Traditional Medicine Research 2025;10(7):41. https://doi.org/10.53388/TMR20241025001

A systematic review of neem flower (*Azadirachta indica*): a promising source of bioactive compounds with pharmacological and immunomodulating properties

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Author contributions

Palanivelmurugan M collected the articles, interpreted the data, and wrote the paper. Mary Saral A supervised, validated and revised the article for publishing.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

The authors would like to thank VIT University management for their support and library facilities in accomplishing this part of the study.

Peer review information

Traditional Medicine Research thanks Shah Nawaz Khan and other anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations

AIP, autoinducing peptide; QR, quinone reductase; MIC, minimum inhibitory concentration; OFT, open field test; BDNF, brain-derived neurotrophic factor; FST, forced swimming test; AuNPs, gold nanoparticles; IC_{50}, half-maximal inhibitory concentration; DPPH, 2,2-diphenyl-1-picrylhydrazyl; PM, phosphomolybdenum; FRAP, ferric reducing antioxidant power; MENF, methanol extracts of neem flowers.

Citation

Mohanasundaram P, Antoneyraj MS. A systematic review of neem flower (*Azadirachta indica*): a promising source of bioactive compounds with pharmacological and immunomodulating properties. *Tradit Med Res*. 2025;10(7):41. doi: 10.53388/TMR20241025001.

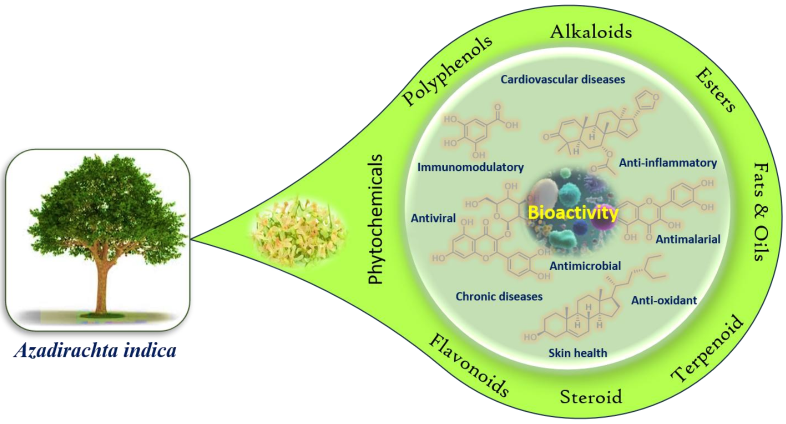
Executive editor: Jian Jia.

Received: 25 October 2024; **Revised:** 19 December 2024; **Accepted:** 11 March 2025; **Available online:** 12 March 2025.

Abstract

The neem flower (*Azadirachta indica*), a well-known element of Ayurvedic medicine, has attracted considerable interest due to its abundance of bioactive compounds. This systematic review examines its pharmacological and immunomodulatory properties in detail, emphasizing its potential role in contemporary healthcare. Neem flowers are rich in various bioactive components, such as flavonoids, terpenoids, and glycosides, known for their antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. Recent studies indicate that extracts from neem flowers can influence immune system activity by boosting both innate and adaptive immune functions while reducing excessive inflammation. These properties suggest that neem flowers could serve as valuable therapeutic agents for treating immune-related disorders. Additionally, neem flowers have demonstrated promise in addressing conditions such as diabetes, cardiovascular issues, and microbial infections through their ability to regulate metabolic functions and inhibit the growth of harmful pathogens. Preclinical research highlights their protective effects against oxidative damage and their capacity to induce apoptosis in cancer cells. However, challenges such as the lack of standardized extraction processes and limited clinical research hinder broader utilization. This review emphasizes the need for more robust studies to fully harness the pharmacological potential of neem flowers and facilitate their incorporation into evidence-based medicinal properties.

Keywords: neem flower; pharmacological activity; phytochemical investigation; immunomodulating property; secondary metabolites



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Highlights

The study opens up a critical review of the phytochemistry and pharmacology of the neem flower.

Discusses the complete pharmacological applications of neem flower.

A complete review of neem flower from 1975–2024 using PRISMA guidelines.

This is the first exclusive report on medicinal properties, bioactive compounds, and immunomodulatory properties of the edible part of *A. indica* A. Juss.

Medical history of objectives

Neem flowers have long been used in traditional medicine systems like Ayurveda, Siddha, and Unani for their therapeutic benefits. Kautilya's *Arthashastra* (4000 B.C.E.) is one of the earliest known texts documenting Neem's medicinal properties and its role in India's traditional healing practices. Similarly, *Brihat Samhita* by Varahamihira (600 C.E.) includes a section on plant-based remedies, advising the planting of Neem trees near homes for their benefits. Saralamp et al. documented their use in treating digestive issues in *Medicinal Plants in Thailand* (1996 C.E.). Neem flower extracts have also been shown to improve insulin sensitivity and lower blood sugar levels, as reported by Puri HS in *Neem: The Divine Tree Azadirachta indica* (1999 C.E.). Additionally, neem flowers are included in Ayurvedic formulations for liver detoxification and metabolic support, as noted in *Indian Materia Medica* (*Vol. 1*). These flowers exhibit various pharmacological properties, such as antioxidant, anti-inflammatory, and antimicrobial effects, which are widely practiced in folk medicine.

Introduction

Neem, or *Azadirachta indica*, is a plant from the Meliaceae family that has been used for medical purposes. The neem tree is known by various names across different languages, including Indian lilac (English), Azadirakhta (Persian), Margosa and Neeb (Arabic), Tamar (Burmese), Kohomba (Sinhala), Pokok Semambu (Malay), Dogon Yaro (Nigerian languages), Neem (Hindi and Bangla), Nimba (Sanskrit and Marathi), Arya-Veppu (Malayalam), Vaypum (Tamil), Bevu (Kannada), and Nimtree, Vepu, Vempu, Vepa (Telugu). Neem is a renewable resource that provides a variety of valuable domestic and agricultural products, such as cattle feed, medicines, soap, nitrification inhibitors, slow-release nutrient manure, fuel, energy, pest control, and more [1, 2]. A wide range of neem-based pest control and healthcare products have been developed internationally, many of which are now used in health and agriculture. Due to its diverse activity and relative safety for non-target organisms, neem is currently recognized as the most significant source of bio-pesticides and related medicinal products. The neem tree thrives in tropical and semi-tropical climates worldwide, and its various components, including seeds, leaves, flowers, and bark, are widely used for numerous applications. Different phytochemicals such as quercetin, azadirachtin, and liminoids like nimbin, nimbinin, and nimbidin have been isolated from various parts of the plant [3, 4]. Neem leaves contain various compounds, including nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, amino acids, and nimbiol. Neem oil, derived from *A. indica* seeds, possesses numerous medicinal and biological. Its antimicrobial and antioxidant properties make neem oil suitable for food preservation (Figure 1) [5].

Among all the parts of the neem tree, flower is the only edible part of the plant. Neem flower possesses numerous phytochemicals including quercetin, rutin, kaempferol, gallic acid, neeflone, nimbin, nimbidin, azadirone, melicitrin, myricetin which are responsible for its pharmacological activities. Since ancient days, neem flowers have been used as home remedies for gut microbiome improvement, skin diseases, anti-viral agents, and antipyretic agents. In traditional medicine, neem flower has been used to detoxify the liver and enhance its function. It is used in remedies to expel intestinal worms and other parasites. Neem flowers are applied in decoctions or pastes for their anti-inflammatory and antimicrobial properties, especially for skin conditions. Neem flower paste or extracts are applied to the scalp to promote healthy hair growth and treat dandruff, face masks to treat acne, reduce blemishes, and cleanse the skin [6–9]. Neem flowers are available only from February to April, and this was used in rituals. Since it is a seasonal flower, potential applications of neem flower were not studied well compared to other parts of neem. Although neem flowers hold great promise in medicine and wellness, research on their bioactive compounds is limited, leaving much of their therapeutic potential untapped. The lack of standardized methodologies often results in inconsistent findings, complicating efforts to build a cohesive understanding. Additionally, clinical trials evaluating the safety, efficacy, and pharmacokinetics of neem flower formulations are notably rare. To harness their full potential in pharmaceuticals, nutraceuticals, and cosmetics, it is crucial to address these gaps through comprehensive chemical studies, standardized protocols, and rigorous clinical validation.

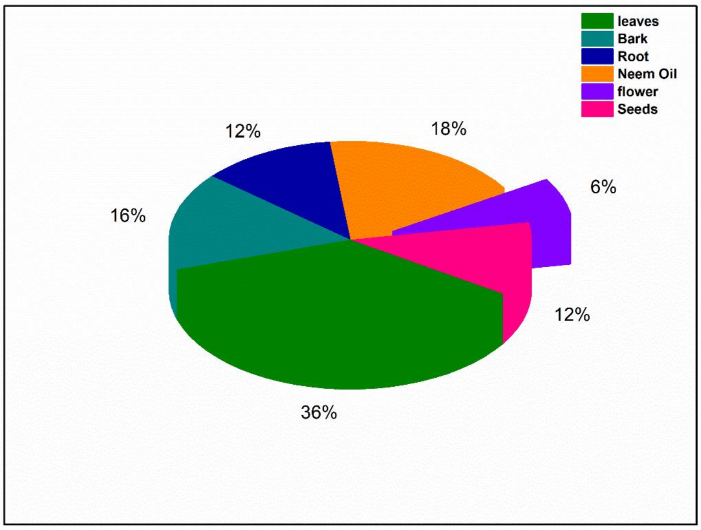

Figure 1 Research work carried out on different parts of the neem tree

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This review emphasizes the immunomodulatory properties and nanotechnology applications of bioactive compounds. While many existing studies explore the general therapeutic benefits of phytochemicals, this review specifically highlights how these compounds influence the immune system by modulating inflammatory pathways, cytokine production, and adaptive immune responses. This focus provides deeper insights into the role of bioactives like gallic acid, quercetin, and neem-derived compounds in managing immune-mediated disorders, including autoimmune diseases, allergies, and infections. Moreover, the review uniquely integrates the emerging role of nanotechnology in enhancing the delivery, stability, and bioavailability of these bioactive. This perspective not only bridges the gap between phytochemistry and advanced drug delivery but also opens new avenues for precision medicine applications in pharmaceuticals, nutraceuticals, and cosmetics. Neem flower research is uniquely poised to address global health challenges by developing accessible and cost-effective therapies. Advances in nanotechnology-based delivery systems can improve the bioavailability and targeted action of neem flower compounds, making treatments more efficient. Additionally, insights from traditional uses can inspire innovative applications, blending ancient wisdom with modern science. The focus on standardization, clinical trials, and safety profiling will be critical for translating neem flower bioactives into commercially viable and widely accepted therapeutic products. By exploring these avenues, neem flower research can significantly contribute to both preventive and curative healthcare solutions [10–14].

Comparative study on different parts of neem

The unique pharmacological activities of various parts of the tree, including its leaves, bark, seeds, flowers, fruits, and roots, are reservoirs of bioactive compounds like azadirachtin, nimbin, nimbolide, quercetin, and other limonoids, flavonoids, and terpenoids. These compounds contribute to neem's wide-ranging biological activities, including antimicrobial, antidiabetic, anti-inflammatory, antioxidant, hepatoprotective, antifungal, and anticancer effects. However, each part of the neem tree exhibits distinct pharmacological properties based on its specific chemical composition. Neem bark is known for its potent antimicrobial and anti-inflammatory properties, making it highly effective in managing infections and inflammation. Additionally, it exhibits anticancer and antimalarial potential, further enhancing its therapeutic applications. Neem leaves, on the other hand, are the most extensively studied and powerful part of the tree, offering notable antimicrobial, immunomodulatory, and antidiabetic effects. These properties make the leaves particularly beneficial for managing chronic conditions such as diabetes and skin ailments. In contrast, neem flowers are relatively underexplored due to their limited availability. However, they stand out for their mild yet balanced effects, which are especially advantageous for therapeutic applications.

Despite the wealth of traditional knowledge surrounding neem, the comparative pharmacological evaluation of its different parts remains essential. By comparing these properties, we aim to provide a comprehensive overview of neem's medicinal value and contribute to the evidence-based integration of this ancient remedy into contemporary medicine (Table 1). This exploration helps identify the most effective part for specific therapeutic applications, enabling the development of optimized neem-based formulations for modern healthcare. Moreover, understanding the distinct activities of various parts can shed light on potential synergies or complementary effects when used in combination. This review aims to shed light on the pharmacological properties and bioactive compounds found in neem flowers, encouraging further research to uncover their untapped biological potential.

Table 1 Comparison of important pharmacological activities reported on different parts of the neem tree

S. No	Study	Leaves	Bark	Seed	Oil	Flower
01	Antibacterial activity	✓	✓	✓	✓	✓
02	Antifungal activity	✓	✓	✓	✓	✓
03	Antibiofilm activity	✓	✓	✓	✓	✓
04	Neuroprotective activity	✓	✓	✓	✓	✓
05	Hepatoprotective activity	✓	❋	✓	✓	❋
06	Cardioprotective activity	✓	❋	✓	✓	❋
07	Antitumor activity	✓	✓	✓	✓	✓
08	Immunomodulatory activity	✓	✓	✓	✓	✓
09	Nephrotoxicity	✓	❋	✓	✓	❋
10	Antioxidant activity	✓	✓	✓	✓	✓
11	Free radical scavenging	✓	✓	✓	✓	✓
12	Cytotoxicity	✓	✓	✓	✓	✓
13	Antiviral activity	✓	✓	✓	✓	✓
14	Antidiabetic activity	✓	✓	✓	✓	✓
15	Antipyretic activity	✓	✓	✓	✓	✓
16	Wound healing activity	✓	✓	✓	✓	❋
17	Insecticidal activity	✓	✓	✓	✓	✓
18	Gastroprotective effect	✓	✓	✓	✓	✓
19	Antimalarial activity	✓	✓	✓	✓	✓
20	Antiparasitic effects	✓	✓	✓	✓	✓
21	Dermatological applications	✓	✓	✓	✓	✓
22	Hemostatic	✓	✓	✓	✓	❋
23	Antiulcer activity	✓	✓	✓	✓	❋
24	Analgesic/sedative	✓	✓	✓	✓	❋
25	Cardiovascular benefits	✓	✓	✓	✓	❋

❋ reports not available.

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It is evident that the neem flower is the under-explored part of the neem. From the literature, it is observed that only one review article focuses on the neem flower, but it does not cover all aspects of it [15]. There are about 31 articles which are published during the period of discussion about various benefits of different parts of the plant. The details of the review articles which are published in the recent past are given in Supplementary Table S1. Hence, this present literature review comprehensively discusses the complete phytochemical evaluation and pharmacological aspects of neem flowers. It also deals with the compounds that are isolated and identified for their immunomodulatory properties.

Botanical aspects and taxonomial classification

Azadirachta indica, or neem, is native to the Indian subcontinent and parts of Indochina, including Cambodia, Laos, Myanmar, Thailand, and Vietnam (Figure 2a). A member of the Meliaceae (mahogany) family, it is one of two species in the genus *Azadirachta* and has been widely introduced to tropical and subtropical regions globally (Table 2, Figure 2b).

Materials and methods

Literature search strategy – databases

We have used PRISMA guidelines for the literature search [16]. We systematically assessed the literature from Scopus, Web of Science, and Sci-finder from the year 1975 to 2024. All the literature with the keywords, neem flower, *Azadirachta indica* flower, and Pharmacological Application of Neem flower have been evaluated for this study (Figure 3–5).

Study design

The initial set of articles underwent a rigorous selection process. Titles and abstracts were first screened for relevance to the research questions, narrowing the pool to potentially relevant studies. These were then thoroughly reviewed to confirm their relevance and evaluate their quality.

Table 2 Taxonomical classification of neem tree

Kingdom	Plantae (Plants)
Sub-kingdom	*Tracheobionta* (Vascular plants)
Super-division	*Spermatophyta* (Seed plants)
Division	*Magnoliophyta* (Flowering plants)
Class	*Magnoliopsida* (Dicotyledons)
Sub-class	*Rosidae*
Order	*Sapindales*
Family	*Meliaceae* (Mahogany family)
Genus	*Azadirachta* A. Juss (*Azadirachta*)
Species	*Azadirachta indica* A. Juss (Neem)

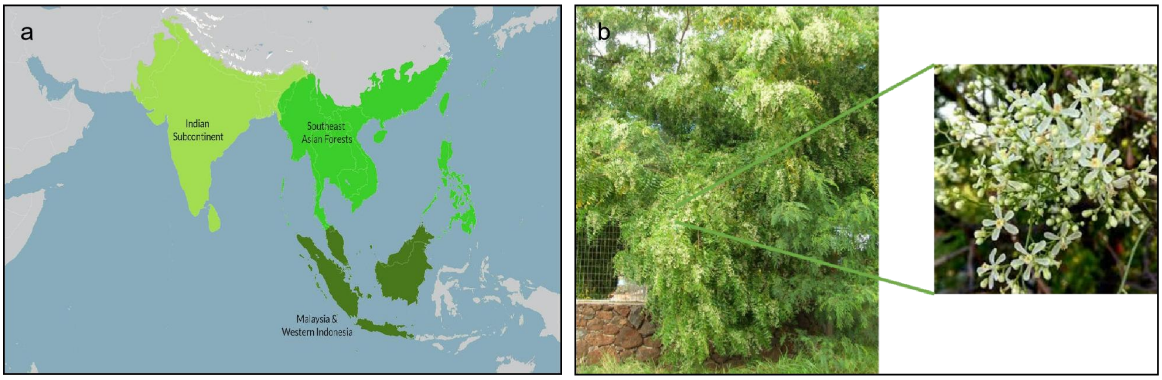

Figure 2 Dominant distribution of *Azadirachta indica* in the world map and image of neem flower. (a) Distribution of neem plant in Indian subcontinent and southeast asian countries (Image adapted from Google). (b) Image of neem tree and neem flower.

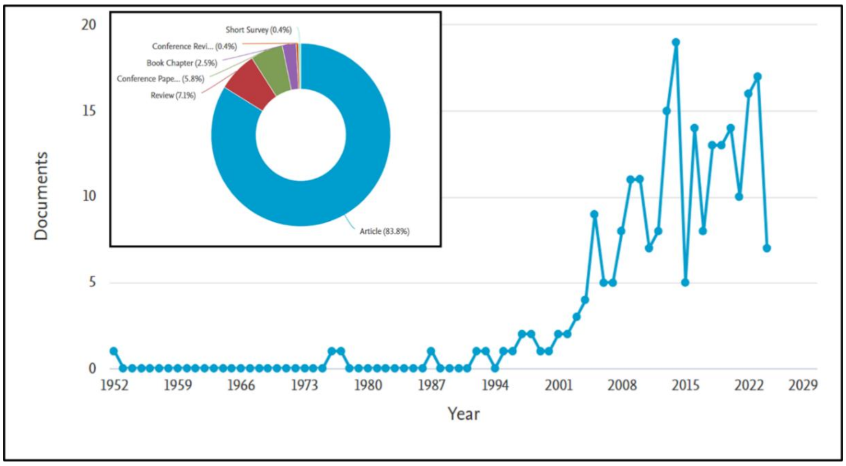

Figure 3 No. of publications per year (Source: Scopus)

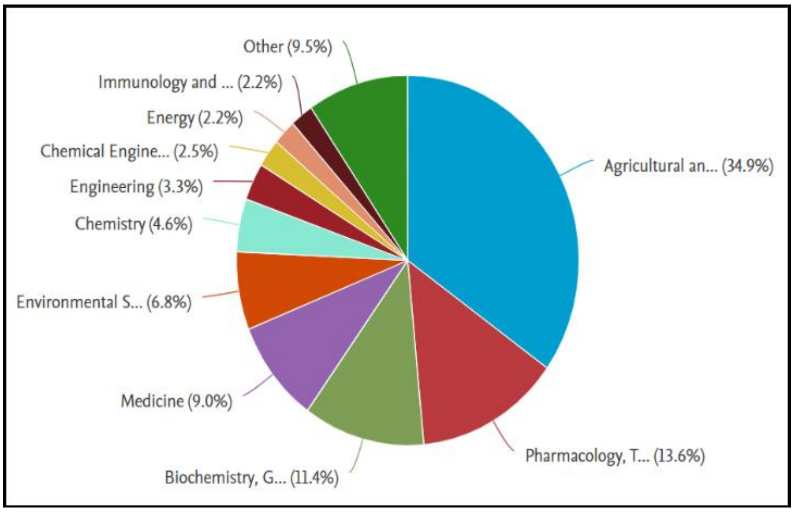

Figure 4 Different types of research work carried out on neem flower (Source: Scopus)

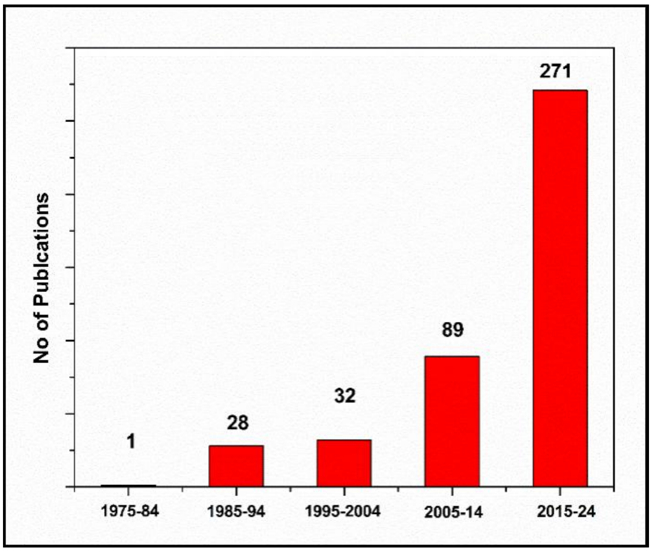

Figure 5 The year-wise publication of review articles. Source: Scifinder; Keywords: Neem flower, *Azadirachta indica* flower, pharmacology of neem flower.

Inclusion and exclusion criteria

Article titles, abstracts, and keywords were manually screened to exclude the articles which are not appropriate to the topic. We strictly restrict the articles that have Neem flower/*Azadirachta indica* flower/Pharmacological application of neem flower as either in abstracts or titles or in keywords (Figure 6).

Inclusion criteria. (a) Articles that primarily focus on neem flowers. (b) Articles discussing the pharmacological properties of neem flowers. (c) Articles that examine in-vivo and in-vitro studies involving neem flower extracts or isolated compounds. (d) Articles that clearly specify the concentration, dosage forms, and methodologies.

Exclusion criteria. (a) Articles not focused on neem flowers or providing minimal/no information on them; (b) Articles lacking clear context regarding methodologies and dosage forms; (c) Articles unavailable as full text; (d) Articles discussing neem flowers without supporting evidence; (e) Articles not aligned with the keywords used in the literature survey; (f) Articles that include additional materials alongside neem flowers in formulations.

Results and discussion

Phytochemical investigations on neem flower

Phytochemical screening is a process used in the field of pharmacognosy, which is the study of medicinal drugs derived from plants. It involves the identification and qualitative analysis of various chemical compounds present in plant extracts. These compounds are often referred to as phytochemicals and can have a range of biological activities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer properties, etc. The primary objective of phytochemical screening is to identify the presence of specific classes of compounds within a plant extract, which can provide insights into its potential pharmacological activities. Some common classes of phytochemicals that are frequently screened for include alkaloids, flavonoids, phenols, terpenoids, tannins, saponins, and glycosides [17]. Phytochemical screening plays a crucial role in the discovery of natural products for drug development, nutraceutical, and functional foods, contributing to the exploration of new sources of therapeutic agents from the plant kingdom. Investigation of neem flower extracts can provide further insight into the specific phytochemical composition and relative abundance of these compounds, facilitating the identification of potential bioactive molecules for further research and development of therapeutic applications.

Earlier reports on the phytochemical composition of neem flowers and other plant parts have identified the presence of phenols and flavonoids in aqueous extracts. However, the role of saponins in these extracts remains underexplored. Additionally, researchers in Thailand have analyzed bioactive compounds such as rutin and quercetin in neem flower extracts obtained through various preparation methods

[18, 19]. They also assessed the total phenolic and flavonoid contents, along with the free radical scavenging capacity, of the crude neem flower extract [20–22]. All these studies were carried out with respect to the crude extract obtained from neem flowers. The hydrocarbon content of the neem flower was determined [21]. The study showed that neem flower extract could be a cost-effective source of antioxidants with antimicrobial and larvicidal properties. Additionally, it was found to lower cholesterol by inhibiting intestinal absorption, suggesting its potential as a nutraceutical for preventing hypocholesterolemia [23, 24]. The constituents of natural products differ based on the time of harvesting, maturity of the plant, parts of the plant chosen, and also the place of collection. There are reports of seasonal variations affecting the composition of the plants as well. The total phenolic and flavonoid content of neem flower extracts using different solvents has been quantified. Based on these results it is evident that different solvents, geographical locations, and soil quality influence the concentration of bioactives in the neem flower (Table 3) [13, 25–29]. The literature highlights that the rutin and quercetin content in neem flowers varies significantly across different regions in Thailand [20]. A quantitative analysis was conducted on samples collected from 14 locations, revealing that factors such as geographical region, soil quality, and solvent type play crucial roles in determining the bioactive properties of plants. Similarly, the antioxidant activity of neem flower extracts has been evaluated (Table 4) [19, 20, 30–34]. From the literature, it is evident that there is a strong correlation between antioxidant activity and bioactive compounds such as phenols and flavonoids. The variations in the results show that different solvent and extraction methods play a vital

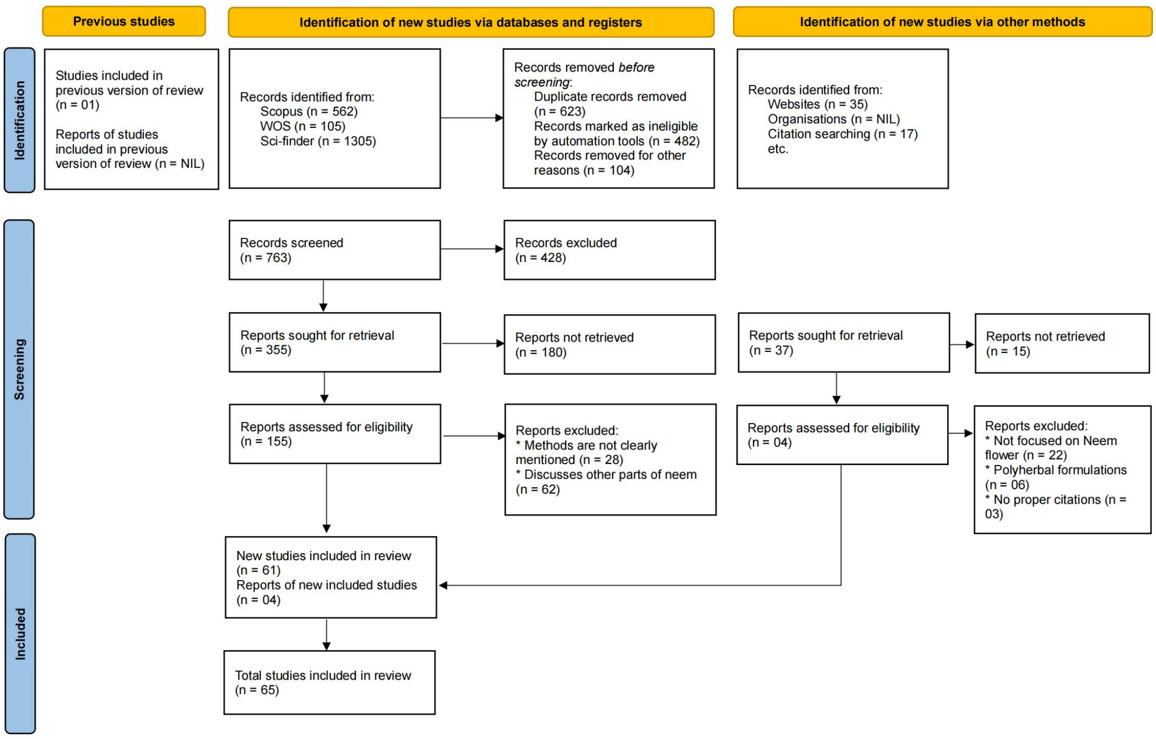

Figure 6 Representation of inclusion and exclusion criteria with response to PRISMA guidelines

Table 3 Variations in bioactive composition based on different solvents

S. No	Study	Solvent	Amount (μg/mL)	Reference
01	Total phenolic content	Methanol	23.73	[29]
			86	[25]
			62.05	[27]
		Water	50	[26]
			18.63	[28]
			15	[25]
		Ethanol	34	[25]
			85.44	[13]
		Pet. ether	3.56	[27]
		Acetone	7.52	
		80% alcoholic Aq	83.88	
02	Total flavanoid content	Water	412.04	[28]
		Pet. ether	1.56	[27]
		Acetone	11.25	
		80% alcoholic Aq	57.36	
		Methanol	20.56	

Table 4 Summary of the anti-oxidant activity of the neem flower extracts

S. No	Extract/compound	Species/method	Dose	Effect/inference	Reference
01	Flower powder	DPPH assay Reducing power assay	0.6 to 2 mg 4 to 32 µg	58.50% 78.53%	[30]
	Ethanol Water Methanol	DPPH assay	40, 80, 120, 160, 200 µg/mL	102 µg/mL < IC_{50} values 140 µg/mL	[32]
	50% EtOH (maceration) 50% EtOH (percolation) 50% EtOH (soxhlet) Water (decoction) Water (ultrasound 50% EtOH (ultrasound) Water (microwave)	DPPH assay	50–1,000 µg/mL	50.16 ± 1.32 µg/mL 39.14 ± 0.67 µg/mL 25.74 ± 0.37 µg/mL 11.36 ± 0.52 µg/mL 18.00 ± 0.31 µg/mL 33.90 ± 1.71 µg/mL 27.85 ± 1.40 µg/mL	[19]
	Water	DPPH	50–1,000 µg/mL	27–133 µg/mL	[20]
	Water	DPPH	100–500 µg/mL	26.29 µg/mL	[33]
	Pet. ether	DPPH, FRAP, PM H_2O_2, NO, Metal chelation	20–100 µg/mL 100–500 µg/mL	IC_{50} – 37, 76, 28 µg/mL IC_{50} – 178.02, 238.00, 76.00 µg/mL	[31, 34, 35]
	Acetone	DPPH, FRAP, PM H_2O_2, NO, Metal chelation	20–100 µg/mL 100–500 µg/mL	IC_{50} – 42, 41, 21 µg/mL IC_{50} – 102.35, 108.20, 51.56 µg/mL	[31, 34, 35]
	Hydroalchol	DPPH, FRAP, PM H_2O_2, NO, Metal chelation	20–100 µg/mL 100–500 µg/mL	IC_{50} – 29, 30, 18 µg/mL IC_{50} – 62.69, 48.20, 32.23 µg/mL	[31, 34, 35]
	Methanol	DPPH, FRAP, PM	20–100 µg/mL	IC_{50} – 40, 42, 34 µg/mL	[35]

DPPH, 2,2-diphenyl-1-picrylhydrazyl; PM, phosphomolybdenum; FRAP, ferric reducing antioxidant power; IC_{50}, half-maximal inhibitory concentration.

role in the extraction and quantification of the bioactive. The concentration of bioactive compounds in neem flowers is shaped by factors like location, soil, and seasons. Geographic differences in climate, altitude, and environmental conditions affect bioactive synthesis. Soil quality, including nutrients and pH, impacts metabolic processes in the plant. Seasonal variations, such as changes in temperature, humidity, and light, influence flowering and compound accumulation. These dynamic factors lead to variations in the consistency and effectiveness of neem flower-derived products [35–43].

Biological importance of bioactive compounds from neem flower. Neem flower bioactive compounds, such as flavonoids, terpenoids, and phenolics, are integral to health due to their antioxidant, anti-inflammatory, antimicrobial, and antifungal effects (Figure 7). They help strengthen the immune system, reduce oxidative stress, and may prevent chronic diseases. These bioactives also show potential in managing diabetes, improving lipid metabolism, and supporting digestive health. Their versatile therapeutic properties highlight their significance in traditional remedies and modern applications across pharmaceuticals, nutraceuticals, and cosmetics (Table 5) [44–69]. The structures of biologically important bioactive have been given in Figure 8.

Pharmacological evaluation of neem flowers

Research in India has shown that neem and its products are highly medicinal, often referred to as a "healer of all ailments". While many studies focus on its leaves, fruit, and bark, few examine neem flowers. A study at the CSIR-CFTRI Resource Center in Hyderabad reported that neem flower crude extract contains significant amounts of volatile oils and lipids and that the extracts were evaluated for their antioxidant potential [30]. One of the studies in Chengalpattu found that neem flower powder effectively treats worm infestations in preschool children (3–6 years) and offers a cost-effective treatment option [70]. In a separate study, flavonol glycosides were isolated from neem collected from the Thirumala Hills; however, the specific plant part used was not mentioned [71].

Anti-bacterial activity. Neem (*Azadirachta indica*) has a long history of use in various traditional medical systems, particularly in South Asia. The use of hot water extract from neem flowers and leaves internally as an anti-hysteric remedy suggests its potential calming or sedative effects, which might have been observed anecdotally over generations. In our laboratory, studied the bacterial activity of neem flower extracts against several clinically important pathogens, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, and *Shigella flexneri* [35]. The fact that the minimum inhibitory concentration (MIC) for these pathogens was as low as 50 µg/mL indicates potent antibacterial activity.

Bioactives can integrate into the lipid bilayer, disrupting membrane integrity and increasing permeability. This leads to the leakage of cytoplasmic contents, including ions, nucleotides, and proteins. Compounds such as phenolics and peptides interact with membrane lipids, causing structural disintegration, loss of membrane potential, and cell lysis (Figure 9). These mechanisms often act synergistically, with bioactive compounds combining membrane disruption, enzyme inhibition, and oxidative stress induction to exert potent antibacterial effects [72, 73].

Anti-fungal activity. Antifungal activity was conducted using *Candida albicans* and *Aspergillus fumigatus*. The two common fungal pathogens The MIC of 50 µg/mL suggests that neem flower extract effectively inhibits the growth of these fungi at relatively low concentrations, indicating its strong anti-fungal potency [35]. This finding underscores the therapeutic potential of neem flowers in combating fungal infections.

In another study by the same authors, the antifungal properties of hydroalcoholic extract of neem flower against five clinically important fungal pathogens were evaluated (Table 6) [35, 31, 74]. This study supports that neem flower could be a potent anti-fungal agent. *Candida albicans* is a yeast-like fungus that can cause various infections, including oral thrush and vaginal yeast infections, while *Aspergillus fumigatus* is a common airborne fungus that can cause invasive aspergillosis, particularly in immunocompromised individuals. The antifungal activity of neem flower extracts is due to bioactive compounds like nimbidin, nimbin, and other phytochemicals with established antifungal properties. These compounds may disrupt fungal cell membranes, inhibit fungal enzymes, or interfere with fungal replication, thereby preventing fungal growth.

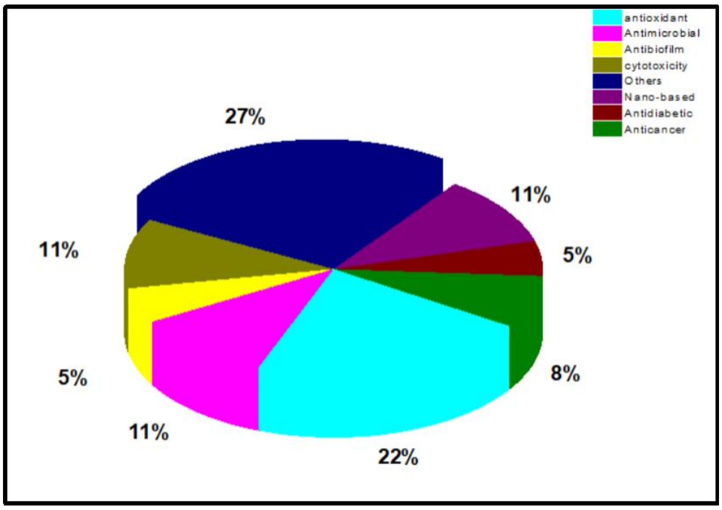

Figure 7 Various pharmacological activities reported on neem flowers

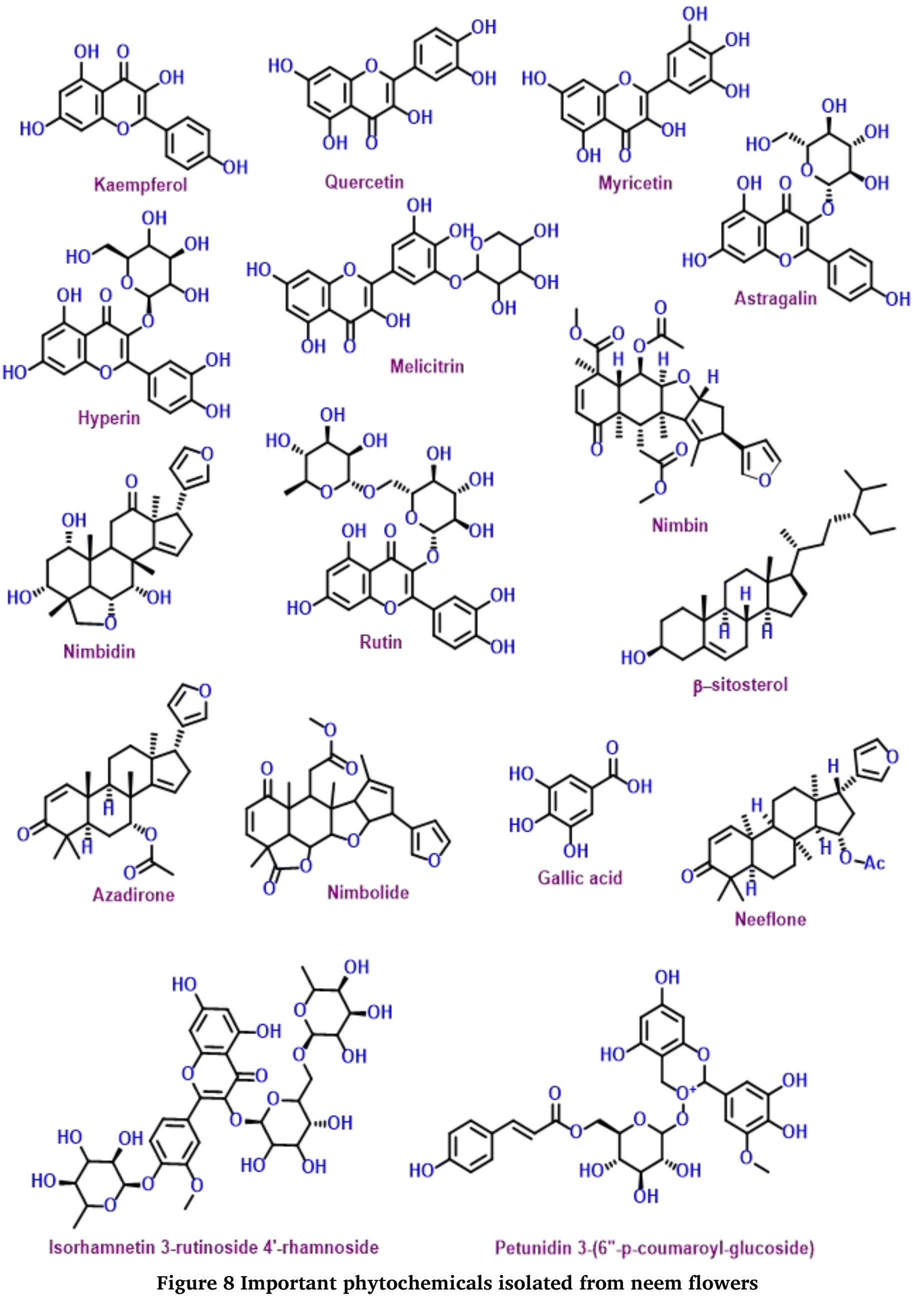

Figure 8 Important phytochemicals isolated from neem flowers

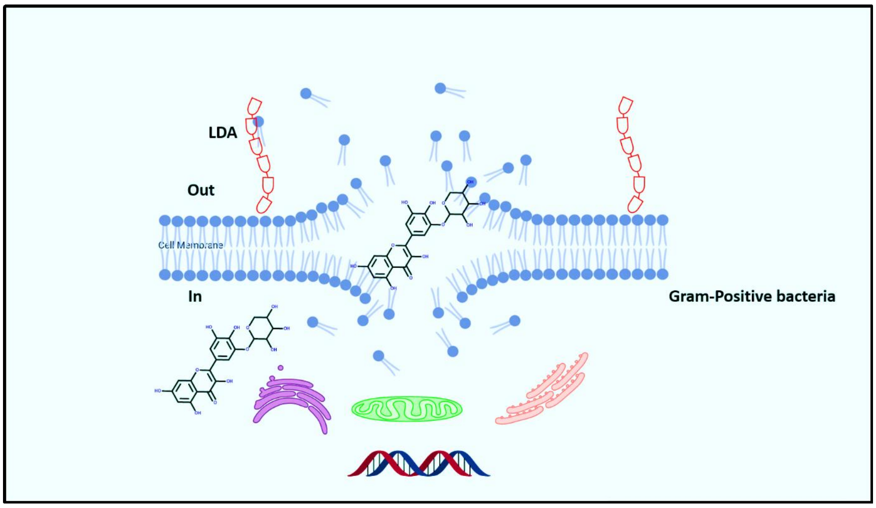

Figure 9 Diagrammatic representation of inhibition of bacterial cell through cell wall disruption

Table 5 Biological activities of some important phytochemicals derived from neem flower

S. No	Compound	Biological activity	Reference
01	Quercetin	Anti-microbial, anti-inflammatory and anticancer.	[44, 45]
02	Myricetin	Anti-oxidant, anti-cancer, neuroprotective effects, antidiabetic.	[28]
03	Kaempferol	Anti-oxidant, anti-inflammatory, anti-cancer, gut health.	[20, 46, 47]
04	Astragalin	Anti-microbial, anti-inflammatory, anti-diabetic and anticancer.	[48–51]
05	Melicitrin	Anti-microbial, anti-inflammatory, neuroprotective effects, and dermatological applications.	[52–56]
06	Hyperin	Cardiovascular diseases, neurodegenerative disorders, anti-cancer and anti-microbial.	[57, 58]
07	Nimbin	Anti-oxidant, anti-malarial and anti-inflammatory.	[59]
08	Nimbidin	Anti-inflammatory, hepatoprotective effects, anti-microbial and anti-cancer.	[60]
09	Rutin	Anti-inflammatory, antidiabetic, neuroprotective, anticancer, cardiovascular, and hepatoprotective properties.	[61–63]
10	β-sitosterol	Cholesterol management, cardiovascular diseases, chronic inflammation, and improving insulin sensitivity and glucose homeostasis.	[64–66]
12	Azadirachtin	Anti-malarial, hepatoprotective, and anticancer.	[67]
13	Gallic acid	Anti-inflammatory, anti-cancer, oxidative stress and anti-viral.	[68, 69]

Table 6 Summary of the microbial, biofilm, larvicidal, and infertility activity of the neem flower

S. No	Study	Extract/ compound	Species/method	Dose	Duration	Effect/inference	Reference
01	Anti-bacterial	Pet. ether Acetone Hydroalcoholic	*S. aureus*, *S. pyogenes*, *K. pneumoniae*, and *S. flexneri*	25, 50, 75, 100 μg/mL	–	50 μg/mL	[35]
02	Anti-fungal	Pet. ether Acetone Hydroalcoholic	*C. tropicalis, A. flavu, C. glabrata, C. parapsilosis, C. albicans, A. fumigatus*	25, 50, 75, 100 μg/mL	–	50 μg/mL	[31, 35]
03	Anti-biofilm	Pet. ether Acetone Hydroalcoholic	*S. aureus*, *S. pyogenes*, *K. pneumoniae*, and *S. flexneri*, *C. albicans, A. fumigatus*	25, 50, 75, 100 μg/mL	–	100 μg/mL (98%)	[35]
04	Anti-larvicidal	Pet. ether Acetone Hydroalcoholic	*An. Stephensi*, and *Ad. Aegyti*	0, 6.25, 12.5, 25, 50, 100 ppm	24 h	107.49 (93%), 142.88 (85%) ppm	[31]
05	Anti infertility	Alcoholic	Sprague-Dawley rats	1 g/kg	3 weeks	No anti-implantation, abortifacient, or teratogenic effects were observed.	[74]

Anti-biofilm activity. The study in our lab explored the anti-biofilm properties of neem flower extract against clinically significant bacterial and fungal strains [35]. The results revealed strong anti-biofilm activity with increasing concentrations of neem flower extracts. Specifically, at a concentration of 100 μg/mL, the inhibition of biofilm formation was observed to be equal to or greater than 98% (Table 6). This indicates that neem flower extract may have the ability to inhibit biofilm formation, which consists of resilient microbial communities resistant to antimicrobial agents and host defenses.

By disrupting biofilm formation, neem flower extract may offer a promising avenue for combating infections caused by bacterial and fungal biofilms, which are often associated with chronic and difficult-to-treat infections. Quorum sensing inhibition, also known as quorum quenching, refers to the enzymatic inactivation or disruption of quorum sensing signals. This mechanism interferes with microbial cell-to-cell communication by targeting autoinducer signaling molecules, their receptors, and downstream signaling pathways through quorum sensing inhibitory compounds (Figure 10). Quercetin has been shown to effectively inhibit biofilm formation and virulence factors in *Pseudomonas aeruginosa*. Additionally, it acts as a potent inhibitor of SrtA, significantly reducing biofilm formation in *Streptococcus pneumoniae* by suppressing sialic acid expression [75, 76].

Anti larvicidal activity. Anti-larvicidal activity of neem flower extracts was investigated by our group against *An. Stephensi*, and *Ad. Aegyti* [31]. The LC_{50} and LC_{90} values were calculated at 24 h for hydroalcoholic extract of neem flower. According to this study, as the concentration increases, the percentage of mortality increases (Table 6). This is due to the presence of metabolites which are present in it. This trend was further supported by the previous works of literature that show the phytochemicals that possess anti-larvicidal activity.

Anti-infertility activity. The research was conducted on investigating the antifertility activity of neem flower extracts in Sprague-Dawley rats, by administering an alcoholic extract of neem flower was found to disrupt the estrous cycle in the rats (Table 5) [74]. Additionally, the extract caused a partial block in ovulation. These findings suggest that neem flower extract has the potential to develop into a female contraceptive. By disrupting the estrous cycle and partially inhibiting ovulation, the extract may offer a means of preventing pregnancy. This highlights the potential of neem flower extract as a natural option for contraception. However, additional research is required to fully understand its mechanism of action and potential side effects before it can be considered for human use.

Anti-oxidant activity. Narsing Rao et al. analyzed shade-dried neem flower powder (NFP) for composition, fatty acids, volatile oil, and antioxidant activity. NFP contained 17.34% protein, 12.32% fiber, 9.16% ash, and 0.07% volatile oil (85% caryophyllene). The 12% lipid content was evenly split between saturated and polyunsaturated fatty acids [30]. The results indicate that the volatile fraction and lipids from neem flowers could be further investigated for pharmaceutical or food applications. A study on the antioxidant activity of neem flowers and seed oil found that ethanolic extracts at 200 mg/mL exhibited the highest free radical scavenging activity, with flowers at 64.17 ± 0.02% and seed oil at 66.34 ± 0.06%. Neem oil also had the highest total phenol content (132 mg/mL), contributing to significant DPPH radical inhibition. Overall, neem flower and seed oil show potential for human health applications, including use in food, diabetes management, and various pharmaceutical industries [32]. From the literature, it is noted that the young leaves and flowers of the Siamese neem tree are commonly consumed as a bitter tonic which has active secondary metabolites such as the flavonoids rutin and quercetin [19, 20]. EC_{50} of the scavenging activity of the extracts was found to be 27–133 mg/mL and no sign of toxicity. These results show the good scavenging ability of neem flowers. In another study, the authors Ram Bindurani and Kamlesh Kumar investigated the antioxidant activity of different parts of the neem tree. From the results, it is noted that neem flower extract shows less antioxidant activity than other parts. This might be due to lower phenolic content [33]. The antioxidant activity of neem flowers has been investigated in our lab by using DPPH assay, Phospomolybdenum assay, FRAP assay, Hydrogen peroxide assay, NO-scavenging assay, and metal chelating activity. From the results, it is observed that hydroalcoholic extract possesses the lowest half-maximal inhibitory concentration (IC_{50}) value which supports the higher antioxidant activity. This may be due to the phytochemicals such as polyphenols, flavonoids, and terpenoids present in major amounts, and other constituents viz., carbohydrates and amino acids [31, 34, 35]. This is further evident in the correlation analysis which was studied between the antioxidant activity and respective phenolics and flavonoids of different extracts (Figure 11).

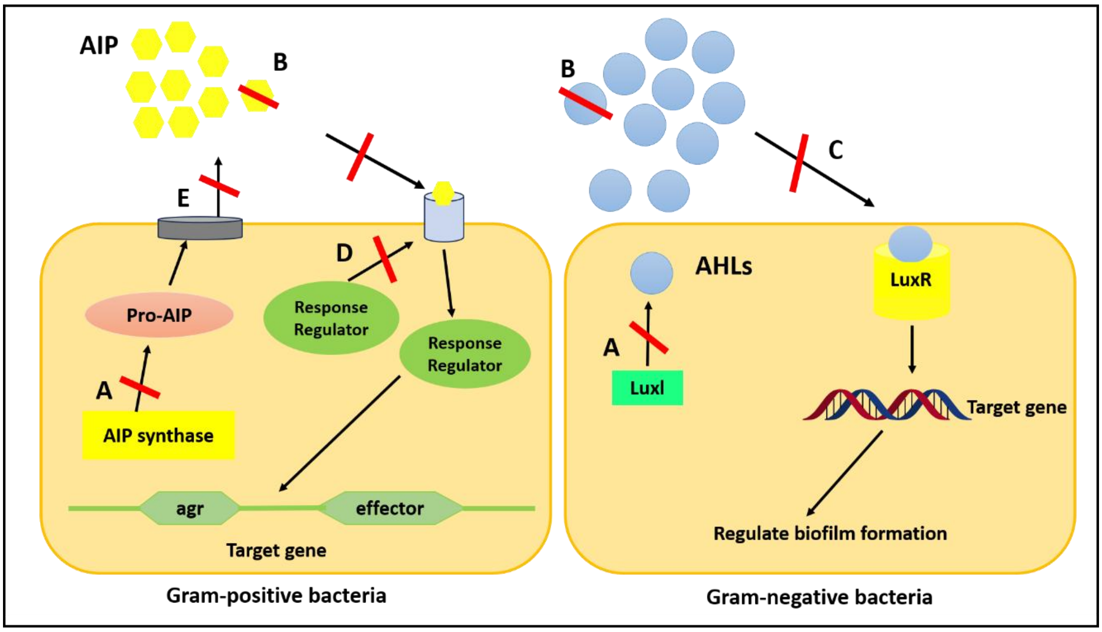

Figure 10 Mechanism of quorum sensing inhibition against biofilm formation. Adapted and modified from Asma Behzadnia et al., 2024 (https://creativecommons.org/licenses/by/4.0/) [76]. A, Inhibition of non-diffusible autoinducing peptides (AIP); B, Degrading of AIPs; C, Interference of signal receptors by antagonists; D, Interference of response regulators; E, Inhibition of cell-to-cell signaling. AIP, autoinducing peptide.

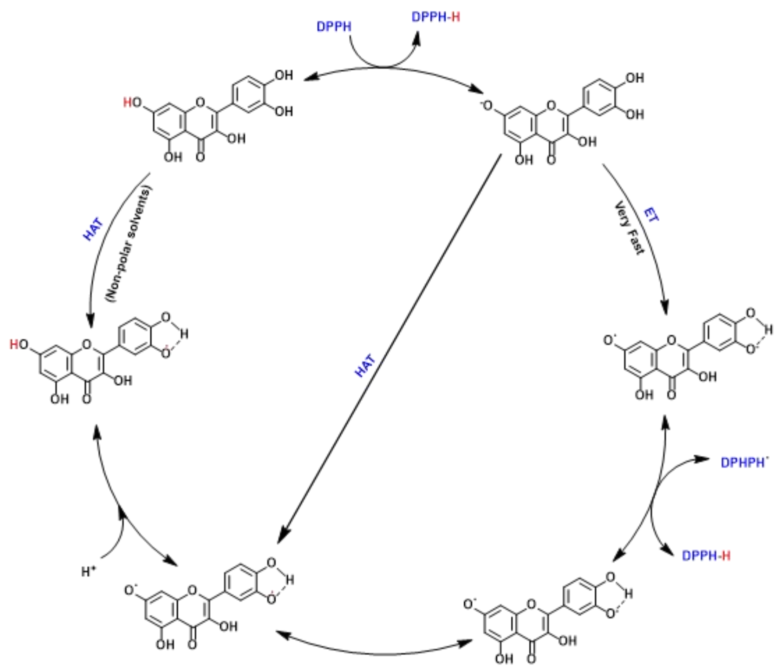

Figure 11 Possible mechanisms for quercetin flavonoid with DPPH radicals [77] . ET, electron transfer; HAT, hydrogen atom transfer.

Neuroprotective activity. The neuroprotective activity of neem flowers on rats subjected to prolonged stress has been investigated by Hawiset et al [78]. In this study, restraint stress was induced in the rats, and neem flower extracts were administered to evaluate their cognitive enhancing effects. Cognitive function was evaluated using the novel object recognition test, while spontaneous locomotor activity was assessed with the open field test (OFT). Brain and blood samples were analyzed for brain-derived neurotrophic factor (BDNF), cortisol levels, and neuron survival. Stressed rats treated with donepezil and neem flower extract showed significantly higher BDNF and neuron survival, and lower cortisol levels compared to the control. Neem flower extract may enhance cognitive function and protect against stress-induced neuronal damage, suggesting neuroprotective potential (Table 7). The BDNF/TrkB signaling pathway is crucial for neurogenesis and synaptic plasticity in the central nervous system. Recent studies indicate that BDNF is involved in cognitive processes, particularly in memory acquisition and consolidation. Recent studies have demonstrated that intracellular signaling associated with the BDNF/TrkB pathway regulates glucocorticoid receptor activity. High levels of glucocorticoids suppress BDNF expression, leading to a reduction in neurogenesis. Quercetin present in *Azadirachta indica* flower extract may help safeguard neurons from damage caused by chronic stress. Quercetin and its metabolites have been shown to have a neuroprotective effect [79, 80].

Chemopreventive agents. The chemopreventive potential of neem flowers, along with a few vegetables, was evaluated by WH Kusamran in 1998 [81]. Diets containing 12.5% neem flowers and Thai bitter gourd significantly increased GST activity after two weeks. This suggests that both contain monofunctional phase II enzyme inducers and compounds that inhibit certain monooxygenases involved in activating chemical carcinogens, indicating their potential chemo-preventive properties. In continuation of this work, the investigation of inhibitory effects of neem flowers on mammary gland carcinogenesis induced by 9,10 dimethyl-1,2-benzanthracene (DMBA) in female Sprague Dawley rats, as well as on hepatocarcinogenesis induced by aflatoxin B1 (AFB1) in male Wistar rats were evaluated [82, 83]. The results of their research show that flowers contain chemo-preventive agents capable of inhibiting both AFB1-induced liver carcinogenesis and DMBA-induced mammary gland carcinogenesis in rats. This suggests that neem flowers may possess compounds with anti-carcinogenic properties, which could potentially be utilized in the development of preventive or therapeutic interventions against liver and mammary gland cancer. These findings highlight the potential health benefits of neem flowers and underscore the importance of further research to identify and characterize the specific chemo-preventive agents present in neem flowers, as well as to elucidate their mechanisms of action.

Quinone reductase (QR) inducing property. Nimbolide and chlorophyll as QR inducers were isolated from neem flowers and characterized using spectroscopic techniques and assessed their QR activity in hepa 1c1c7 cells. QR induction was determined by comparing the enzyme-specific activities of cells treated with the compounds to those of control cells. The results indicate that both nimbolide and chlorophyll may play an important role in the chemopreventive activity of neem flower extracts. This suggests that these compounds could potentially be utilized in the development of preventive or therapeutic interventions against certain diseases, given their ability to induce QR activity, which is associated with detoxification and protection against carcinogens [82].

Cholesterol lowering activity. Acharaporn et al. investigate the cholesterol absorption and intestinal motility in rats using extracts of neem flower [84]. They discovered that *A. indica* ethyl acetate extract enlarges cholesterol micelles, whereas aqueous, myricetin, and quercetin decrease cholesterol solubility within micelles. Both the extract and quercetin inhibited KCl-induced contractions by 29% and 18%, respectively, and also reduced $CaCl_2$-induced contractions. These results support the traditional use of *A. indica* for lowering cholesterol and regulating gastrointestinal motility. Also, the same authors, analyzed the cholesterol-lowering effect of neem flower extracts interfering with NPC1L1 and micellar properties in vitro and intestinal Caco-2 cells [85]. The prepared cholesterol micelles were introduced into the Caco-2 cell lines and the uptake of the micelles was evaluated. Also, the effect of cholesterol synthesis in cells by *A. indica* extracts was estimated by HMG-CoA reductase activity. According to the results, *A. indica* extracts inhibit cholesterol intake without any toxicity. Also, the expression of NPC1L1 was inhibited. This might be due to the presence of neem flower extract consequently inferes the cholesterol intake. From the HMG-CoA assay, it is evident that neem flower extract directly inhibits the 3-hydroxy-3-methylglutaryl-CoA reductas (HMGR) activity resulting in endogenous cholesterol synthesis.

Anti-diabetic activity. The in-vitro antidiabetic activity of gold nanoparticles (AuNPs) synthesized by utilizing neem flower extract as a reducing agent was evaluated [86]. The synthesized AuNPs were evaluated for their inhibition of α-glucosidase and α-amylase, key enzymes in carbohydrate digestion. The study results demonstrated that the nanoparticles effectively inhibited both α-glucosidase and α-amylase enzymes. The IC_{50} values obtained were 45.72 μg/mL for α-amylase inhibition and 58.74 μg/mL for α-glucosidase inhibition. These findings suggest that the neem flower-induced synthesis of AuNPs holds potential as a natural approach for managing diabetes by regulating carbohydrate metabolism through the inhibition of key digestive enzymes involved in glucose absorption. The hyperglycaemic activity of neem bark and neem flower has been evaluated [87]. Albino mice were introduced for this study. Blood glucose levels in both normal and streptozotocin-induced diabetic mice decreased significantly. The study findings indicate that neem flowers exhibit greater hypoglycaemic activity compared to neem bark.

Clastogenic and anti-clastogenic activity. The clastogenic and anticlastogenic effects of neem flower extract were assessed using a rat liver micronucleus assay [85]. Methanol extracts of neem flowers (MENF) were administered orally to male Sprague Dawley rats, and the incidence of micro nucleated hepatocytes was assessed using fluorescence microscopy. The results indicated that MENF did not induce clastogenic effects in rat hepatocytes. Instead, at higher doses, MENF significantly reduced micronucleus formation in rat livers compared to the control group. Thus, it was concluded that MENF at doses of 100 and 500 mg/kg body weight showed no clastogenic effects and demonstrated anticlastogenic potential, especially at higher doses (Table 7) [78, 81–86, 88–94].

Anthelmintic activity. Rani et al., have investigated the anthelmintic activity of neem flower extracts on earthworms (*Pheretima posthuman*) [89]. Different concentrations of plant extract were administered to earthworms, and paralysis and death time were observed. From the results, it is observed that pet ether extract shows a higher mortality rate and lower paralysis time i.e., 40 μg/mL (Table 7).

Antitubercular activity. The antitubercular activity of neem flower ethanol extract was evaluated against the standard drug rifampicin [90]. From the result, they have concluded that the ethanol extract of neem flower shows 25 μg/mL of MIC whereas the standard drug shows 12.5 μg/mL (Table 7).

Skeletal muscle activity. The skeletal muscle activity of neem leaves and flowers has been investigated with acetylcholine on frogs [91]. In this study, the authors reported the individual muscle response when

Table 7 Summary of the biological activity of the neem flower

S. No	Study	Extract/compound	Species/method	Dose	Duration	Effect/inference	Reference
01	Chemopreventive activity	12% neem flower	Male Wistar rats	12%	2 weeks	Increase in phase II enzymes.	[81]
		Methanol	Female Sprague Dawley rats; Male Wistar rats		20 weeks	35.2% (mammary gland), 61.7% (liver gland)	[82]
						80.1% (liver gland)	[83]
02	QR inducer	Pet ether Chloroform Ethyl acetate Methanol Nimbolide Chlorophyll	Hepa 1c1c7	–	–	20.3 μg/mL 8.3 μg/mL 32.5 μg/mL > 50 μg/mL 0.42 μg/mL > 25 μg/mL	[82]
03	Cholesterol-lowering activity	Aqueous (NF-A) Ethyl acetate (NF-E) Insoluble (NF-G)	Male Wistar rats	100 μg/mL	–	14.54 ± 3.34 7.94 ± 6.52 0.33 ± 2.16	[84]
		Methanol	Human Caco-2 cells	12.5–100 μg/mL	3, 24, 48 h	33%	[88]
04	Anti-diabetic activity	50% ethanol extract	Male albino mice	10 mg/mice	72 h	3 h – 54.39% 6 h – 70.21%	[87]
05	Clastogenic and anticlastogenic activity	Methanol	Male Sprague Dawley rats	100, 500 mg/kg of bw	6 weeks	No clastogenic @ 100, 500 mg/kg, anticlastogenic @ higher doses.	[85]
06	Anthelmintic activity	Pet ether	Indian earthworm (*Pheretima posthuma*)	10, 20, 40 mg/mL	–	For 40 mg/mL Paralysis @ 9 min (< std); Death @ 13 min (<std).	[89]
07	Antitubercular activity	Ethanol	*M. tuberculosis*	0.79, 1.56, 3.125, 6.25, 12.5, 25, 50 μg/mL	–	MIC – 25 μg/mL.	[90]
08	Skeletal muscle activity	Water	Frogs (species not mentioned)	1, 10, 100 μg/mL	–	Muscle activity increases.	[91]
09	Neuroprotectivity	Water	Male Wistar rats	250, 500, 1,000 mg/kg of bw	31 days	Increases in BDNF levels preventing neural loss on chronic stress.	[78]

Table 7 Summary of the biological activity of the neem flower (*continued*)

S. No	Study	Extract/compound	Species/method	Dose	Duration	Effect/inference	Reference
10	Anxiolytic and antidepressant-activity	Water	Male Wistar rats	250, 500, 1,000 mg/kg of bw	31 days	EMPT method shows an anxiolytic effect. The FST method shows antidepressant activity.	[92]
11	Melanogenesis inhibitory	Methanol Ethyl acetate soluble Water soluble	B16 mouse melanoma cell line	10 & 100 μg/mL	–	78.7%, 20.6% 20.9%, 4.3% 79.8%, 71.8%	[93]
	Cytotoxicity	Methanol Ethyl acetate soluble Water soluble	HL 60 A549 AZ521 SK-BR-3 HL 60 A549 AZ521 SK-BR-3 HL 60 A549 AZ521 SK-BR-3	(1×10^{-4} to 1×10^{-6} g/mL)	48 h	30.9 μg/mL > 100 μg/mL > 100 μg/mL > 100 μg/mL 66.7 μg/mL 72.9 μg/mL 22.3 μg/mL 73.3 μg/mL > 100 μg/mL > 100 μg/mL > 100 μg/mL > 100 μg/mL	
		Isoazadironolide, 4'-O-methyl-8-prenylnaringenin, euchrestaflavanone A, 3-methoxy-3'-prenylnaringenin	HL 60, A549, AZ521, SK-BR-3	(1×10^{-4} to 1×10^{-6} g/mL)	48 h	4.5–9.9 μM	[94]

BDNF, brain-derived neurotrophic factor; EMPT, elevated plus maze test; FST, forced swimming test; MIC, minimum inhibitory concentration; QR, quinone reductase.

testing drugs (leaves and flower extract) and standard drugs. It is noted that the standard drug shows higher muscle activity followed by leaves and flowers. Notably, when the concentration increases the skeletal muscle activity of leaves and flowers also increases, since it is lower than the control group. Further, they have evaluated the muscle activity of combination test drugs (Acetylcholine + neem leaves, acetylcholine + neem flowers). Herein, acetylcholine-induced leave extract shows higher skeletal muscular activity in higher concentrations than flowers.

Anxiolytic activity and antidepressant-like activity. A study conducted by Hawiset T et al. investigated the anxiolytic and antidepressant-like activities of neem flowers (Table 7) [92]. Behavioral assessments were conducted using the elevated plus-maze test, the forced swimming test (FST), and the OFT. In the elevated plus-maze test, both the control group and the neem flower-treated group exhibited good activity. However, no significant results were observed in the FST and OFT. Additionally, dopamine and serotonin levels in the brain were higher in the treated groups compared to the control group. The neem flower extract also showed no toxicity in rat liver. As a preliminary study, these findings will aid future research.

Anti-cancer activity. The anti-cancer activity of the neem flower was analyzed. Neem flowers contain monofunctional phase II enzyme inducers and compounds that can inhibit certain monooxygenases, particularly those involved in the metabolic activation of chemical carcinogens. A diet including 12.5% neem flowers fed over 2 weeks significantly increased GST activity by nearly 2.7-fold and notably reduced phase I reaction levels. According to the literature limonoids and flavonoids from the neem flower possess to have anti-cancer properties [93]. The study found that flavonoids isolated from Siamensis neem flowers inhibit melanogenesis in B16 melanoma cells. Additionally, their cytotoxic effects were evaluated against HL60, A549, AZ521, and SK-BR-3 human cancer cell lines, showing promising cytotoxic activity within the range of 4.5–9.9 μM (Table 7).

Quercetin (Qu) regulates the cell cycle by interacting with molecular targets, blocking progression at G2/M or G1/S depending on the cell type and tumor origin. At the G1/S transition, Qu upregulates p21, p27, and p53. p21 inhibits CDKs, particularly CDK2-cyclin E, preventing phosphorylation of pRb and E2F1 activation, thereby halting S-phase entry. It also inhibits CDK2-cyclin A and CDK1-cyclin B, essential for S-phase and G2 progression. p27 can inhibit CDK4/6-cyclin D complexes under specific conditions. p53, activated by Qu, induces growth arrest and apoptosis by upregulating p21 and p27. Qu stabilizes p53 at both mRNA and protein levels. Additionally, Qu promotes apoptosis via the mitochondrial pathway by disrupting mitochondrial membrane potential, leading to cytochrome c release and activation of caspases, including caspase-3 and caspase-7 (Figure 12) [95].

Acute and subacute toxicity studies. Acute toxicity was assessed by administering MENF at doses of 6, 9, and 12 g/kg body weight. For subacute toxicity testing, MENF, suspended in 0.5% tragacanth, was given at doses of 150, 750, and 1,500 mg/kg body weight for 90 consecutive days [94]. The results indicated that the LD_{50} value of the methanol extract is higher than 12 g/kg body weight (Table 7). In the subacute toxicity study, the methanol extract significantly reduced growth in male rats but not in female rats, and it significantly increased relative liver weights in rats receiving 750 and 1,500 mg/kg body weight. Histopathological analysis revealed elevated blood chemical levels in female rats, followed by male rats and control groups.

Immunomodulatory activities of neem flower

This present scenario of a highly polluted environment and an increasing number of diseases demands need-based research concerning human health. From the literature, it is understandable that, rather than identifying and developing a drug to fight against the diseases it is worthwhile to educate each human to develop an immune system within the body. This is generally achieved through a healthy lifestyle, especially through a healthy diet. In this context, the neem flower with its excellent antioxidants and its secondary metabolites such as flavonoids and phenolics can be a promising source of a natural immunomodulating compound. Several studies have investigated the immunomodulatory effects of Neem flower extracts, revealing promising results:

Enhancement of immune response. The studies have suggested that Neem flower extracts possess immunostimulatory properties, meaning they can enhance the activity of the immune system [96–98]. This may involve increased production of certain immune cells or cytokines that play a role in immune defense. Neem oil from flowers was also found to have immunomodulating properties [99].

Anti-inflammatory effects. Neem flower extracts have also been found to exhibit anti-inflammatory activity. By reducing inflammation, these extracts may indirectly modulate the immune response, as inflammation is closely linked to immune function.

Regulation of immune cell function. Neem flower extracts may influence the function of specific immune cells, like macrophages, lymphocytes, and dendritic cells. These effects can vary depending on the concentration and formulation of the extract used.

Antioxidant properties. Additionally, Neem flower extracts are known to possess antioxidant properties, which can help protect immune cells from oxidative damage and support overall immune function.

Potential therapeutic applications. Based on these immunomodulatory properties, Neem flower extracts are being investigated for their potential therapeutic applications in various immune-related disorders, such as autoimmune diseases, allergies, and infections [100, 101]. It's important to note that while preliminary research suggests the immunomodulatory activity of Neem flower extracts, further studies, including clinical trials, are needed to fully understand their mechanisms of action and potential therapeutic benefits. Additionally, the efficacy and safety of Neem-based treatments should be carefully evaluated before widespread clinical use. Indeed, the scarcity of studies specifically focusing on the isolation of bioactive constituents from Neem flowers is notable, especially considering their potential health benefits, including hepatoprotective and immunomodulating properties. While there is extensive research on other parts of the Neem tree, such as the leaves, seeds, and bark, the flowers have received comparatively less attention in scientific literature. Also, Neem flower extracts hold significant promise in managing autoimmune disorders, chronic inflammation, and infections due to their rich array of bioactive compounds, including flavonoids, terpenoids, and glycosides. Neem flower bioactives can regulate immune responses, reducing excessive inflammation and preventing immune cells. The compounds in neem flowers help modulate pro-inflammatory and anti-inflammatory cytokines, potentially mitigating the overactive immune response characteristic of autoimmune conditions. It also inhibits inflammatory mediators such as cyclooxygenase (COX) enzymes and reduces the release of cytokines like interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α). These properties are valuable in managing chronic conditions like arthritis, and inflammatory bowel disease [102–104].

Neem flowers possess immunomodulatory properties by regulating cytokines and macrophage activity, largely due to their bioactive compounds, including flavonoids, terpenoids, and phenolics. They suppress pro-inflammatory cytokines like IL-6 and TNF-α by inhibiting NF-κB, reducing inflammation linked to chronic or autoimmune conditions while enhancing anti-inflammatory cytokines like IL-10 to promote immune balance. Neem bioactives boost macrophage phagocytosis, facilitate M2 polarization for tissue repair, and suppress the pro-inflammatory M1 phenotype. Additionally, they regulate reactive oxygen species and nitric oxide production, balancing pathogen destruction with oxidative stress prevention. These effects highlight neem flowers' potential in managing immune-related disorders and merit further research.

To expand the applications of neem flower bio actives and derived products, efforts should focus on developing neem flower-based immunomodulators, anti-inflammatory medications, and topical formulations for managing infections. Additionally, neem flower extracts hold potential for inclusion in dietary supplements aimed at boosting immune function and reducing systemic inflammation. These bioactives can serve as complementary therapies, enhancing the efficacy of existing treatments for autoimmune disorders, chronic inflammation, and infectious diseases. Rigorous research, encompassing clinical trials and advanced formulation studies, is crucial to transform these possibilities into practical and effective therapeutic solutions [105, 106].

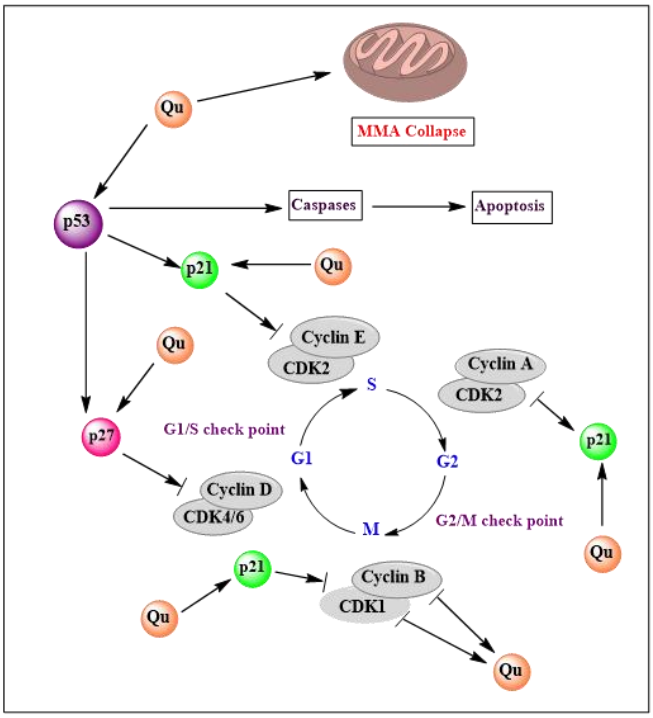

Figure 12 Mechanism of action of quercetin on anticancer

Nano material-based pharmacological application of neem flower
Nanotechnology has attracted many researchers from almost all fields of science. Due to the promising biological applications, the synthesis of nanoparticles is being attempted by many researchers by various approaches. In this context, bio-mediated synthesis of nanomaterial is widely appreciated in green chemistry. This involves the use of biomass/biowaste as the natural reducing, capping, and stabilizing agents in the biosynthesis of nanomaterial. Countless reports are available in the literature that exploit bioderived products (whole plant, flower, seed, root, stem, etc.) for nanomaterial synthesis. In line with this, neem flowers were also experimented for this study. The study was conducted which involved the synthesis of gold AuNPs using the aq. extract of neem flower. The optimization of the synthesis process included varying parameters such as pH, temperature, extract volume, and Au (III) concentrations. Characterization of the synthesized AuNPs was performed using techniques such as UV-visible spectral analysis, HRTEM, and XRD which confirmed the presence and characteristics of the AuNPs. Furthermore, the antibacterial activity of the synthesized AuNPs was investigated, yielding promising results [107]. Additionally, the authors explored the antimicrobial properties of Ag-nanoparticles (NPs) against *S. aureus* and *E. coli* [108]. Tetracycline was utilized as a standard drug for comparison purposes. The MIC of the synthesized AgNPs was calculated as 100 μg/mL, indicating their effectiveness against the tested bacterial strains. Moreover, the zone of inhibition exhibited by the AI-Ag NPs was comparable to that of the standard drug tetracycline, further demonstrating their antimicrobial potential. Also, they have investigated the anti-oxidant potential of Ag NPs, and it was found that the radical scavenging activity of neem flower extract is lower than the synthesized nanomaterial. The synthesis, characterizations, and antibacterial activity of Cu NP were reported by the same authors [109]. They have investigated the anti-bacterial properties against 4 different pathogens, and the MIC was reported to be 100 μg/mL. In continuation with this, the authors were trying to explore the anti-oxidant and invitro-antidiabetic activity of Au, Ag, and Cu NPs [86, 110]. They have investigated antioxidant activity by DPPH, ABTS, NO Scavenging, and OH radical scavenging methods. Among all the three NPs, Cu NPs show higher inhibition activity than Au and Ag NPs. From the anti-diabetic activity, it came to know that Cu NPs show good enzyme inhibition ability (Table 8) [86, 108–111].

Nanoparticles enhance the stability, absorption, and targeted delivery of bioactive compounds by encapsulating them in protective systems. These systems shield bioactives from degradation caused by environmental factors like heat, light, or oxidation while improving their solubility for better biological uptake. They can also allow for controlled, site-specific release, reducing side effects and increasing therapeutic efficiency. Research with compounds like curcumin shows that nanoparticles can enhance both stability and effectiveness, indicating promising applications for neem flower bioactives in pharmaceuticals and nutraceuticals [112].

Table 8 List of NPs synthesized using neem flower extract

S. No	NP's	Extract used	Size	Study	Species	Doses	Inference	Reference
01	Au	Water	20–50 nm	Anti-bacterial	*E. faecalis, P. mirabilis, K. pneumonia*, and *S. aureus.*	20, 30, 40 μg/mL	Intermediate activity was reported	[110]
				Anti-diabetic	Non-enzymatic glycosylation of hemoglobin assay, α-glucosidase enzyme assay, alpha-amylase enzyme assay.	20–100 μg/mL	72.17, 58.66, 45.72 μg/mL	[86]
				Anti-oxidant	DPPH, NO scavenging, ABTS assay, OH^- radical assay.	20–100 μg/mL	69.77, 65.67, 60.04, 57.86 μg/mL	
							69.77, 65.57, 50.64, 51.83 μg/mL	[110]
02	Ag	Water	100 nm	Anti-bacterial	*E. coli* and *S. aureus.*	25, 50, 75, 100 μg/mL	MIC – 100 μg/mL	[108]
				Anti-diabetic	Non-enzymatic glycosylation of hemoglobin assay, α-glucosidase enzyme assay, alpha-amylase enzyme assay.	20–100 μg/mL	61.42, 48.01, 44.07 μg/mL	[86]
				Anti-oxidant	DPPH, NO scavenging, ABTS assay, OH^- radical assay.	20–100 μg/mL	74.19, 66.87, 58.54, 55.96 μg/mL	
03	Cu	Water	5 nm	Anti-bacterial	*E. faecalis, P. mirabilis, K. pneumonia*, and *S. aureus.*	20, 30, 40 μg/mL	MIC – 100 μg/mL	[109]
				Anti-diabetic	Non-enzymatic glycosylation of hemoglobin assay, α-glucosidase enzyme assay, alpha-amylase enzyme assay.	20–100 μg/mL	61.43, 44.85, 37.41 μg/mL	[86]
				Anti-oxidant	DPPH, NO scavenging, ABTS assay, OH^- radical assay.	20–100 μg/mL	61.90, 58.52, 52.70, 51.92 μg/mL	
04	CuO	Water	10–17 nm	Toxicity	H9c2.	5, 10–50 μg/mL	Selective apoptosis on H9c2	[111]
				Ecotoxicity	Zebrafish.	5, 10, 20 μg/mL	75 μM	

MIC, minimum inhibitory concentration; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

Metal oxide nanoparticles exert their cell-damaging effects through a variety of mechanisms that target bacterial or eukaryotic cells. These mechanisms involve interactions with the cell membrane, intracellular components, and metabolic processes, leading to oxidative stress, structural disruption, and eventual cell death. The interaction of metal/metal oxide nanoparticles with targetted cells will lead to cell damage via multiple mechanistic pathways. The mechanism involves oxidative stress induction, membrane damage, protein dysfunction, DNA damage, disruption of cellular metabolism, induction of apoptosis/necrosis, biofilm, and quorum sensing disruption, mechanical & electrostatic interactions, and autophagy & immune response (Figure 13) [113].

Conclusion

This review systematically explores the phytochemical composition, pharmacological properties, and immunomodulatory potential of neem flowers, emphasizing their therapeutic applications. It highlights key bioactive compounds identified in neem flowers and their pharmacological relevance, suggesting their suitability for medicinal purposes. The review underscores the need for further research to harness the therapeutic potential of neem flowers, particularly for developing herbal medicines or supplements. Potential applications include formulations for treating skin cancer, promoting wound healing, and offering UV protection, with scope for industrial-scale production. Overall, this comprehensive analysis provides insights into unlocking the full medicinal value of neem flowers.

Gaps identified

Although the forgoing reports address the potential applications of neem flowers, a thorough study related to the bioactive constituents and their identification is almost scanty.

The limited availability of studies on Neem flowers could be attributed to several factors:

Historical focus: Traditionally, the leaves, seeds, and bark of the Neem tree have been more commonly used in medicinal preparations, leading to a greater emphasis on research related to these parts.

Research priorities: Research priorities and funding allocation may have favored investigations into other medicinal plants or parts of the Neem tree perceived to have higher therapeutic potential or commercial value.

Technical challenges: Isolating bioactive compounds from plant materials can be technically challenging, requiring specialized equipment and expertise. The complex chemical composition of neem flowers may pose additional challenges compared to other plant parts. Analytical techniques have to be improved for the further analysis of the natural compounds [114].

Cultural practices: Cultural practices and traditional knowledge may influence research directions with certain parts of the Neem tree being more commonly used or valued in different cultures or regions [115].

Limitations attributed to the usage of neem flower

(a) Seasonal and limited availability: Neem flowers bloom seasonally, usually in spring or early summer, making them unavailable for use throughout the year in culinary and medicinal practices.
(b) Limited scientific research: Neem flowers have received less research attention compared to leaves and bark, resulting in limited scientific evidence supporting their effectiveness for various conditions.
(c) Toxicity: Excessive consumption of neem flowers may have toxic effects, particularly on the liver, due to the presence of certain bioactive compounds.
(d) Unidentified active compounds: While neem flowers are known to have some bioactive compounds, their pharmacological properties are not as well-characterized, leading to uncertainty about their full range of benefits and potential side effects.

Suggestions and directions for future research

Advancing research in chemical analysis, toxicology, and comparative studies can uncover valuable applications for neem flowers. Improved preservation and cultivation methods can ensure consistent accessibility and availability. Developing scientifically backed guidelines for safe consumption can help address toxicity concerns. Additionally, documenting and promoting traditional uses can help preserve cultural practices while inspiring innovative applications. Research should prioritize creating standardized extraction methods to ensure consistent quality and potency of neem flower products. Pharmacokinetic studies are needed to explore the absorption, distribution, metabolism, and excretion of bioactive compounds, clarifying their safety and effectiveness. Clinical trials can then confirm these findings by evaluating neem flowers' therapeutic potential for specific conditions, supporting evidence-based medical applications.

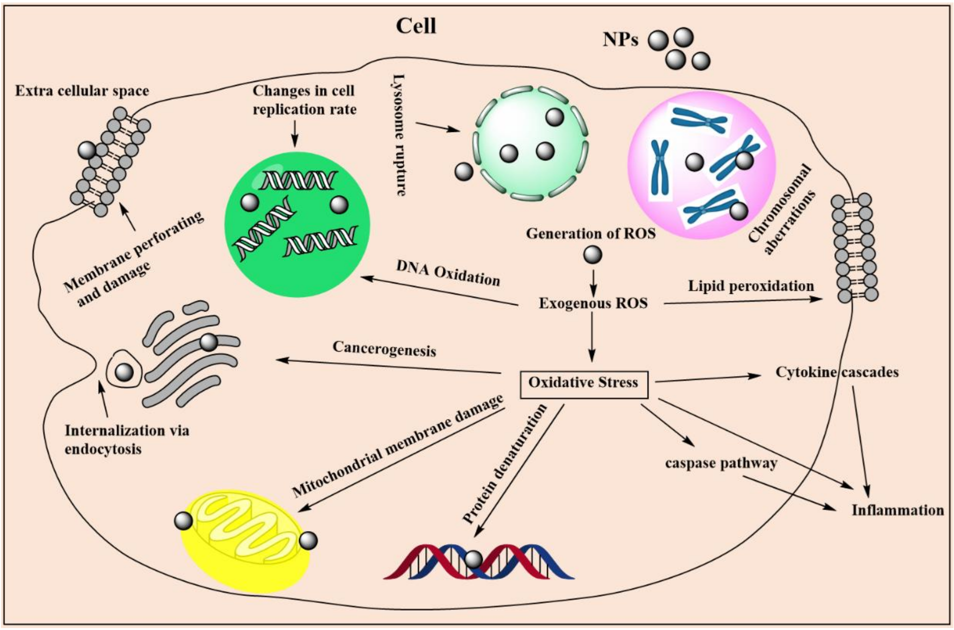

Figure 13 Schematic representation of multiple delivery pathways and cell damage by metal oxide nanoparticles. Adapted and modified from Maria P. Nikolova et al., 2020 (https://creativecommons.org/licenses/by/4.0/) [113]. ROS, reactive oxygen species.

Neem flowers hold significant translational potential in pharmaceuticals, nutraceuticals, and cosmetics. Their bioactive compounds could be harnessed to develop novel drugs for managing various health conditions. In nutraceuticals, neem flowers can be used to create supplements or functional foods that promote wellness and prevent diseases. Additionally, their antioxidant, antimicrobial, and anti-inflammatory properties make them valuable for cosmetic formulations aimed at skincare and haircare, offering natural solutions for common concerns. Exploring these applications through rigorous research could unlock their commercial and therapeutic potential.

Their real-world applicability lies in their potential for development into therapeutic agents targeting diverse health conditions. It is reported that neem flower extracts possess various phytochemicals that show promising antioxidant activities [19, 20, 31, 34, 35]. These properties can be harnessed in developing treatments for oxidative stress-related disorders such as neurodegenerative diseases, cardiovascular conditions, and diabetes. Neem flower holds significant antimicrobial and antibiofilm applications [31, 34, 35]. This potential activity can make them candidates for the development of novel antimicrobial agents via formulations like ointments to combat drug-resistant pathogens. From the earlier reports, it is known that neem flower possesses anti-inflammatory, antidiabetic, and neuroprotective properties. This suggests that their use in treating chronic inflammatory conditions such as arthritis and inflammatory bowel disease, regulates blood sugar levels, presenting opportunities for their integration into therapies for inflammatory and diabetes management. The real-world application of neem flowers can be facilitated by focusing on clinical trials, standardizing extraction methods, and integrating them into formulations like nutraceuticals, pharmaceuticals, and topical agents. Their natural origin and multifaceted benefits underscore their value as a resource in modern medicine.

Collaboration between researchers, funding agencies, and traditional healers or practitioners knowledgeable about the traditional uses of Neem flowers may also facilitate research efforts in this area. Additionally, interdisciplinary approaches that combine traditional knowledge with modern scientific methods could offer novel perspectives on the therapeutic properties of Neem flowers and other medicinal plants.

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