, β is the full width at half maximum, and D is the mean crystalline domains length corresponding to the reflecting surfaces. The dimension of the nanomaterials was measured and their form was observed using transmission electron microscopy (TEM) analysis. Double-distilled water was used for dispersing the material. A small dispersion spray was placed on a "staining mat." The coated side of the carbon-coated copper panel was placed within the drop. The grid was taken out and allowed to air dry in around ten minutes. The JEOL JEM 2100 Transmission Electron Microscope (JOEL Corp, Tokyo, Japan) was then used to examine particles.

Antibacterial activity

The agar well diffusion method, as described by [36], was employed to assess the antibacterial activity in this study. The test pathogens included two strains of bacteria: *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). These bacterial strains were obtained

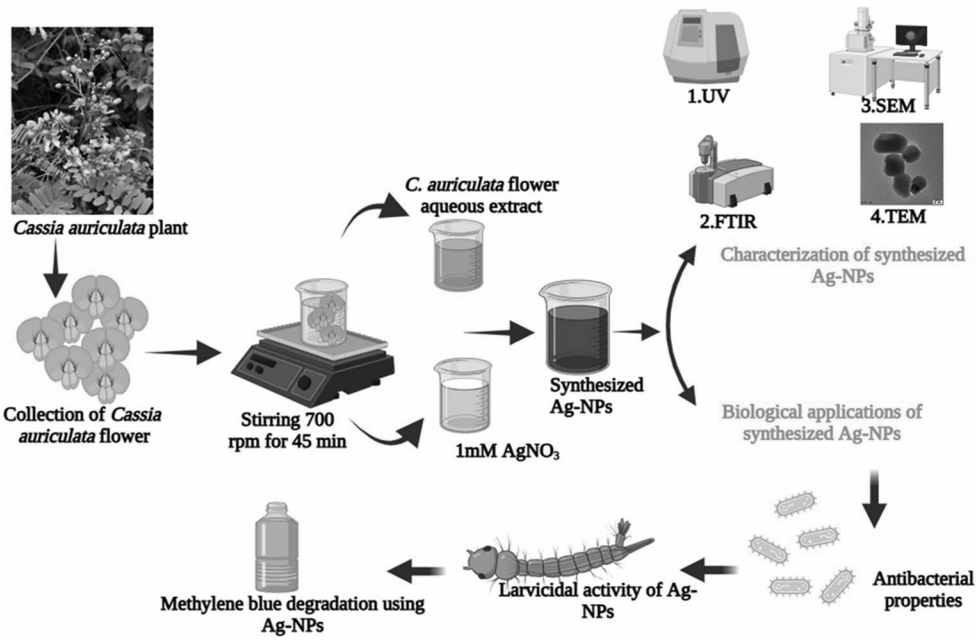

Fig. 1 Schematic diagram shows the synthesis and biological properties of silver nanoparticles using the flower extract of *C. auriculata*

from the Green Laboratory Unit of Saveetha Dental College and Hospitals in Chennai. The achieved pure strains of this organism were sub-cultured in Mueller-Hinton broth and maintained for six hours at 35 °C on a rotary shaker rotating at 200 rpm per minute. Following the cultivated cultures' washing in 0.9% saline solution, the strain intensity was evaluated at 570 nm at 0.5 optical density (OD). Afterwards, sterile cotton swabs were used to properly swab each strain on each Mueller-Hinton culture agar plate, and gel puncture was used to make a well on the agar plates. Subsequently, solution samples containing two distinct amounts of silver nanoparticles (50 and 100 µg/mL) were added to that well. After a 24-h incubation period at 37 °C, the zone of inhibition was evaluated using a zone scale.

Larvicidal activity

The modified WHO methodology was applied to assess the *An. stephensi* and *Ae. albopictus* larvicidal properties of the Ag-NPs developed from *C. auriculata* flowers (World Health Organization, 2005). Various concentrations of examined samples (50, 100, 150, 200, and 250 mg/L) were produced and analysed using the Ag-NPs stock solution (5 mg/mL) in accordance with the modified [37] protocol. Twenty IVth instar larvae (each with a known concentration) were placed in a 100 mL glass beaker along with 99 mL of dechlorinated water and 1 mL of Ag-NPs. Each dose was kept in five replicates, totalling 100 larvae. The observed larval mortality 24 h post-exposure. Throughout the test period, the larvae received no food. The experimental setup described involves the use of control groups and duplicates for each concentration in toxicity testing. Probit analysis, as proposed by Finney in 1971, was employed to determine the deadly concentration levels (LC_{50} and LC_{90}). Additionally, (Abbott, 1925) formula from was applied to calculate the observed/corrected mortality of five replicates for each concentration.

Histopathological study

The histopathology impact of Ag-NPs was evaluated by analysing mosquito samples to notice alterations in morphological features. Furthermore, the 4th instar larvae were treated with the produced Ag-NPs after the control larvae had first been preserved with 10% formalin. Following ethyl alcohol dehydration, the tissues were cleaned with xylene, fixed with para-plast, and sliced at a thickness of 6 µm. Hematoxylin and eosin (HE staining) was applied to the sections following the conventional staining protocol [35]. Finally, pictures were captured and a light microscope with a 40x resolution was used to examine the midgut regions of the control and treated larvae.

Photocatalytic properties of bioengineered Ag-NPs

In order to investigate the photocatalytic property of silver nanoparticles, the present investigation used dyes, namely methylene blue. After carefully mixing 2.0 mg of dye into 100 ml of distilled H_2O, the dye stock solution was produced. Ag-NPs (10 mg) were measured from the start of the photocatalytic activity and combined with 100 ml of an aqueous dye reagent as a control. After 45 min of careful stirring, the dye reaction mixture was exposed to sunlight to produce photocatalytic activity. The resultant mixture (3 ml) was aliquoted at several time periods (20, 40, 60, 80, 100 and 120 min) and utilized to assess the photocatalytic efficiency of the nanoparticle treatment via UV-Vis spectroscopy at different wavelengths for dyes. Using the following formula, the percentage of photocatalytic dye oxidation ability was determined:

$$Dye\,degradation\,(\%) = 100 \times \left(\frac{Co - C}{Co}\right)$$

where C denotes the concentration following photocatalytic degradation and C0 indicates a starting dosage.

Data analysis

The data collected was subjected to probit analysis for chi-square values, 95% confidence intervals, LC_{50}, and LC_{90}. The statistical package for the social sciences (SPSS) 20.0 software was used to analysed the data and evaluate the significance ($p < 0.05$) level.

Results and discussion

UV spectral analysis of biosynthesized Ag-NPs

Growing becoming a significant area of nanotechnology is the invention of experimental procedures for nanoparticle production that are inspired by biology [39]. There have been studies recently about the use of flowers as a bio factory for synthesizing metallic nanoparticles. At the moment, metallic nanoparticles are used in a wide range of industries, including packaging, electronics, cosmetics, coatings, and medical research [40]. Because it avoids the need to maintain cell cultures, biosynthesis of nanomaterials using flower extract is superior to other biological methods notably those involving bacteria and fungus. It is also more appropriate for large-scale nanoparticle manufacturing [41, 42]. The process of biogenesis of silver nanoparticles using *C. auriculata* flower aqueous extract is addressed in this paper. The current investigation focused on *C. auriculata* flower since

it is known to possess flavonoids and phenolic compounds that are accountable for its antibacterial, antioxidant, and reducing power [33]. Plant flower extract contains phenols and other compounds that effectively decrease silver salts and offer exceptional resistance to agglomeration. The proteins and enzymes in floral extract may help to cap, which would increase the stability of the silver nanoparticle. *C. auriculata* aqueous flower extract was utilized for reducing $AgNO_3$ to Ag^o, and its reduction was detected by a change in color from colorless to yellowish dark brown. Ag-NPs distinct surface plasmon resonance (SPR) peak shows up in the extract UV–visible spectrum evaluation at a maximum absorbance of 380 nm (Fig. 2a-d). This is due to the abundance of phytochemicals found in plant extracts, which function as a capping agent and help reduce Ag-NPs. The surface plasmon resonance phenomenon is facilitated by a free electron that is available due to the linked vibration of electrons in metallic nanoparticles, mainly Ag-NPs.

The synthesis procedure is environmentally friendly because the reducing agent used in *C. auriculata* flower extract is a natural, plant-based molecule. Because no harmful chemicals are used, approach adheres to the principles of green chemistry. In terms of green synthesis, biocompatibility, and cost-effectiveness, using flower extract as a reducing agent might be beneficial. It includes a variety of bioactive components that may contribute in the reduction process and possibly enhance the characteristics of the produced nanoparticles [23]. The aqueous flowers extract of *C. auriculata* was used in this work to investigate the plant-mediated formation of Ag-NPs, which was triggered by the extract phenolic and flavonoid constituents. It has been found that *C. auriculata* contains metabolites such as alkaloids, flavonoids, phenolic compounds, proteins, and carbohydrates. Proteins and some other phytochemicals act as Ag-NPs binding agents, whereas flavonoids and phenolic substances are efficient reducing agents [43]. By breaking the O-H bond, the flavonoid and phenolic molecules can liberate electrons, which can then be utilized to reduce Ag + to Ag^o. The proposed approach that indicates the participation of –OH and $–NH_2$ groups in Ag-NPs synthesis and capping may be supported by the IR analysis. Two resonance components help transform the enol types into stable quinonoid forms [44]. Moreover, it's considered that the protein molecule in *C. auriculata* serves as a stabilizing and capping agent [45]. Likewise, [46] observed a broad resonance spectrum with a significant resonance peak at 451 nm, which

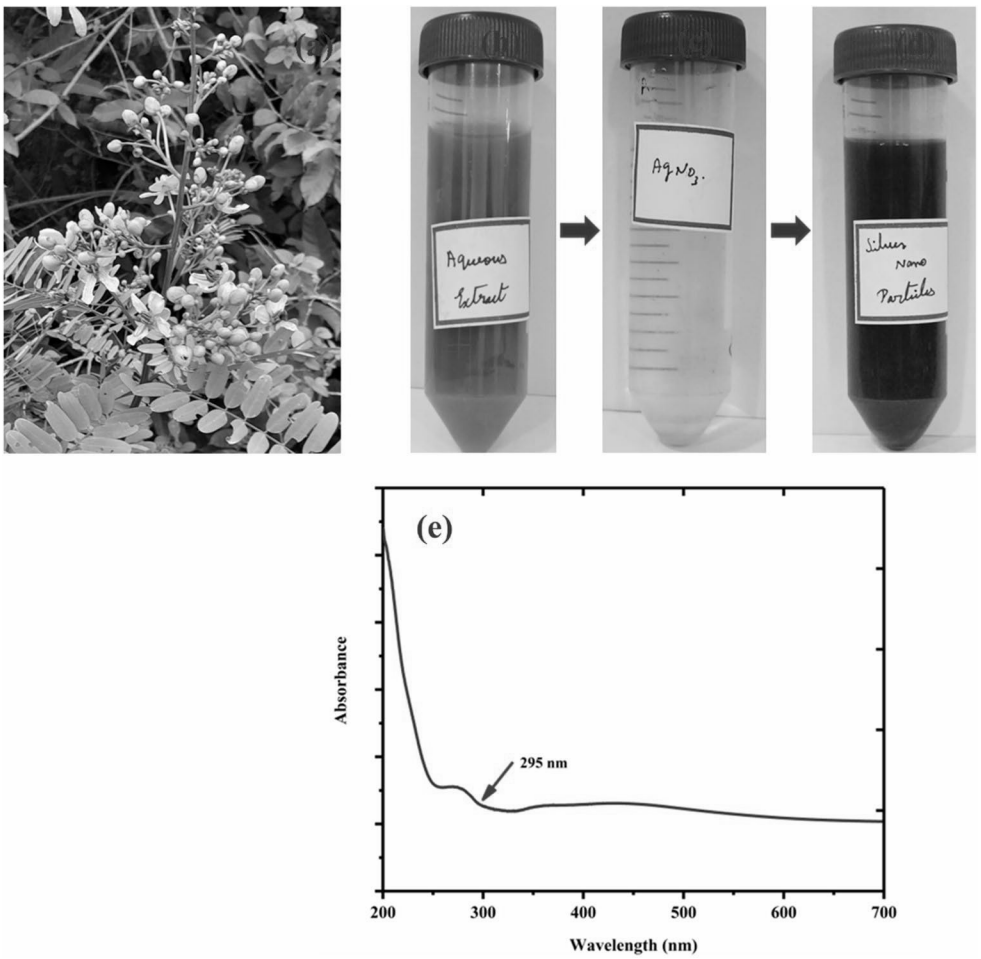

Fig. 2 **a** *Cassia auriculata* morphology, **b** *C. auriculata* flower aqueous extract, **c** 1 mM silver nitrate, **d** Synthesized Ag-NPs and **e** UV spectrum of synthesized Ag-NPs

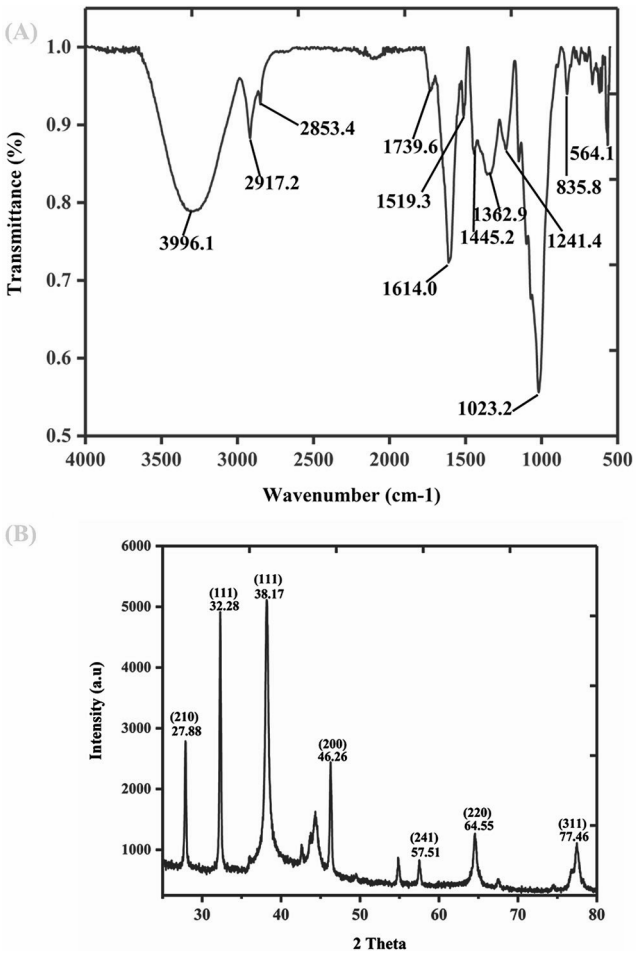

Fig. 3 **A** FTIR analysis of biosynthesized Ag-NPs, **B** XRD analysis of Ag-NPs

Table 1 FTIR spectrum of *C. auriculata* flower extract biosynthesized Ag-NPs

S.No	Observed wave numbers (cm-1)	Functional groups	Bonding pattern
1	3996.1	O-H stretch, free hydroxyl	Sharp, Sharp
2	2917.2	O-H stretch, H-bonded, alcohols or phenols	Broad, medium
3	2853.4	C-H stretch, alkanes	Broad, medium
4	1739.6	C = O bend, aldehydes	Weak, medium
5	1614.0	N-H bend, primary amines	Sharp, Strong
6	1519.3	N-O asymmetric stretch, nitro compounds	Sharp, Strong
7	1362.9	C-H rock, alkanes	Sharp, medium
8	1023.2	C-O stretch, alcohols or carboxylic acids	Sharp, strong
9	835.8	C-Cl stretch, alkyl halides	Sharp, medium

suggested that the silver nanoparticles were arranged in an aggregated manner. The surface plasmon resonance (SPR) function that the produced silver nanoparticles triggered and the existence of free electrons on the silver nanoparticles as a result of $AgNO_3$ reduction are considered to be the reasons for a change in color [47, 48]. Numerous phytochemicals, including tannins, terpenoids, flavonoids, saponins, steroids, and cardiac glycosides, are present in *C. auriculata* flowers [49]. The cardiovascular and anti-inflammatory properties of *C. auriculata* flower extract may be due to the presence of steroidal and cardiac glycosides.

FTIR study

The FTIR spectrum revealed the potential interaction of silver nanoparticles with capping agents. In this sense, we obtained many distinctive peaks, displayed in Fig. 3; Table 1. The following prominent peaks were also found in bio-synthesized silver nanoparticles: 835, 1023, 1362, 1519, 1614, 1739, 2853, 2914, and 3996 cm-1. The peak patterns all matched molecules that might be involved in the formation and stability of silver nanoparticles. As previously mentioned by [49], the synthesis and stability (capping) of the synthesis of silver nanoparticles were facilitated by the interaction between the metal ion and amide group in the *C. auriculata* extract. The peaks at 3996.1 cm-1 belong to the amide group, and aromatic rings were considered as functional groups from flavonoids, triterpenoids, and polyphenols. The stretching frequency vibration of the O-H group may be the cause of a prominent peak in the biosynthesized Ag-NPs spectra located at 2917.2 cm-1. A peak found about 1739.6 cm-1 for the symmetric and asymmetric C = O vibrations of the aldehydes group, respectively. The notable spectral shift observed at 1614.0 cm-1 was assigned to the primary stretch of amine, likely associated with the protein molecules within the aqueous extract of the flowers. This presence of proteins is potentially involved in both the production and capping of silver nanoparticles during synthesis [50]. In addition, the intense vibrational state at 1519.3 cm-1 found in Ag-NPs may be the stretching state of (N-O) in the silver nitrate N bond [51]. The wavelength at 1362 cm-1 is connected to the C-H bending vibration of alkane/alkene. Further FT-IR observations of the synthesized Ag-NPs band at 1023 cm-1 are identified as a carboxylic acid C-O group. The overall spectrum data indicated that the floral extracts included phytochemicals such as polyphenolic components of flavonoids, tannin, and coumarins, which may be involved in the reduction and stabilization of NPs production [52].

XRD analysis

Figure 3B shows the X-ray diffraction of the synthesized silver nanoparticles. Various Bragg reflections have been identified at 2θ = 27.88º, 32.28°, 38.17°, 46.26°, 57.51°, 64.55°, and 77.46°, respectively, with silver nanomaterials and the Miller index (210), (111), (200), (241), (220), and (311). The results reported agree with the previous research [53, 54]. The pattern of XRD exhibits a few additional peaks, which may be driven by crystalline extract impurities that are still present on the surface of the nanoparticles, even if the primary phase of silver was confirmed by the results. The synthesized silver nanoparticles have a face-centered cubic structure, according to the XRD pattern investigation [55]. In addition, [56] work on the environmentally friendly production of silver nanoparticles using *Vernonia amygdalina* revealed that, according to XRD, four strong peaks occurred at 38.10°, 44.14°, 64.47° and 77.37°.

SEM-TEM and EDAX analysis

The scanning electron microscopy (SEM) technique was used to comprehensively analysed the shape of the Ag-NPs. The produced nanoparticles showed a well-defined shape, with an approximate cross-sectional diameter of 65 nm, and were long, square, and spherical, as can be observed in the findings shown in Fig. 4A displayed SEM was used to examine the structural properties of the nanoparticles extensively and visually. This provided for important insights into the size and form of the particles, which can be useful for future study and applications [57, 58]. The EDAX instrument analysis may be used to identify the chemical composition of the produced silver nanoparticles. The ZAF method was used to determine the samples % chemical composition. The elements atomic numbers are represented by Z, absorbance by A, and fluorescence by F. The sample's weight and atomic percent are determined by this algorithm. Figure 4B shows the percentage of Ag-NPs that were obtained in terms of chemical makeup. According to the Ag-NPs Energy-Dispersive spectroscopy (EDAX)

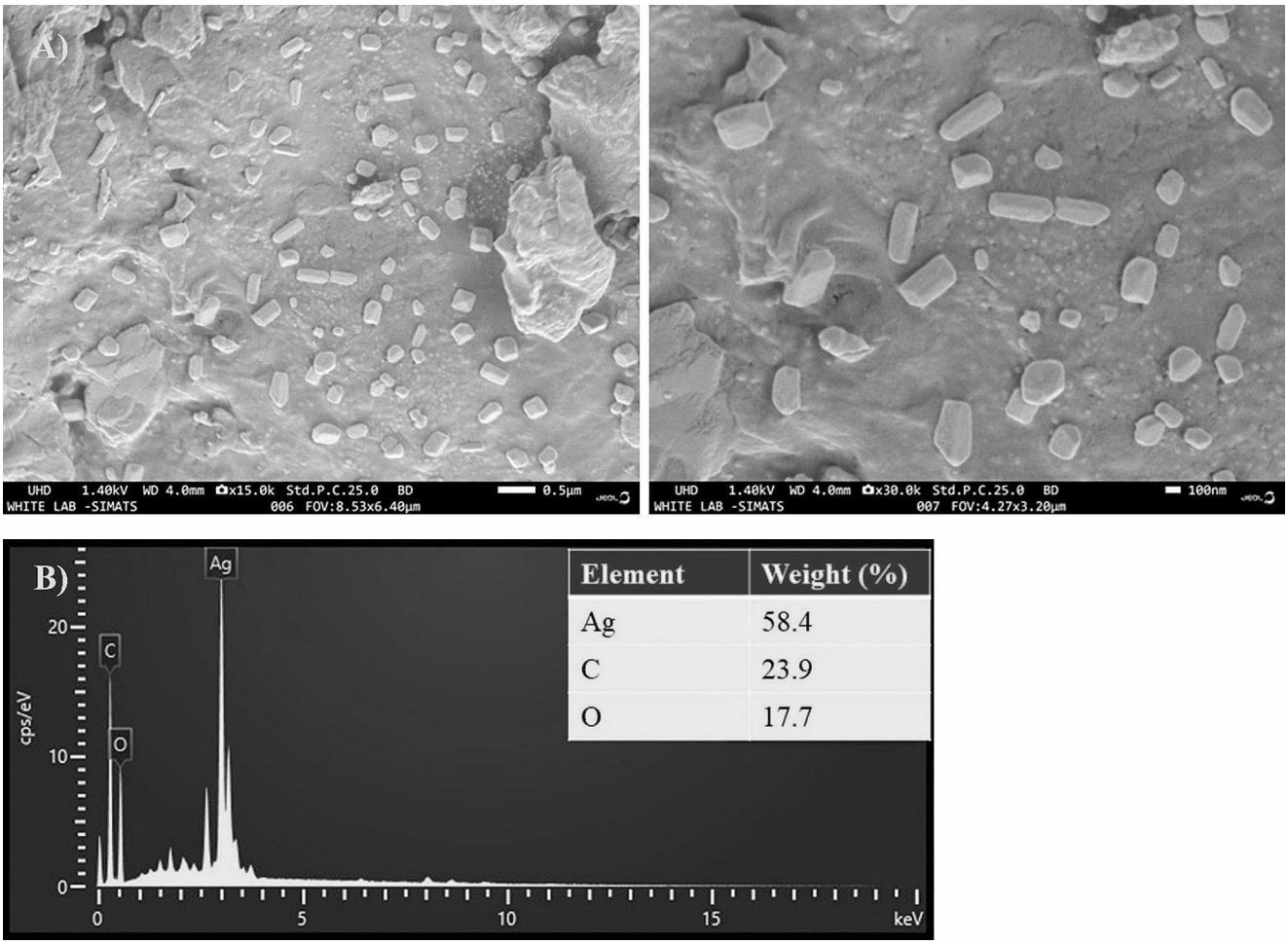

Fig. 4 **A** FE-SEM micrographic pictures of synthesized Ag nanoparticles, **B** EDAX analysis of Ag NPs

estimation, the Ag-NPs development mainly occurs in the (111) plane at 3.2 keV. This, according to EDAX, confirms that the peak characteristic of metallic Ag-NPs is 58.4% by weight. Similarly, Dua et al., (2023) investigated the formation of Ag-NPs by *E. adenophorum* extract, which was validated by EDAX data at 3.1 keV. The presence of silver nanoparticles was confirmed by EDAX analysis, which also often revealed strong signal energy peaks for silver atoms in the 2–4 keV region [59]. The morphological and structural properties of the synthesized Ag-NPs were confirmed using TEM, as shown in Fig. 5A-B. TEM micrographs show that Ag-NPs are spherical and clear of aggregation. The particle size varied from 10.3 to 83.1 nm. Similarly, [58] found that Ag-NPs produced from *Aloe barbadensis* miller leaf extracts had a predominantly spherical shape, as shown by FE-SEM analysis. Additionally, it is clearly suggested that the biogenic Ag-NPs are single crystals by the clear hexagonal point in the SAED pattern (Fig. 5D) from one of the Ag nanoparticles. The mean size distributions of NPs were compared with a normal histogram and shown in Fig. 5C. The hexagonal scattering positions, observed with the top plane facing the electron beam, clearly indicate that the silver nanoprisms exhibit an essentially spherical shape along the (111) crystal plane.

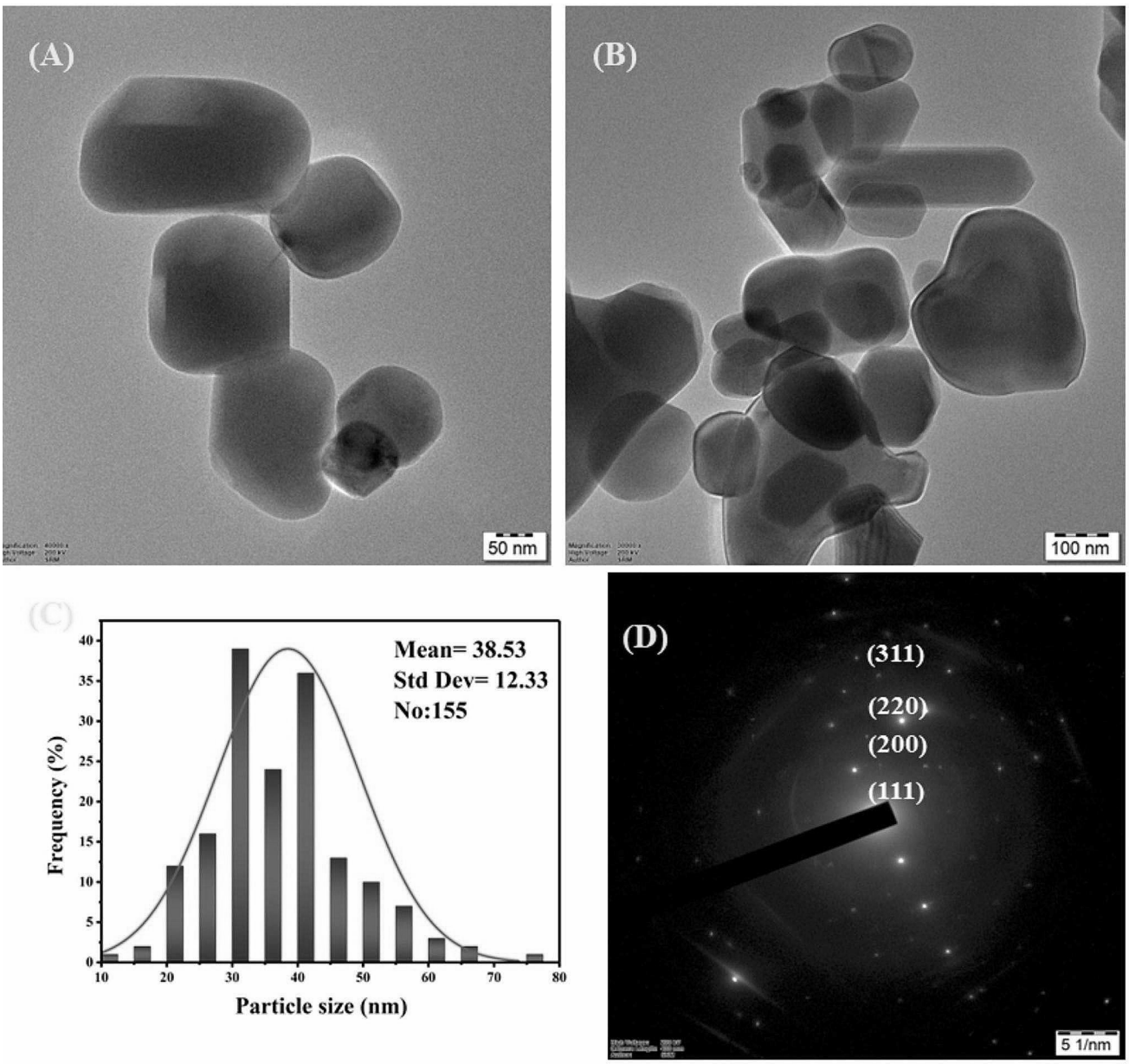

Fig. 5 Electron microscopic analysis of Ag-NPs. **A** TEM image at 50 nm, **B** TEM image at 100 nm, **C** Particle size distribution of Ag-NPs and **D** SAED pattern of Ag-NPs

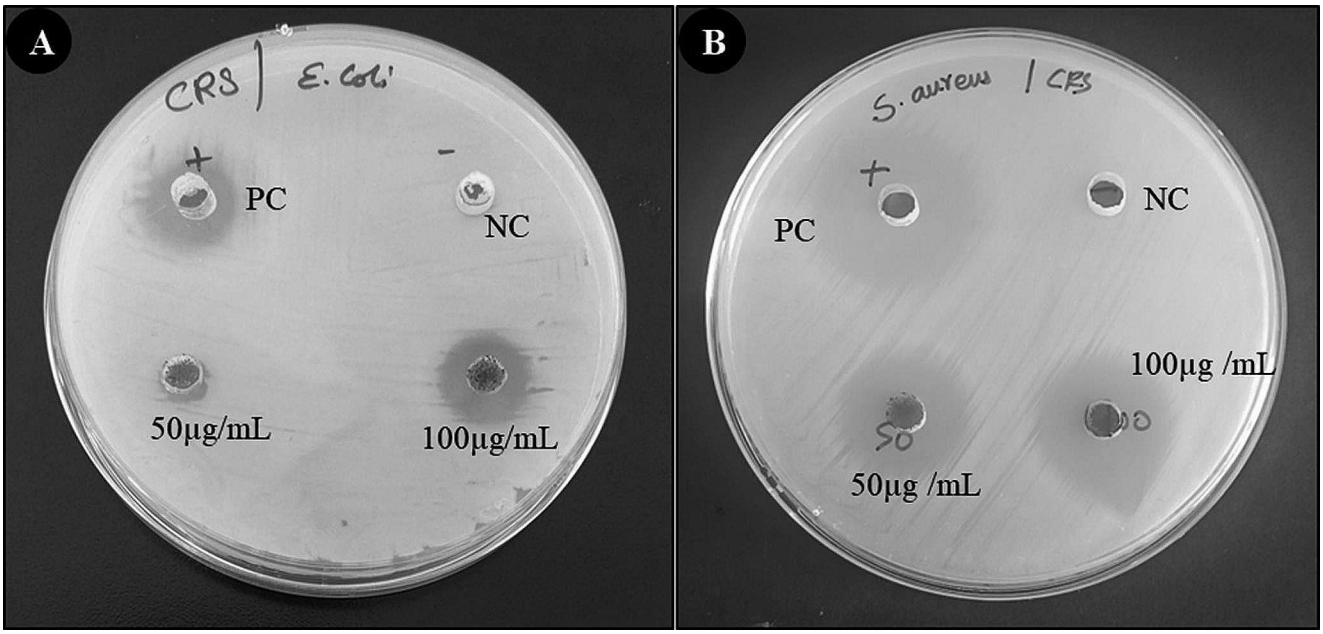

Fig. 6 Antibacterial activity of *C. auriculata* flower synthesized Ag-NPs against **A** *E. coli* and **B** *S. aureus*

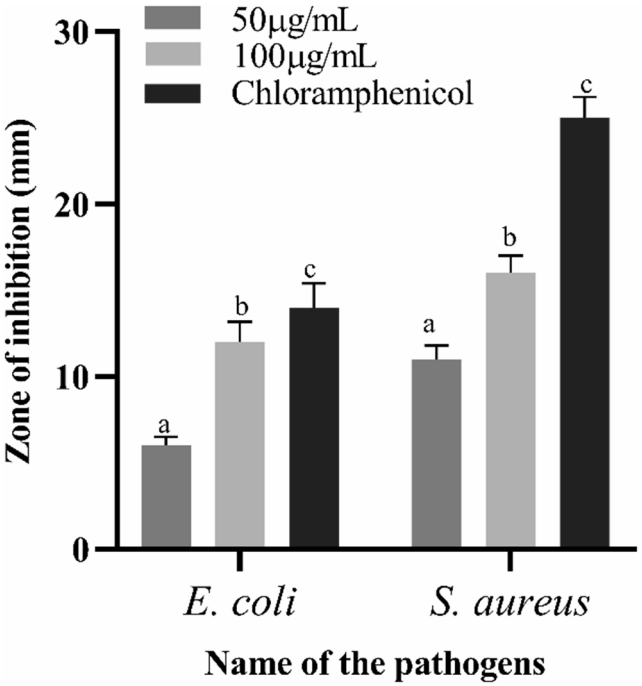

Fig. 7 Bar diagram represents in zone of inhibition (mm). Mean values within the column followed by the same letter in superscript are not significantly different at $P < 0.05$ level

Antibacterial activity

The necessity to investigate novel natural and inorganic alternatives to commercially existing antimicrobial agents and antibiotics has increased due to human pathogen resistance to these drugs [60]. Silver is one of the inorganic antibacterial agents that has been utilized extensively from ancient times to combat illness [61]. Numerous studies have examined the antimicrobial properties of silver, silver ions, and silver compounds [62]. Surveys have also demonstrated the exceptional antibacterial activity of green produced Ag-NPs [63, 64]. In the view present study *C. auriculata* flower extract synthesized Ag-NPs was evaluated against two clinically important pathogenic microorganisms: *E. coli* and *S. aureus*. As summarized in Fig. 6, the Ag-NPs had a dose-dependent effect on both of the bacteria. A concentration of 100 µg/mL of Ag-NPs produced strong antibacterial activity (18 mm) against *S. aureus*, similar to the effect exhibited by 10 µg/mL of chloramphenicol, a commercial antibiotic (Fig. 7). Furthermore, the diameter of the zone of inhibition against *E. coli* width of 6.5 and 11.5 mm at Ag-NPs concentration of 50 µg/mL and 100 µg/mL, respectively. A zone of inhibition is the region that becomes bacteria-free by the use of Ag-NPs or chloramphenicol. Ag-NPs interfere with membrane permeability when they puncture bacterial cell membranes, leading to intracellular ATP leaking and bacterial cell death. AgNP-released silver ions serve as a source of antibacterial action [65]. Significant variations in the diameter of the zone of inhibition between the two species of bacteria were computed using ANOVA, with $p < 0.05$, as seen in Fig. 6 The unique cell wall structure characteristic of Gram-ve bacteria, distinguishing them from Gram-+ve bacteria, could be a contributing factor. The periplasmic space, referred to as the periplasm, features an exposed arrangement of peptidoglycan chains, forming the peptidoglycan layer between the cytoplasmic membrane and the outer membrane, facilitating the penetration of Ag-NPs [66].

Gram-negative bacteria possess a cytoplasmic membrane, a thin peptidoglycan layer, and an outer membrane containing lipopolysaccharide [67, 68].

Larvicidal activity of synthesized Ag-NPs

Aedes albopictus are the mosquito vectors of significant arboviruses from the Flavivirus and Togavirus genera worldwide [69]. Mosquitoes become the main vectors of deadly diseases such as malaria, chikungunya, filariasis, and yellow fever. Dengue fever has spread to tropical and subtropical areas in recent years, with an estimated 390 million cases globally, millions of which will exhibit clinical symptoms [70]. A promising substitute for using bioactive compounds directly obtained from plants as larvicides are silver nanoparticles (Ag-NPs), which are produced from plant extracts [71]. Plant material is excellent for the production of Ag-NPs because it can include secondary metabolites that function as reducing, capping, and stabilizing agents, resulting to related shortened responses [72]. Low-cost, biocompatible technologies can be used to manufacture green nanoparticles [73]. The use of green synthesized nanoparticles with minimal larvicidal properties has proven effective in reducing mosquito populations during their early instars [70, 74].

In the present study, the toxicity of *C. auriculata* flower synthesized Ag-NPs tested against IVth instar larval of *An. stephensi* and *Ae. albopictus* resulted the least LC_{50} and LC_{90} values: (LC_{50} = 24.14; LC_{90} = 62.58, mg/L) and *Ae. albopictus* (LC_{50} = 35.53; LC_{90} = 65.27 mg/L), respectively (Table 2). Following the application of Ag-NPs, a notable decrease in larval survival against *Ae. albopictus* was observed. Although *Ae. albopictus* exhibited a reduced mortality rate, its larvae experienced significant malformations. Notably, at a concentration of 250 mg/L of Ag-NPs, strong activity against *An. stephensi* larvae was demonstrated, with a mortality rate higher than that observed for *Aedes*. These results were in agreement with earlier studies by Elumalai al [75]. finding that Ag-NPs produced from *Pila virens* shell were lethal to *An. stephensi* larvae and pupae, the malarial vector larval stage from the Ist to the IVth instar [76]. obtained similar findings when they investigated the bioactivity of Ag-NPs generated from the latex producing plant *Azolla pinnata* against the larval instars of *Ae. albopictus* and *Ae. stephenesi*. Ag-NPs made from phyto-synthesized can attach to proteins or DNA and then enter a mosquito cell, breaking through the exoskeleton and destroying the insect. Additionally, they alter enzymes and cause DNA mutations, but their effects on non-target species like fish and beneficial arthropods are less severe [77]. Because of their small size (100–200 nm), silver nanoparticles have a favourable surface area to volume ratio with high potency, even at extremely low concentrations [78].

Table 2 Larvicidal activity of Ag-NPs against two mosquito vectors *An. stephensi* and *Ae. albopictus* 4th instar larvae

Species	Concentration (mg/L)	Mortality Percent	LC_{50} (mg/L) (LCL-UCL)	LC_{90} (mg/L) (LCL-UCL)	χ^2
An. stephensi (24 h)	50	33.45 ± 1.57	24.14 (18.46–45.60)	62.58 (46.39–97.48)	11.18
	100	46.00 ± 1.20			
	150	67.00 ± 1.00			
	200	81.43 ± 1.55			
	250	100 ± 1.00			
	control	00.00 ± 0.00			
Ae. albopictus (24 h)	50	27.53 ± 1.50	35.53 (25.48–55.14)	65.27 (38.50-98.44)	14.47
	100	39.52 ± 1.26			
	150	57.56 ± 1.21			
	200	69.42 ± 1.50			
	250	87.00 ± 1.57			
	control	00.00 ± 0.00			

Histology profiles of 4th instar larvae

The midgut epithelial columnar of 4th instar *An. stephensi* larvae were severely damaged after being treated with *C. auriculata* flower mediated Ag-NPs. In the control groups, the lumen was surrounded by a thin peritrophic membrane (pm) containing food particles. However, in the treated larvae, the midgut contents, epithelial cells, and peritrophic membrane were observed to be collapsed (Fig. 8b). The midgut, hindgut, muscles, brush border, and epithelial cells of the control larvae appeared normal (Fig. 8a). Similarly, 4th instar *Ae. albopictus* treated with biosynthesized Ag NPs showed significant damage to the midgut, hindgut, muscles, and nerve ganglion tissues, as well as disorganized and damaged epithelial cell layers (hyperplasia in some areas). Furthermore, swelling, enlarged, and deformed epithelial cell lesions were discovered. Additionally, blebbing cells, missing microvilli, and cells protruding into the lumen were seen (Fig. 8d). In contrast, the control larvae had typical epithelial cell morphology, with feeding bolus, strong brush border, basophilic nuclei, and acidophilic cytoplasm (Fig. 8c). Likewise, [79] reported on the environmentally friendly creation of Ag nanostructures utilizing *Pedalium murex* plant seed extract exhibited histopathological alteration of *Ae. aegypti* larvae. Similarly, [80] found that *Vernonia anthelmintica* induced histological anomalies in the head, thorax, abdomen, and siphon areas of mosquito larvae treated with silver NPs (Ag-NPs), while Ag-NP accumulation was found in the gut region of *Ae. aegypti* Linn. and *Cx. quinquefasciatus* Say.

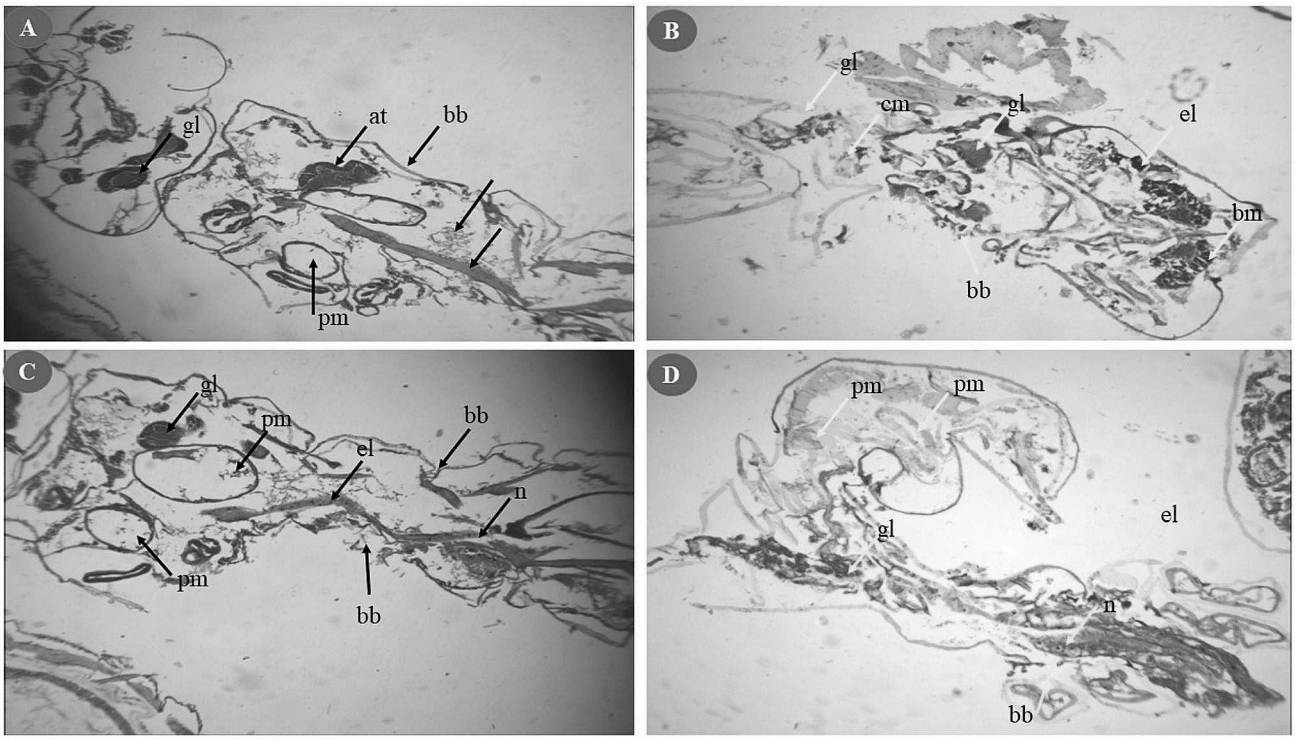

Fig. 8 Histological profiles of 4th instar larvae of *An. stephensi, Ae. albopictus* **A**, **C** control and **B**, **D** after exposure of Ag-NPs at 250 mg/L (at, adipose tissue; m, muscles; el, epithelium layer; gl, gut lumen, pm, peritrophic membrane, cm, cytoplasmic masses, bm, basement membrane). 400×

Photocatalytic activity of MB

The many dyes, such as methylene blue, safranin, crystal violet, and green malachite, create a risk to humans and the environment. Due to its continuous discharges through the wastewater of textile industries, it is important that it be degraded [81]. The produced Ag-NPs photocatalytic activity has been evaluated by degrading MB dyes in the presence of sunlight. The visual monitoring of dye degradation was made possible by the constant change of the necessary color into a colorless dye solution. Figure 9a demonstrates the absorption peak measured in the aqueous solution at different times during the action of Ag-NPs, displaying the photocatalytic degradation of MB dyes by produced Ag-NPs. The most significant absorption peak at 665 nm gradually decreased with an increase in exposure duration, indicating dye degradation via photocatalytic reactions. Without the nano-catalyst (control), the process came to an end. Degradation was evident at 15% after the first 20 min in sunlight. On the other hand, when the photoperiod was extended, the degradation efficiency of MB dye increased substantially and reached at 92% after 120 min, as shown in Fig. 9b Gaps, superoxide anion radicals, and hydroxide ions were the main active ingredients during the photocatalytic oxidation of the dye, as demonstrated by the study carried out by [82]. The results of photocatalytic studies indicate that these Ag-NPs are effective for removing MB from sunlight, indicating their great potential for use in the textile and wastewater treatment industries. In order to evaluate the ability of the derived Ag-NPs for dye degradation, a comparison of the findings from published research [83]. The photocatalytic activity of the Ag-NPs synthesized from the floral extract of *C. auriculata* was validated by the UV-visible spectrum. This could be the result of the extract's OH group-containing components directly interacting with one another. Following 8 min of photocatalytic degradation with Ag-NPs (0.2 mg/mL) produced by *Echinophora platyloba* extract, it showed that 90.11% of the dye had been eradicated [51]. Furthermore, [84] found that Ag-NPs significantly enhanced the rate at which sunlight degraded the MB dye solution. Ag-NPs made from an extract from *Cynara cardunculus* leaf extract demonstrated more than 90% photocatalytic degradation of methylene blue reactive when exposed to UV light, based on research by [85]. The ability to scavenge free radicals is due to the presence of flavonoids. Redox catalysis and the electron transition from donor to MB dye are both facilitated by the flower extract electron relay action. The catalytic competence of phenolic or flavonoids is determined by the extract's reduction potential. A variety of electronic transferring chemicals found in

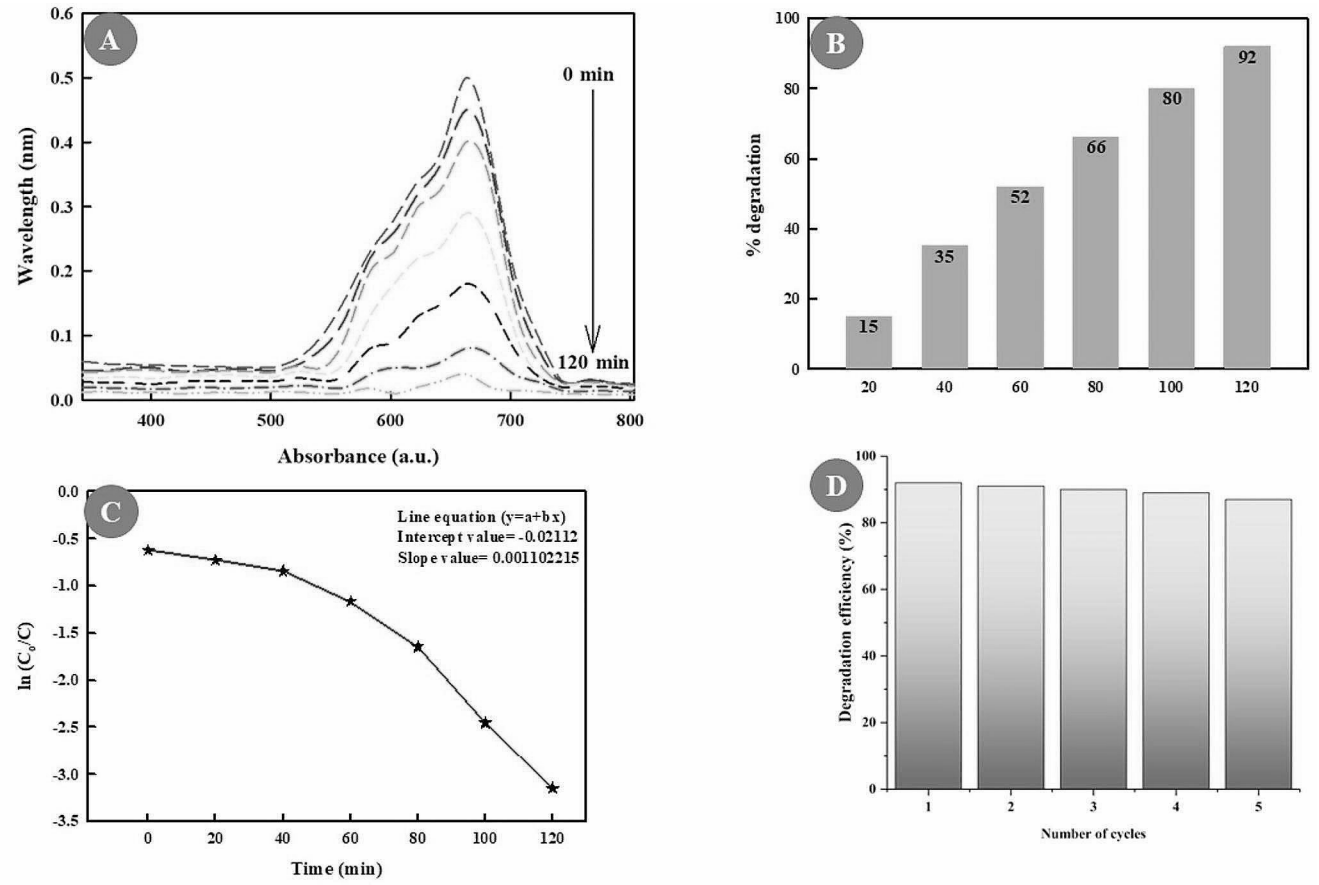

Fig. 9 Absorbance spectra of **a** methylene blue (MB) in the presence of synthesized Ag-NPs. **b** Percentage of degradation MB, **c** Kinetic linear fits that are pseudo-first-order, **d** Test for Recyclability

the *C. auriculata* flower extract may lead to the MB dye reduction [86]. The catalyst's increased catalytic efficiency is caused by a significant increase in surface area when size is reduced to nanoscale levels. Research on the relationship between alcohols and silver metal has shown that the alcohol's C–O bond is easily broken on the metal's surface, producing oxide molecules and hydrocarbons [17].

Figure 10 shows the possible process for Ag NPs to photodegrade methylene blue (MB) dyes. The Surface Plasmon Resonance (SPR) effect is responsible for the photogeneration of electron–hole pairs on the surface of Ag-NPs during solar light illumination. Notably, since the reaction is confined to the nanoparticle surface, electrons are elevated from the valence band (VB) to the conduction band (CB), creating holes in the VB (h_{VB}^{+}) and conduction electrons $\left(e_{CB}^{-}\right)$ in the CB, as elucidated by [1]. Equation 1 demonstrates that these photogenerated molecules produce highly reactive radicals, which aid in the breakdown of MB in the ensuing solution. Equation 1 shows that the radicals that are highly reactive induced through these photogenerated molecules causes the breakdown of MB in the reaction mixture. The e_{CB}^{-} forms anions of superoxide radicals left $\left(O_2^{\bullet-}\right)$ by reaction with surface adsorbed dissolved oxygen (O_2) (Eq. 2). Meanwhile, the surface adsorbed hydroxyl ion and the photogenerated holes h_{VB}^{+} combine to produce the very reactive hydroxyl radical $(HO^{\bullet})$ (Eqs. 3,4). Moreover, $O_2^{\bullet-}$ also interacts with the water molecule, resulting in the hydroperoxyl radicals $HO^{\bullet}$ and $(HO_2^{\bullet})$ (Eqs. 5 and 6) [87]. Equation 7 points out that the photocatalytic degradation of MB dye, which produces carbon dioxide (CO_2), water (H_2O), and other breakdown products, is mostly facilitated by these strongly oxidizing radicals. From the aforementioned considerations, it can be concluded that the Ag NPs synergistic impacts on the SPR behaviors are causing them to display improved photocatalytic ability.

$$Ag\,NPs + h\nu \rightarrow Ag\,NPs\left(e_{CB}^{-} + h_{VB}^{+}\right) \tag{1}$$

$$O_2 + e_{CB}^{-} \rightarrow O_2^{\bullet-} \tag{2}$$

$$H_2O + h_{VB}^{+} \rightarrow HO^{\bullet} + H^{+} \tag{3}$$

$$HO^{\bullet} + HO^{\bullet} \rightarrow H_2O_2 \tag{4}$$

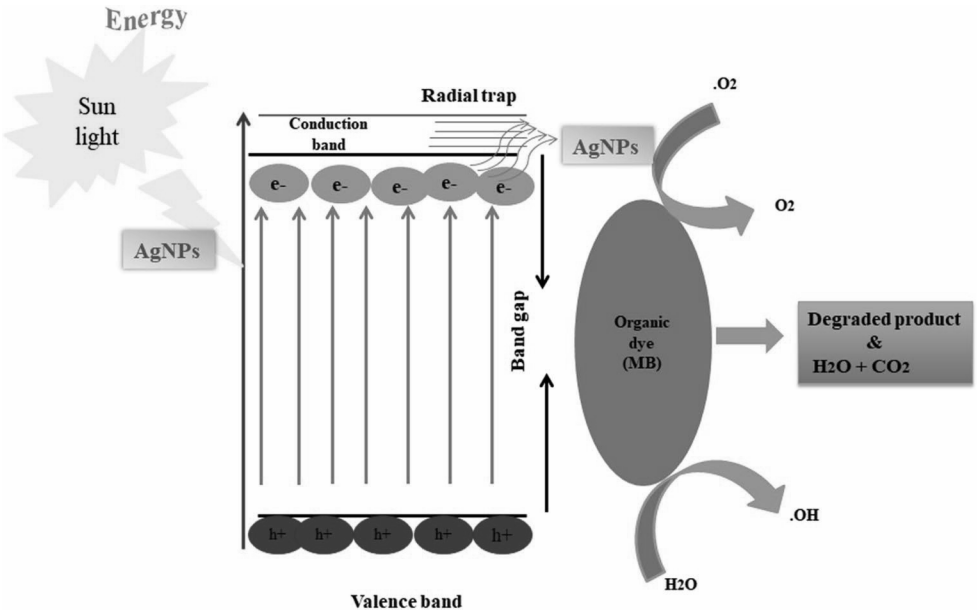

Fig. 10 Proposed photocatalytic degradation mechanism of MB using Ag-NPs

$$H_2O_2 + O_2^{\bullet -} \rightarrow HO^{\bullet} + HO^{-} + O_2 \quad (5)$$

$$H_2O_2 + e^{-} \rightarrow HO^{\bullet} + HO^{-} \quad (6)$$

$$Ag\,NPs\,(O_2^{\bullet -}, HO^{\bullet}, h_{VB}^{+}\,or\,HO_2^{\bullet}) \rightarrow Degraded\,Products \quad (7)$$

The duration of use of the catalyst is an essential characteristic of the photocatalytic method because using it for an extended period of time results in considerable cost savings for the treatment. As a result, the catalyst was recycled, and as Fig. 9d illustrates, the efficiency of the MB dyes decreased from 92% in the first cycle to 90% in the third. According to these findings, Ag-NPs catalyst continued to function well and be reused in sun light.

Conclusion

The fabrication of silver nanoparticles using *C. auriculata* flower extract has been confirmed to be an efficient and affordable process. FTIR evidence showed that proteins, carboxylic acids, flavonols, alcohols, and phenols were among the biomolecules responsible in the bio reduction and bio capping of silver nanoparticles. The biologically produced Ag-NPs exhibited a maximum absorption peak at 295 nm, and XRD proven their crystalline nature. TEM examination confirmed the spherical average size of biogenic silver nanoparticles, which ranged from 10.3 to 83.1 nm. In addition to showing photocatalytic dye degradation activity for the dyes commonly used in pathological laboratories (methylene blue) with 90.11% of degradation, respectively, under solar light irradiations, the biogenic Ag-NPs displayed strong antibacterial efficacy towards both Gram + ve and Gram -ve bacterial strains, with zones of inhibition of 18 mm for *S. aureus* and *E. coli* (11.5 mm), respectively. Ag-NPs produced from *C. auriculata* flowers exhibited 90% mortality against *An. stephensi* and *Ae. albopictus* within 24 h at a dosage of 250 mg/L. Ag-NPs employ secure substances and natural agents, which lead to a high yield, biocompatibility and potential applications and advancements as effective and eco-friendly nano catalysts for sewage treatment. This method can be scaled up for large-scale manufacturing and is remarkably friendly on the environment.

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Author contributions CR and MM conceptualized and planned the experiments. CR, ML, MWA, and VK performed the investigations and assessed the results. The data was processed, evaluated, and the text was written by CR and MM. CR and CK revised the manuscript. The final manuscript has been read and approved by all of the authors.

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Declarations

Ethical approval Not applicable.

Consent to publish Not applicable.

Consent to participate Not applicable.

Competing interests The authors declare no competing interests.

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ORIGINAL ARTICLE

Molecular Docking Studies of Schiff Bases with Azetidinone Against Dihydrofolate Reductase Enzyme as Potential Anti-cancer Agents

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Abstract

Background: In recent times, cancer has emerged as a major health concern. It was established that every antagonist of the dihydrofolate reductase exhibits anti-cancer activity. For anti-cancer action, several Schiff-based derivatives with azetidinone rings were designed and docked against the dihydrofolate reductase protein (PDB id:6CXK) in the current work. The ligands were compared to those of standard antagonists of dihydrofolate reductase, that is, trimethoprim and pyrimethamine. **Materials and Methods:** The ligands were drawn in.mol format using ChemSketch software and converted to.pdb format using Avogadro software. The iGEMDOCK software was utilized to conduct molecular docking investigations, and Discovery Studio Visualizer was ultimately used to visualize the results. **Results and Discussion:** Most compounds have demonstrated a better affinity for binding to the dihydrofolate reductase. Most of the ligands have demonstrated nearly the same binding affinities as that of the standard dihydrofolate reductase, such as trimethoprim (−102.1 kcal/mol) and pyrimethamine (−91.8 kcal/mol). The top 2 compounds 3A8B (−100.6 kcal/mol) and 3A9B (−94.6 kcal/mol) were chosen for visualization. **Conclusion**: Schiff base derivatives with azetidinone ring have the potential to be a promising class of drugs for the treatment of anti-cancer action since they have a higher binding affinity to the dihydrofolate reductase than standard antagonists.

Key words: Anti-cancer, dihydrofolate reductase, discovery studio visualizer, iGEMDOCK software, molecular docking, Schiff base

INTRODUCTION

Schiff base

Schiff bases are a significant class of medications, for the therapy of numerous diseases. They have been gaining importance since Hugo Schiff originally characterized Schiff's base 160 years ago. A ketone or an aldehyde that contains a carbonyl group and has a nitrogen-based moiety is called a Schiff base. It is created by condensing a primary amine with the carbonyl group and substituting the carbonyl group with an imine group known as azomethine.[1-3] Particularly adaptable compounds with C = N (imine) groups are aniline-Schiff bases, which have been shown to exhibit a wide range of biological functions,[4-7] antibacterial, antifungal,[8,9] anti-cancer,[10] and anti-inflammatory.

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Azetidinone

Since the discovery of penicillin by Sir Alexander Fleming in 1928 and the subsequent discovery of cephalosporin, both of which were employed as effective antibiotics, the chemistry of ß-lactams has assumed a significant role in organic chemistry. The emergence of bacterial resistance to commonly used antibiotics of this kind continues to encourage research in this field. Functionalized ß-lactams or novel active principles in the ß-lactam series are required. ß-lactam has antiviral,[11,12] antifungal,[13,14] antibacterial,[15,16] and anti-cancer activities.[17-20] Penicillins, cephalosporins, carbapenems, nocardicin, and monobactams are among the broad spectrum ß-lactam antibiotics[21,22] that share the 2-azetidinone (ß-lactam) ring as a structural characteristic.

MATERIALS AND METHODS

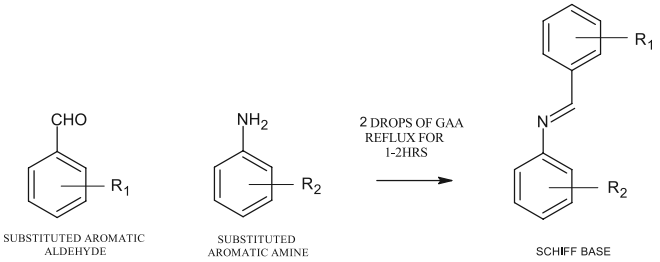
Step 1

Schiff bases are the condensation products of aldehydes and amine compounds in the presence of glacial acetic acid

Table 1: The interactions and binding energies of the top 10 ligands with the enzyme dihydrofolate reductase

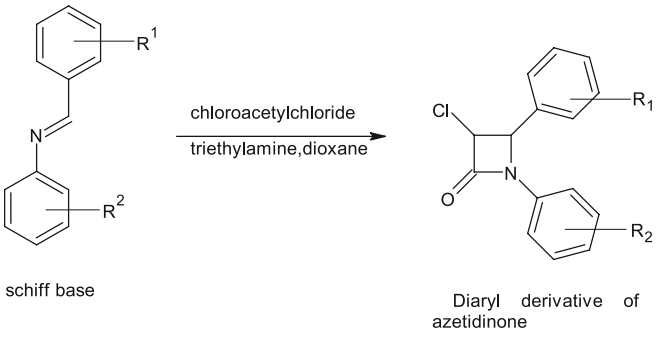
Compound Code	Binding Energy Kcal/mol	Interacting active site amino acid residues
3A8B	−100.6	ARG: 57 [2.61], ASN: 18 [3.10], TYR: 100 [2.59], ALA: 7 [2.94], LEU: 28, LEU: 54, MET: 16, PHE: 31, and ALA: 6
3A9B	−94.6	ASN: 18 [2.80], ARG: 57 [2.77], LEU: 28, LEU: 54, ILE: 94, MET: 16, ASP: 27, and PHE: 31,
3A5B	−93.6	ASN: 18 [3.88], ARG: 57 [6.23], TYR: 100 [7.33], ILE: 94 [4.87], LEU: 54, LEU: 28, MET; 16, PHE: 31, LYS: 32, PRO: 55, MET: 20, GLU: 17, SER: 49, ILE; 50, ILE: 5, ALA; 6, and THR: 46
2A5B	−90.6	ASN: 18 [4.12], ARG: 57 [6.28], ILE; 94 [4.39], LEU: 54, LEU: 28, PHE: 31, MET: 16, TYR: 100, THR: 46, ILE: 50, MET; 20, and LYS; 32
2A10B	−90.2	ASP: 27 [4.76], THR: 113 [4.31], PHE: 31, ALA: 6, ALA; 7, ILE; 5, LEU: 28, TRP; 30, ILE: 50, THR; 46, MET: 20, GLU; 17, and LEU: 54
4A8B	89.8	ARG: 57 [6.34], LEU: 28, PHE: 31, ILE: 50, LEU: 54, ARG: 52, ASN: 18, ILE: 94, THR: 46, MET: 16, LYS: 32, ad PRO: 55,
7A8B	89.6	ASN: 18 [4.79], ILE: 50 [4.98], ARG: 57 [6.48], PHE: 31, LEU: 54, ARG: 52, ILE: 94, THR: 46, MET: 16, LEU: 28, LYS: 28, and PRO: 55
3A4B	−89.5	ASN: 18 [3.79], ARG: 57 [6.05], TYR: 100 [7.63], PHE: 31, MET: 16, LEU: 28, LEU: 54, LYS: 32, PRO: 55, MET: 20, GLU: 17, SER: 49, THR: 46, ILE: 50, ALA: 6, ALA: 7, and ILE: 94
4A10B	−88.9	ASN: 18 [4.21], ARG: 57 [6.19], MET: 16, LYS: 32, PRO: 55, LEU: 54, LEU: 28, ILE: 50, GLU: 17, MET: 20, ALA: 6, ILE: 5, TYR: 100, ALA: 7, ad ILE: 94
3A3B	−88.7	ASN: 18 [4.21], ARG: 57 [6.19], MET: 16, PHE: 31, ASP: 27, LYS: 32, PRO: 55, LEU: 54, LEU: 28, ILE: 94, ALA: 7, TYR: 100, MET: 20, ALA: 6, ILE: 5, GLU; 17, and ILE: 50
Trimethoprim	−102.1	ASP: 27 [2.82], THR: 113 [3.26], PHE: 31, ALA: 7, ILE: 5, ILE: 50, MET: 16, and LEU: 28
Pyrimethamine	−91.8	TYR: 100 [2.60], ILE: 5 [2.60], PHE: 31, ILE: 50, ILE: 94, and ALA: 7
Co-crystalized ligand (Dihydrofolate)	−101.8	ASP: 27 [3.90], ILE: 5 [3.81], MET: 16 [3.59], ARG: 57, LYS: 32, PHE: 31, ALA; 6, ALA: 7, TRP: 22, MET: 20, GLU: 17, ASN: 18, THR: 46, LEU: 54, PRO: 55, LEU: 28, GLU: 95, TRP: 30, THR: 113, ILE: 94, and TYR: 100

and ethanol refluxed for 4 h after cooling the product and recrystallized by ethanol.



Step 2

Schiff base in the presence of chloroacetyl chloride, triethylamine, and dioxane gives azetidinone-derived Schiff base.



The Schiff base synthesis techniques were derived from the literature.[23-25] There have also been reports of alternative Schiff base synthesis techniques using azetidinone.[26-29] The method indicated above was used to select several substituted aromatic aldehydes and aromatic amines. Schiff bases have been designed by adding an azetidinone moiety, and the final products were designed by the approach. Using Swiss ADME software,[30-32] the ADME properties of designed ligands were predicted after they were screened using TopKat software[31-33] for *in silico* toxicity. Designed compounds with good ADME properties and anticipated non-carcinogenic and non-toxic compounds were chosen for molecular docking.

Molecular docking

The target was chosen based on the SWISS target prediction software.[31,34,35] Most of the compounds have shown dihydrofolate reductase as a potential target. Hence, dihydrofolate reductase is used for molecular docking.

ChemSketch software was used to sketch the ligand's 2D structures, which were then saved in.mol format. Using the Avogadro tool,[31,35,36] the ligand structures in .mol format were converted into the .pdb format. Docking studies were conducted for the safe, non-carcinogenic developed compounds with good ADME features to evaluate binding poses and interactions. Hence, the present study aims to evaluate Schiff base derivatives for anti-cancer activity.

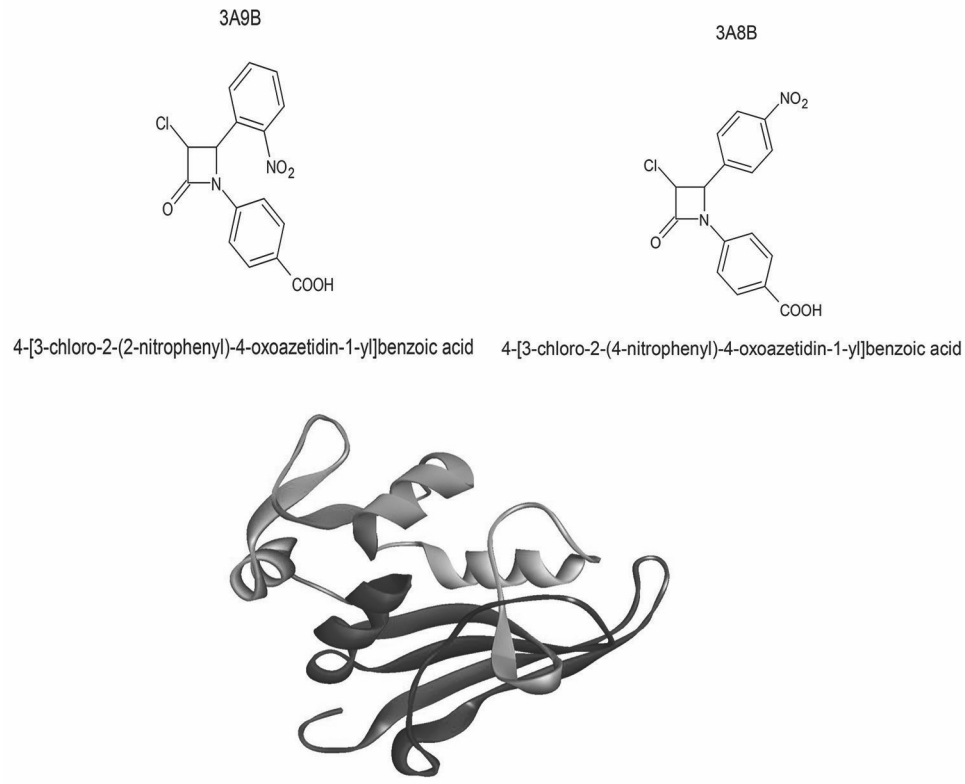


Figure 1: Cleaned dihydrofolate reductase enzyme – PDB ID: 6CXK

Table 2: Docking and visualization data of standard antagonist trimethoprim against dihydrofolate reductase enzyme

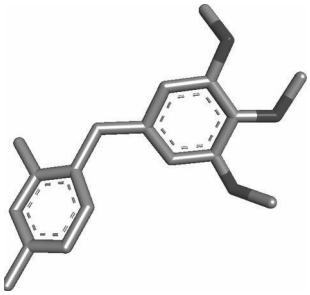
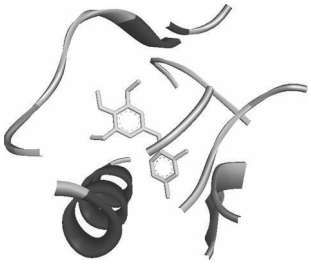
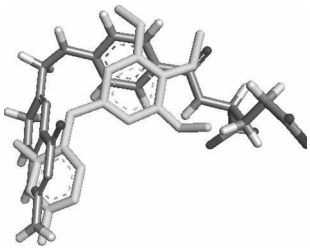
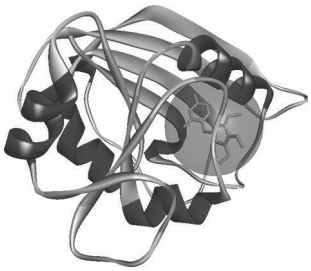
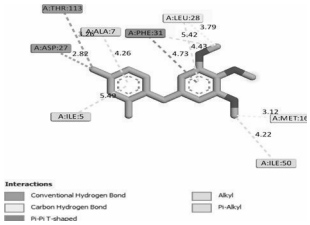
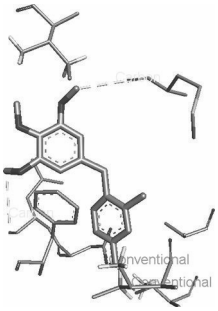
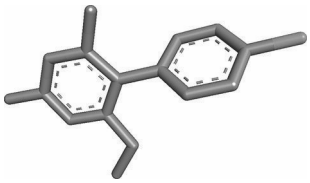
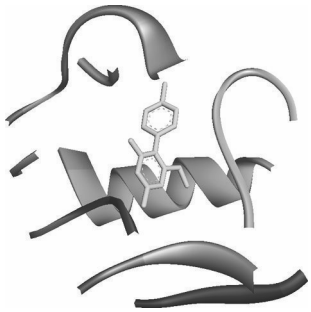
	
Trimethoprim ligand	Trimethoprim ligand+dihydrofollate reductase enzyme complex
	
Trimethoprim ligand+co-crystal ligand overlap	Trimethoprim ligand+whole dihydrofolate reductase enzyme
	
Trimethoprim ligand+2D interaction with dihydrofolate reductase protein	Trimethoprim 3D interactions with dihydrofolate reductase enzyme

Table 3: Docking and visualization data of standard antagonist pyrimethamine against dihydrofolate reductase enzyme

	
Pyrimethamine ligand	Pyrimethamine ligand+dihydrofollate reductase enzyme complex

(Contd...)

Table 3: *(Continued)*

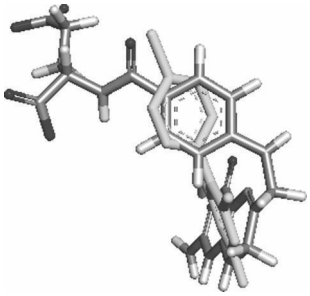
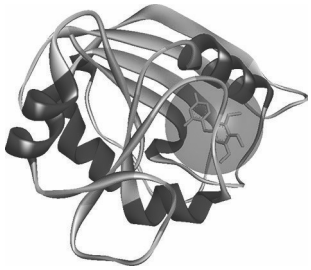
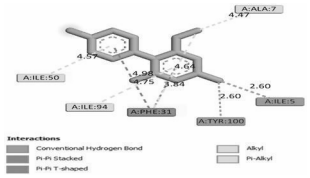
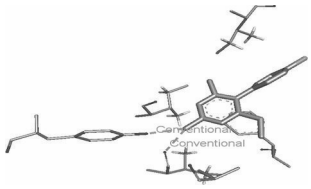
	
Pyrimethamine ligand+co-crystal ligand overlap	Pyrimethamine ligand+whole dihydrofolate reductase enzyme
	
Pyrimethamine ligand+2D interaction with dihydrofolate reductase enzyme	Pyrimethamine 3D interactions with dihydrofolate reductase enzyme

Table 4: Docking and visualization data of 3A8B ligand against dihydrofolate reductase enzyme

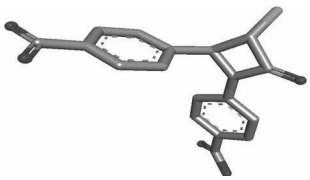
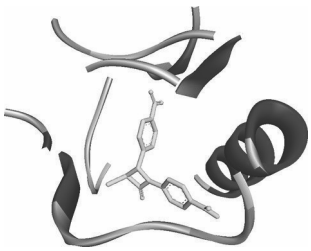
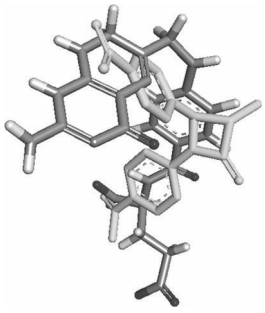
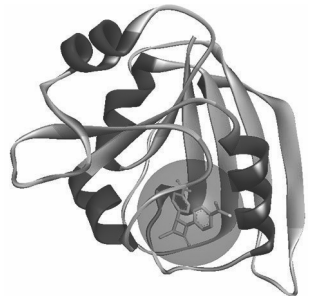
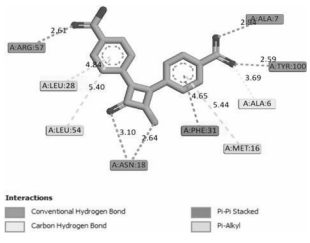
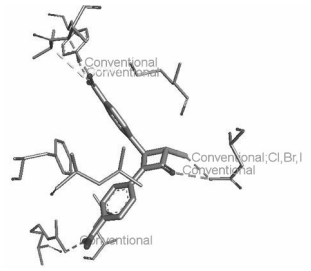
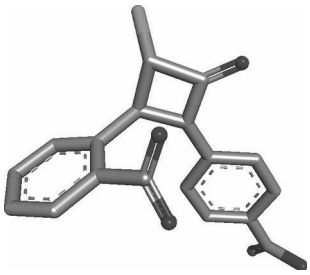
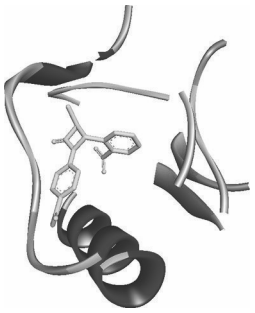
	
3A8B Ligand	3A8B Ligand+dihydrofollate reductase enzyme complex
	
3A8B Ligand+co-crystal ligand overlap	3A8B Ligand+whole dihydrofolate reductase enzyme
	
3A8B ligand 2D interaction with dihydrofolate reductase protein	3A8B ligand 3D interactions with dihydrofolate reductase enzyme

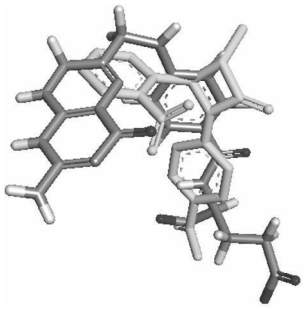
Table 5: Docking and visualization data of 3A9B ligand against dihydrofolate reductase enzyme



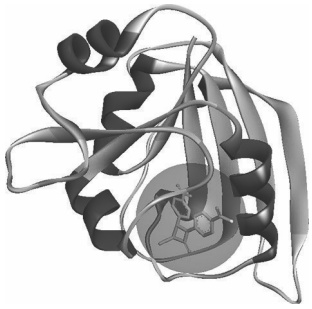
3A9B ligand



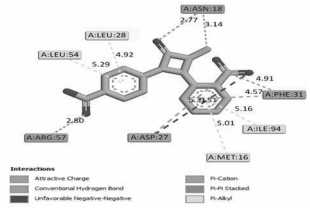
3A9B ligand+dihydrofollate reductase enzyme complex



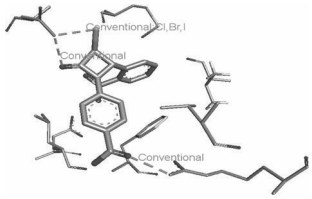
3A9B ligand+co-crystal ligand overlap



3A9B ligand+whole dihydrofolate reductase enzyme

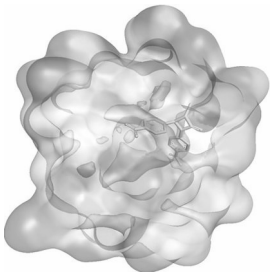


3A9B ligand+2D interaction with dihydrofolate reductase protein

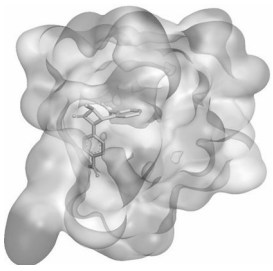


3A9B ligand 3D interactions with dihydrofolate reductase enzyme

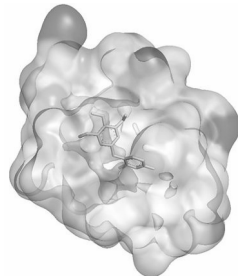
Table 6: Pocket analysis and binding modes of 3A8B, 3A9B, trimethoprim, and pyrimethamine



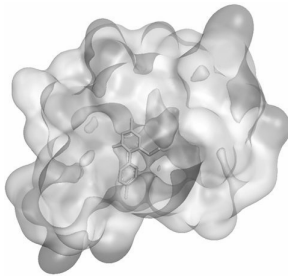
3A8B



3A9B



Trimethoprim



Pyrimethamine

iGEMDOCK was the program utilized for docking.[31,35,37] This software calculates the orientation and structure of ligands concerning the protein's active site. To assess the molecular interactions of the chosen safe chemicals with the dihydrofolate reductase (Figure 1, PDB ID:6CXK) using a co-crystallized ligand inhibitor dihydrofolate that was retrieved from the protein data bank, *in silico* docking simulation studies were carried out.

The Discovery Studio Visualizer (Biovia) was used for visualization. An accurate docking method was chosen, and a standard docking protocol was adhered to. The optimal docking solutions were examined based on the scoring function. The scoring function uses a combination of hydrogen bonding, van der Waals, and electrostatic energies. To determine the interactions between the ligands and the target protein, post-docking interaction profile analysis of the best poses was carried out. Using Insilco toxicity prediction, safe and non-carcinogenic compounds were found and molecular docking was performed along with standard dihydrofolate reductase inhibitors such as trimethoprim[38-44] and pyrimethamine.[45-51] To assess binding affinities and

molecular interactions, docking simulations were performed. For the post-docking interaction investigation, the top 2 compounds were selected based on their superior binding energies and molecular interaction profiles.

RESULTS AND DISCUSSION

The top 2 ligands' structures that have superior binding energies have been chosen for visualization.

CONCLUSION

Table 1 In conclusion, the binding energies of all of the top 10 compounds were nearer to the binding energies of the standard antagonists of the Dihydrofolate reductase enzyme. The binding energies of the top two compounds 3A8B (-100.6 k.cal/mol) and 3A9B (-94.6 k.cal/mol) were nearer to those of the standard Dihydrofolate reductase enzyme Inhibitors, such as Trimethoprim (-102.1 k.cal/mol) and pyrimethamine(91.8 k.cal/mol) and hence selected for visualization.

In the visualization process, the top 2 ligands were compared with the co-crystallized ligand [Dihydro folic acid] for structural similarity. The ligand binding site in the whole protein is also visualized. In 3d interaction, the number of conventional hydrogen bonds was visualized. 2d interaction gives us a clear-cut idea of the interacting amino acid residues and their distance from that of the ligand at the active pocket site.

Table 2 Trimethoprim has Two Hydrogen Bond Interactions Namely ASN:18 [2.80], ARG:57 [2.77] Table 3 Pyrimethamine also has two hydrogen bond interactions namely TYR:100 [2.60], ILE:5 [2.60]. Table 4 Compound 3A8B has four conventional hydrogen bond connections through the amino acid residue ARG:57 [2.61], ASN:18 [3.10], TYR:100 [2.59], and ALA:7 [2.94].Table 5 Compound 3A9B has two conventional hydrogen bond interactions with the receptor through the amino acid residues ASN:18 [2.80], and ARG:57 [2.77]. There are two conventional hydrogen bond interactions displayed by the standard Inhibitors. Trimethoprim and 3A8B has four amino acid residues in common ALA:7, LEU:28, MET:16, PHE:31. Trimethoprim and 3A9B has four amino acid similar ASP:27, PHE:31, MET:16, LEU:28. Pyrimethamine and 3A8B have three amino acid residues in common TYR:100, ALA:7, and PHE:31. Pyrimethamine and 3A9B have two amino acid residues common PHE:31 and ILE:94.

BINDING POCKET ANALYSIS

Table 6 the standard antagonists Trimethoprim, Pyrimethamine and the top ligands 3A8B,3A9B were docked in the centre of the binding pocket. This might have been the reason for their better binding energy. The top compounds 3A8B and 3A9B contains one electron-withdrawing group NO2 and one electron-withdrawing group COOH, which might have contributed to their better binding energies. since the compounds 3A8B and 3A9B had near-binding energies as that of the standard Dihydrofolate reductase enzyme inhibitors Trimethoprim and pyrimethamine, they can be further synthesized and used for further studies.

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