Review Article

PHYTOCHEMICAL AND ANTIMICROBIAL EVALUATION OF *AZADIRACHTA INDICA* AGAINST MULTIDRUG-RESISTANT MICROORGANISMS

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Abstract;

Background – The global rise of multidrug-resistant (MDR) microorganisms poses a significant challenge to public health, rendering many conventional antibiotics ineffective. *Azadirachta indica* (neem), widely known for its medicinal properties, has gained attention as a potential natural alternative due to its phytochemical richness and broad-spectrum antimicrobial activity. This review aims to explore the phytochemical composition, antimicrobial efficacy, and potential applications of A. indica, particularly in combating MDR pathogens.

Methods

Relevant studies were reviewed to summarize the phytochemical profile, antimicrobial activity, and mechanisms of action of A. indica. Data on extraction techniques, compound identification (e.g., TLC, HPLC, GC-MS), and antimicrobial testing (e.g., disc diffusion, MIC) were analysed. Additionally, toxicological studies and the development of neem-based formulations were assessed to evaluate their clinical and industrial viability.

Results

A. indica contains diverse bioactive compounds, including azadirachtin, nimbin, and quercetin, which exhibit potent antimicrobial, anti-inflammatory, and antioxidant properties. In vitro studies demonstrate its efficacy against MDR strains like MRSA, *E. coli,* and *K. pneumoniae*, primarily through mechanisms such as cell membrane disruption and oxidative stress induction. Neem-based formulations, such as antimicrobial coatings and topical preparations, offer promising alternatives to synthetic antibiotics. Furthermore, synergistic effects have been observed when neem is used in combination with conventional antibiotics.

Conclusion

A. indica demonstrates significant potential as a natural solution to address the growing issue of antimicrobial resistance. While its therapeutic efficacy and safety are well-supported by preliminary studies, further clinical research and standardization efforts are essential to unlock its full potential in healthcare and pharmaceutical industries.

Key words: *Azadirachta indica*, Multidrug-resistant microorganisms, Phytochemicals, Antimicrobial activity, Synergistic effects

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INTRODUCTION:

Given the rise of infectious diseases, patients suffer from more bacteria, viruses and other microbes that are becoming temperate to several drugs. Large death tolls, long hospital stays, cost and the global health care system is overwhelmed by Multidrug-resistant (MDR) organisms including *Methicillin-resistant Staphylococcus aureus* (MRSA) extended *Spectrum Beta-lactamase* (ESBL), and carbapenem-resistant *Klebsiella pneumoniae* [1]. AMR is ranked as one of the top ten global public health threats by the WHO, meaning it needs a fast solution. Difficulties using the traditional antibiotic drugs have only compounded the situation. Much more importantly, there is an urgent demand for fresh methods of therapy because antibiotics are used and abused a lot more than they should, and the formulation of latest antibiotics is relatively slow. Thus, plants are potential sources of bioactive components since they exhibit obvious advantages over synthetic antimicrobials in terms of effectiveness against a variety of microbes and low potential for generating resistance [2].

There is, however, a large body of records on traditional uses and potential benefits of Neem, also scientifically known as *Azadirachta indica*. For this reason, neem is valued for the numerous pharmacological properties related to its phytochemical composition which includes azadirachtin, nimbin and nimbidin and quercetin among others. It can combat infections and the pathogens causing these infections, such as those producing multidrug-resistant bacteria, also has anti-inflammatory properties and acts as an antioxidant [3].

The present paper aims to provide the detailed elucidation of phytochemical composition of *A. indica* along with its antibacterial potential specifically against the MDR bacterial strain. It also discusses aspects concerning toxicological safety, interactions with conventional antibiotics, and its mode of working. The purpose of this study is to concentrate on the tangible benefits of *A. indica* in lieu of antibiotic resistance by outlining the current gaps in the existing literature and calling to attention of possible future work [4].

2. Phytochemical Profile of Azadirachta indica

2.1 Major Bioactive Compounds

Antibacterial properties and various other health advantages of Azadirachta indica (Neem) are also ascribed to biochemistry density containing secondary metabolites. At the present, azadirachtin, nimbin, nimbidin, quercetin and other chemicals that have been examined have been identified as important chemicals. A good example of a potent. Reset against bacterial as well as fungal infections is azadirachtin, a complex tetranortriterpenoid with inflammatory, insectical as well as antibacterial property [5]. These include lipophilic agents nimbin and nimbidin, which are important in the plant's antimicrobial, antifungal and anti-inflammatory activities. These chemicals are very essential in combating infections because they dissolve the cell wall of microorganisms and prevent reproduction. There is quercetin – an antioxidant, anti-inflammatory and antibacterial flavonoid present in neem [6]. It helps to support the functions of the immune system to eradicate infections, destroy free radicals and reduce the amount of oxidative stress. One of the most important pharmacological utilities that are essential in the fight against MDR bacteria are antimicrobial abilities offered by these two bioactive compounds. As we know, AMR is a global issue and neem can be

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very effective since its effects are mutually complementary [7].

2.2 Extraction and Analytical Methods

This makes the extraction of usable components from *A. indica* a delicate process that has various methods to ensure the yield is optimised but the medicine value retained. There are several techniques of extraction among them being Maceration, Soxhlet and supercritical fluid extraction [8]. The easiest procedure of recovery of active chemicals from plants is maceration, which entails treating the material with a solvent like ethanol or methanol for a long period. The phytochemicals are extracted more efficiently than by using the simpler and less efficient maceration whereby the solvent rises up through the plant material in a column in a device called a Soxhlet [9]. Super critical fluid extraction (SFE) recently developed a new method of extracting bioactive chemicals with very low level of solvent residues is through applying high pressure carbon dioxide. Some of the chemicals typically go through series of test after the extraction process. Because it can be used to follow the movement of sample components through a fixed phase, thin-layer chromatography, a basic method for preliminary distinction of chemicals, is valuable in the lab [10]. More accurate and a better profiling of the extract and quantization of specific chemicals can be done using High-Performance Liquid Chromatography (HPLC) The extraction method also enables a better separation and quantization of specific chemicals in the extract. For chemical identification of the active components of neem, GC-MS serves as a practical way of analysing volatile compounds and determining chemical content. For understanding the potential of these compounds in the antimicrobial therapy, these approaches ensure the identification of the entire spectrum of the pharmacologically active molecules [11].

2.3 Pharmacological Activities of Phytochemicals

Neem *(Azadirachta indica)* is regarded as a multipurpose medicinal plant because its phytochemicals cause various pharmacological actions. In terms of the pharmacological activity, the neem has been found out to possess antimicrobial action and has been the subject of investigation in great detail. Azadirachtin and nimbidin are compounds that prevent development of infection by MDR organisms through cell membrane perturbations, direct effect on microbial metabolism and blocking of biofilm formation [12]. These chemicals are highly us against many different kinds of bacteria, fungi and viruses Some of the chemicals behind neem include Quercentin and nimbin which are known to have anti-inflammations capabilities. Such chemicals lower the inflammation caused by infections through regulation of the generation of the cytokines and enzymes. Especially in the case of chronic inflammatory diseases which often attend upon infections this is very valuable in minimizing their occurrence [13]. Further, it has been seen that flavonoid, especially quercetin, present in neem, are largely responsible for its antioxidant activity; these chemicals reduce cellular injury due to oxidative stress by propounding ROS. These chemistries help support tissue remain integrity during inflammation and infection by reducing the levels of oxidative stress. The foregoing pharmacological actions view neem's medicinally-active ingredients or phytochemicals especially where there is MDR-pathogen related infection. Prime advantages of the neem are its antibacterial properties, anti-inflammatory properties and antioxidant properties, which together with the threat being posed by antibiotic resistance, make neem a viable subject for development of future antibiotics

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[14].

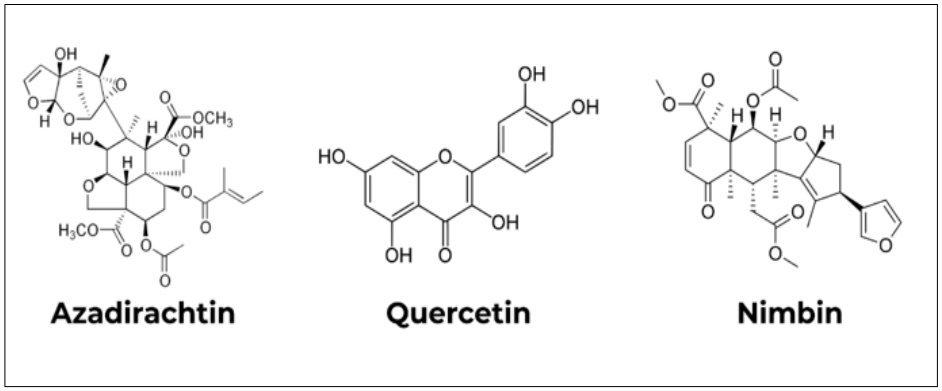

Figure 1: Chemical structures of major phytochemicals in *A. indica*. [15]

Table 1: List of major phytochemicals in *A. indica* with their biological activities. [16]

Phytochemical	Biological Activity
Azadirachtin	Antimicrobial (antibacterial, antifungal, antiviral), insecticidal, anti-inflammatory
Nimbin	Antibacterial, antifungal, anti-inflammatory, antioxidant
Nimbidin	Antibacterial, antifungal, anti-inflammatory, antioxidant
Quercetin	Antioxidant, anti-inflammatory, antimicrobial, anticancer
Salannin	Antibacterial, antifungal, insecticidal
Mahmoodin	Antibacterial, anti-inflammatory
Triterpenes	Antimicrobial, anti-inflammatory, anticancer
Flavonoids	Antioxidant, anti-inflammatory, antimicrobial, anticancer
Steroids	Anti-inflammatory, antimicrobial, immunomodulatory
Limonoids	Antimicrobial, anti-inflammatory, insecticidal
Azadiradione	Antimicrobial, insecticidal
Cinnamyl alcohol	Antibacterial, antifungal

3. Antimicrobial Activity of Azadirachta indica

3.1 In Vitro Studies on Antimicrobial Activity

Neem – *Azadirachta indica* has a pharmacological background of activity in vitro against bacterial and fungal organisms, which includes fungus, both Gram-positive and Gram-negative bacteria and more. Its efficiency can be evaluated by using different methods including disc diffusion, MBC, and MIC [17]. For the disc diffusion method of study, the findings confirms that neem extracts especially those extracted from leaves possess high antimicrobial qualities against many microorganisms such as *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* [18]. The MIC indicates the minimum concentration of neem extract required to prevent microbial growth as supporting the antibacterial effect of neem as would be expected, the neem extract displayed significant antibacterial activity with the following MIC values. The MBC which has been described above as the minimum concentration required to kill the pathogen has also been reported in other studies thereby supporting the use of the plant in treating infection. These researches indicate that neem eradicates microbes and it can be used instead of synthetic antibiotics to cure various sorts of diseases [19].

3.2 Mechanisms of Antimicrobial Action

The antibacterial potential of *Azadirachta indica* is expected to affect many sectors of microbial physio biology through many mechanisms. The neem chemicals which act effectively comes in the following broad categories

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including; disruption of microbial cell membrane [20]. This is instrumental in causing the contents of the cell to leak out and most importantly bringing about cell death. Another study has also shown that neem can immobilise enzymes which are important for microbial existence. If it restrains some enzymes that are required for cell wall synthesis, then cell walls are weak unable to withstand pressures within the environment [21]. The biologically active compound in neem activates the generation of ROS within the target cells of microbes: the second factor is the induction of oxidative stress. These reactive oxygen species (ROS) augment microbial cell killing by altering proteins lipids and nucleic acids all of which are parts of cells. Due to these effects neem is a wide-spectrum antibiotic that can inhibit growth at various stages of microbial existence [22].

3.3 Comparative Efficacy with Synthetic Antibiotics

Studies comparing the effectiveness of the *Azadirachta indica* with synthetic antibiotics established that neem extracts exhibit similar antibacterial activity, if not better, to conventional antibiotics. The antimicrobial zones by neem extracts on the most common pathogens are often wider than that of several standard drugs including fluconazole, tetracycline and amoxicillin [23].

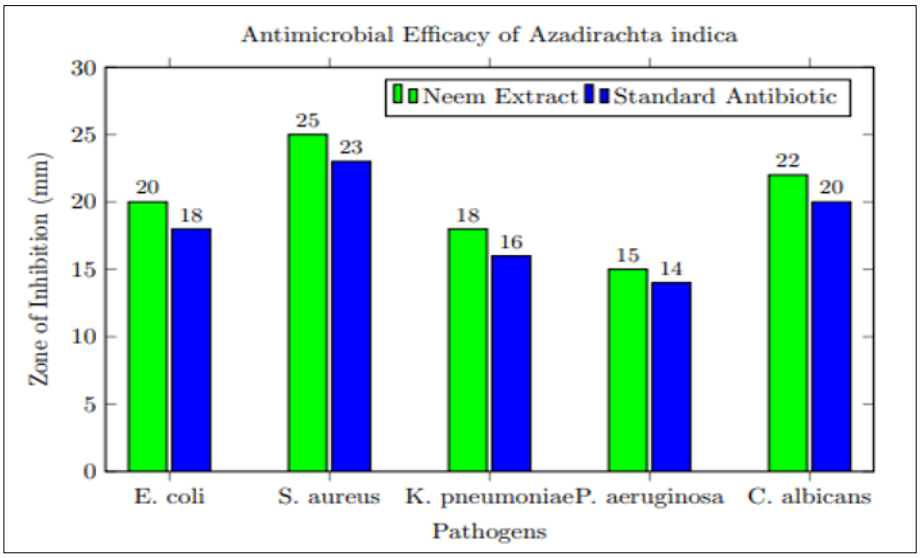

Figure 2: Graphical representation of antimicrobial efficacy (e.g., zones of inhibition). [26]

Table 2: Antimicrobial activity of *A. indica* against specific pathogens (e.g., MIC, zone of inhibition). [27]

Pathogen	MIC (µg/mL)	Zone of Inhibition (mm)	Type of Extract
Escherichia coli	125	20	Neem leaf extract
Staphylococcus aureus	100	18	Neem leaf extract
Pseudomonas aeruginosa	150	22	Neem leaf extract
Candida albicans	200	25	Neem leaf extract
Aspergillus niger	250	28	Neem leaf extract
Salmonella typhi	200	23	Neem leaf extract
Bacillus subtilis	100	21	Neem leaf extract
Klebsiella pneumoniae	125	24	Neem leaf extract
Streptococcus pneumoniae	150	19	Neem bark extract
Enterococcus faecalis	175	20	Neem bark extract
Fusarium oxysporum	300	26	Neem seed extract
Microsporum canis	250	27	Neem seed extract
Trichophyton rubrum	225	23	Neem seed extract

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Helicobacter pylori	200	30	Neem flower extract
Shigella dysenteriae	150	22	Neem leaf extract

These findings show that neem extracts have MIC and MBC values similar to these synthetic medications so it might be a good replacement. Furthermore, while neem acts as an antibiotic substance, its antibacterial performances are functional on MDR strains. Neem is one the many potential solutions to the problem of antimicrobial resistance (AMR), a significant threat to world health [24]. The ability of Neem-based medicines to address a number of facets in the microbial cell and act on many fronts affords the possibility of vastly preferable approaches to resistance of synthetic antibiotics. Despite all the potential of neem, more work must be done to determine its readiness for use in clinical practice: its safety, efficacy, and stability when compared to antibiotics [25].

4. Activity Against Multidrug-Resistant Microorganisms

4.1 Efficacy Against MDR Pathogens

The problem of emergence of new strains of bacteria, viruses, and other microbes that are less sensitive to many drugs is an emerging problem that is widely seen in healthcare facilities globally. New lethal strains of bacteria such as *Klebsiella pneumoniae, Methicillin-resistant Staphylococcus aureus* (MRSA), antibiotic- resistant *Escherichia coli* contribute to health care associated infections, chronic illnesses, and mortality of human beings [28]. The antbacterial potential of Azadirachta indica locally known as neem makes it a potential source of treatment for these chronic infections. The effectiveness of neem in eradicating these multi-drug-resistant bacteria has been tested in a number of studies wanted on the extracts of neem leaf, seed, and bark [29].

Furthermore, an outstanding eradication effect of the extracts from neem on *Methicillin-resistant Staphylococcus aureus* (MRSA) was observed. The compounds in neem inclusive of azadirachtin and nimbidin deter MRSA since they have the ability to physically kill these bacteria through its cell walls and inhibit all its metabolic functions [30]. These include antibiotic-resistant Staphylococcus aureus (Sa) particularly *Methicillin-resistant Staphylococcus aureus* (MRSA), *Klesiella pneumoniae, and Escherichia coli.* It was also found out that neem extracts possess a significantly lower MIC as compared to other conventional antibacterial agents which means that the aforesaid pathogens can be pronounced more sensitive to finite concentrations of neem extract. Due to this, neem is now viewed as a possible future possibility for the treatment of diseases resulting from multidrug resistant bacteria [31].

4.2 Synergistic Potential with Conventional Antibiotics

The uses of traditional antibiotics collectively with *Azadirachta indica* are perhaps the most interesting areas of research on these trees. Thus, adding neem extracts to conventional antibiotics enhances the therapeutic level with a concomitant reduction in antibiotic resistance. Syneregetic effects of neem extracts with antibiotics such as ciprofloxacin, gentamicin, amoxicillin, and tetracycline against MDR bacteria has fascinated many researchers [32].

For example, when tried on the resistant strain of *E. coli* bacteria, the study found that extracts from neem leaves made they antibiotic to be more effective. The cooperation of neem and amoxicillin produced lower MIC of the antibiotic and therefore, lower dosages of amoxicillin were effective to have similar antibacterial effect as the corresponding single antibiotics [33].

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For this reason, extracts of neem also enhanced effects of ciprofloxacin on *Klebsiella pneumoniae* bacteria by overcoming the bacterial barrier. This is because of synergistic effect which works in ways such as affecting bacterial cell membrane permeability, preventing bacterial efflux and blocking of resistance enzymes as well as beta-lactamases [34].

The use of neem together with antibiotics presents an idea that can be adopted to increase the abilities of available antibiotics, reduce usage and the future spread of antibiotic resistance. This synergy can be particularly valuable to address the global rise in AMR, and to target chronic diseases as well as diseases that are caused by MDR bacteria [35].

4.3 Challenges and Opportunities

Thus, while neem specifically *Azadirachta indica*, can enhance the effects of conventional antibiotics has good potential for use as an antibacterial it still has a number of obstacles that must be cleared for it to be used clinically on a large scale. Since the type, part of plant used, extraction method and geographic provenance of neem all impact the concentration of bio active chemicals in neem, the standardization of neem extracts is a very challenging task [36]. Because of this variation, the ant wipe efficacy might differ from one study to another leading to a lot of inconsistencies. Because neem contains a rather complicated combination of bioactive compounds, it is crucial to establish efficient methods of extraction as well as the necessary measures of quality control in order to ensure the activity of the extract [37].

The potential side effects and dangers of neem in medical practice form the next challenge at the same time. Despite its antiquity and wide use in traditional medicinal systems, little effort has been devoted to documenting its effects in the long-term use of neem, or determining its reduced-toll dosage and the side effects of this substance [38]. Some of the side effects of neem extracts include hepatotoxicity and nephrotoxicity especially when high concentrations and or long-term use is employed. The safety and efficacy of neem in standard medical treatments have to be clarified through toxicological and clinical studies before this wonder tree can be incorporated in normal health care procedures [39].

Still, there are various challenges in establishing neem-based therapy for MDR illnesses, however, the potential is quite high. A readily available and even providential substitute that has the specific properties of antibiotics, neem's antibacterial properties are currently selling like hotcakes due to the emergence of the dreaded drug-resistant bacteria [40]. In the case of combination medicines, this makes research into neem as an alternative or complementary medicine to the commonly used antibiotics relevant. The expanded utilization of Neem as a potential new source of botanical medicine that could be a key strategist in combating MDR bacteria relies on clinical trials that address questions of safety, efficacy, and optimal form of Neem [41].

5. Toxicological and Safety Profile of Azadirachta indica

5.1 Overview of Toxicity Studies

The anti-inflammatory, anti-diabetic and anti-bacterial properties of *Azadirachta indica* (neem) are quite established yet side effects are the primary concern when it comes to using neem as a drug in various healings. In this connection, the therapeutic safety and all the potential side effects of neem must be defined by the findings of toxicological research involving both human and animal subjects [42]. Studies concerning the toxic effect of neem

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extracts on an acute level have been conducted. Research like this seeks to confirm chronic signs of toxicity such as fatigue, health complications in organs, or altered physiological markers, and this calculates LD50 based on routes of administration such as oral or intravenous or topical application [43].

Moreover, the study pointed that neem extracts are not greatly toxic in general. This is perhaps better when taken in moderation. Neem oil is also considered safe with low acute toxicity in that its LD50 in rats is above 5 g/kg. Of course, it is important to mention that side effects of neem are possible and include organ damage, in particular liver, unspecified gastrointestinal disorders, and, rarely, severe forms of intolerance to extract—provided that it is taken in large amounts [44]. To read more about the probable side effects in the long run, one can conduct long-term toxicity studies of neem extracts taken by humans. Genotoxicity, categorised by organ toxicity, reproduced toxicity and alteration of biochemical endpoints, are often studied in this research in order to assess the likelihood of any long-term impacts [45].

Moreover, very limited research studies concerning impacts of neem-based medicines on human have been conducted thus, it is fairly said that the safety study of the herb is in the initial phase in human trials. Neem has been used in traditional medicine for many years but to reveal the full consequences of neem use in humans especially in the long run, further large-scale clinical trials should be conducted [46].

5.2 Determination of Safe Dosage

The azadirachtin dose which are safe vary depending on the extract type used, treatment to be given, and the mode of administration. For instance, neem leaves are often used in traditional medicine to regulate inflammation and diabetes but there is no standard measure of this. Literature and folk knowledge, surveys, and clinical trials guide the ideal concentrations of neem extracts for therapeutic purposes [47].

The proposed range of safe neem leaf powder used for oral administration as a daily regimen is 50-100 mg/kg body weight. Exercise some care when using neem oil because it contains strong chemicals that are dangerous when ingested in large quantities. Neem oil should be diluted and then applied externally in cases where it is used for a medicinal effect [48]. This is useful so as to prevent products from absorbing into the bloodstream and hence reduces the toxicity of the drug. Some of the elements of neem and its different forms are said to have exact doses for use in treating parasites in cattle from the veterinary point of view [49].

The main concern with neem-based treatments is setting acceptable toxicity limits when used together with other drugs or therapeutic compounds. Due to a possibility of interference with the metabolism and excretion of the bioactive compounds in Neem, age, health condition, and other diseases may also affect the safe amount of Neem to be used. Such guidelines which would have adequate groundwork setup from preclinical and clinical studies are what is necessary to attain a safe era of neem within the clinics [50].

5.3 Areas for Further Research

Despite the fact that *Azadirachta indica* has demonstrated some medicinal value, there are questions about the genotoxicity and other chronic side effects of the tree. It is important that further research be conducted on the chronic effects of neem use although the overall literature on acute toxicity has provided good data on the agent's short-term safety profile. As the

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chronic toxicity study is missing, it is vital to carry out these studies in relation to neem and its extracts administration, especially for patient having chronic diseases for a prolonged period [51].

Evaluation in genotoxicity is also addressed by limited academic research. Genotoxicity is actually the action of a compound causing a loss of the normal structure of DNA, which in turn may lead to the formation of a neoplastic cell. As it concerns the pharmacological properties of neem there is lack of data evaluating the genotoxic risk of the compound even though it is widely used in folk medicine [52]. To further confirm that the neem-based treatments should not pose a genotoxic risk, extensive genotoxicity studies should be conducted. Such studies should comprise of vitro already known cytogenetics tests, e.g. the Ames test or the chromosomal aberration test as well as the in vitro ones [53].

Another scientific area associated with Neem oil is the effect of neem oil on the organism when it is ingested. Possible side effects of neem oil include therefore: As mentioned earlier, neem oil might have side effects when consumed in large proportions – for instance, liver issues. To prove the safety of neem oil in its therapeutic applications, toxicity in liver and kidney function, and safe internal use must be investigated [54].

Further, despite the fact that studies into neem's toxicity in human beings reveal that it is safe, more studies are needed to establish its cumulative effects, its interactions with other medications, and the optimal therapeutic amounts of neem. Thus, these above-mentioned studies should involve different groups of patients such as those with prior illnesses, children, and elderly people in order to discover appropriate dosage concerning neem [55].

6. Applications of Azadirachta indica

6.1 Formulation and Product Development

Neem or *Azadirachta indica* has been the most versatile tree, used in developing so many products like antimicrobial finish, topical formulations, and even supplements. A natural antimicrobial product formulated with neem would be advisable owing mainly to the broad-spectrum antimicrobial properties. Skin infections, acne, dermatitis, and other skin conditions are treated by using lotions ointments, and creams [56]. Due to its antibacterial, anti-inflammatory, as well as antioxidant properties, these formulations use neem in an effort to realize therapeutic gains while at the same time minimizing side effects [57].

The herb in oral preparations is used for the management of diseases such as diabetes and high blood pressure and to enhance general body health and immune system. Besides, neem used in antimicrobial coatings for medical instruments is becoming popular because of low infection rates and improved patient care [58]. These coatings can be applied to wound dressings, one used in surgeries and another on prosthetic devices. An advantage of these medicines is their potential to prevent the growth of adversative microorganisms in healthcare facilities [59].

6.2 Role in Combating Antimicrobial Resistance

Antimicrobial resistance is rising as a major threat in global health and *Azadirachta indica* is a major tool in combating the rising threats. Most of the conventional antibiotics are either ineffective owing to the presence of MDR bacteria and viruses. Neem is believed to contain various phytochemicals that may get passed the conventional antibiotics' limitations as an alternate or additional remedy. Numerous Mult-resistant bacteria have been

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eradicated with the use of this medicine [60]. They include *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and MRSA among others. To prevent germs that do not die with antibiotics and make neem which can burst bacterial cell membranes, lock enzymes from working and cause oxidation stress [61].

Plant infections can now be fought with neem extracts because these nontoxic extracts have been used as insecticides and fungicides in farming. Current growing emphasis on the search for environment-friendly practices in the health sector and crop production makes the utilization of neem-based formulations a potential area of reducing reliance on synthetic chemicals in combating AMR [62].

6.3 Challenges in Commercialization

Azadirachta indica has several probable applications but there are a number of challenges which hinder its commercialization. The issue of Standard Deviation is a major concern affecting all neem-based goods. Repetition and effectiveness are compromised by the fact that the chemical composition of the neem may differ depending on the places they grow, how the neem extract is prepared, and how the neem is processed. The use of these drugs in therapeutic practice requires their standardization to ensure that they possess therapeutic properties in their effective concentrations when used therapeutically [63].

Another disadvantage is that due to the complexity of regulatory approval of the products, the sale of neem-based products also faces constraints. Many people cannot get neem approved even if this product has been on the market for as long as it is because there are not enough clinical trials, and the safety is uncertain. The inclusion and the dose can provoke certain doubts; it is especially important and urgent to indicate the safe therapeutic concentration is for oral application [64].

7. Conclusion

The powerful antibacterial characteristics are imparted by a wide variety of bioactive chemicals in the *Azadirachta indica* plant, most often known as neem. The wide range of bacterial and fungal infections that it can effectively combat is due in part to its diverse phytochemical profile, which includes substances such as azadirachtin, nimbin, nimbidin, and quercetin. The traditional medicinal use of neem has been backed by modern scientific research that confirms its therapeutic efficacy, especially in combating illnesses caused by germs that are resistant to many drugs. With its ability to damage cell membranes, block enzymes, and induce oxidative stress, neem offers a potential alternative to conventional antibiotics, which is becoming an increasingly pressing worldwide concern.

Given the increasing resistance dilemma, the use of neem in the fight against multidrug-resistant infections is crucial. Combinations of neem-based formulations with topical treatments, dietary supplements, and antimicrobial coatings have demonstrated great potential in healthcare settings. As a supplementary strategy for controlling antibiotic-resistant infections, its possible synergy with traditional antibiotics only adds weight to its therapeutic utility.

Despite the obvious advantages of neem, numerous obstacles and knowledge gaps need to be filled in order to fully utilize it in clinical and industrial settings. Future research should focus on developing strong clinical studies to support regulatory approval, standardizing neem-based products, and

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conducting long-term safety reviews. In addition, neem could be more effectively used to battle MDR infections and other infectious diseases if its large-scale agricultural applications and potential in combination medicines are studied further. This would increase its commercial viability and accessibility.

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REFERENCES

1. Alara JA, Alara OR. An Overview of the Global Alarming Increase of Multiple Drug Resistant: A Major Challenge in Clinical Diagnosis. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders). 2024 May 1;24(3):26-42.

2. Ferri M, Ranucci E, Romagnoli P, Giaccone V. Antimicrobial resistance: A global emerging threat to public health systems. Critical reviews in food science and nutrition. 2017 Sep 2;57(13):2857-76.

3. Islas JF, Acosta E, Zuca G, Delgado-Gallegos JL, Moreno-Treviño MG, Escalante B, Moreno-Cuevas JE. An overview of Neem (Azadirachta indica) and its potential impact on health. Journal of Functional Foods. 2020 Nov 1;74:104171.

4. Shuvo MN, Halder SK, Alam N, Himel MK, Shil A. Developing phytocompound-based new drugs against multi-drug-resistant Staphylococcus aureus. Royal Society Open Science. 2024 Jul 24;11(7):231475.

5. Gupta A, Ansari S, Gupta S, Narwani M, Gupta M, Singh M. Therapeutics role of neem and its bioactive constituents in disease prevention and treatment. Journal of Pharmacognosy and Phytochemistry. 2019;8(3):680-91.

6. Nagini S, Palrasu M, Bishayee A. Limonoids from neem (Azadirachta indica A. Juss.) are potential anticancer drug candidates. Medicinal research reviews. 2024 Mar;44(2):457-96.

7. Shin J, Prabhakaran VS, Kim KS. The multi-faceted potential of plant-derived metabolites as antimicrobial agents against multidrug-resistant pathogens. Microbial pathogenesis. 2018 Mar 1;116:209-14.

8. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med aromat plants. 2015 Jul 6;4(196):2167-0412.

9. Kapadia P, Newell AS, Cunningham J, Roberts MR, Hardy JG. Extraction of high-value chemicals from plants for technical and medical applications. International journal of molecular sciences. 2022 Sep 7;23(18):10334.

10. Uwineza PA, Waśkiewicz A. Recent advances in supercritical fluid extraction of natural bioactive compounds from natural plant materials. Molecules. 2020 Aug 24;25(17):3847.

11. Winnik B, Barr DB, Thiruchelvam M, Montesano MA, Richfield EK, Buckley B. Quantification of Paraquat, MPTP, and MPP+ in brain tissue using microwave-assisted solvent extraction (MASE) and high-performance liquid chromatography–mass spectrometry. Analytical and bioanalytical chemistry. 2009 Sep;395:195-201.

12. Latif MJ, Hassan SM, Mughal SS, Aslam A, Munir M, Shabbir N, Mushtaq M, Perveiz S. Therapeutic potential of Azadirachta indica (neem) and their active phytoconstituents against diseases

* punit.j.rai@gmail.com

prevention. J Chem Chem Sci. 2020;10(3):98-110.

13. Moin MS, Siddiqui JI, Alam MA, Khatoon F, Khan S, Minhajuddin A. Ethnomedicinal potential of widely used plant Azadirachta indica A. Juss: A comprehensive review. The Journal of Phytopharmacology. 2021;10(6):456-67.

14. Ullah Khan S, Saleh TA, Wahab A, Khan MH, Khan D, Ullah Khan W, Rahim A, Kamal S, Ullah Khan F, Fahad S. Nanosilver: new ageless and versatile biomedical therapeutic scaffold. International journal of nanomedicine. 2018 Feb 2:733-62.

15. Sandhir R, Khurana M, Singhal NK. Potential benefits of phytochemicals from Azadirachta indica against neurological disorders. Neurochemistry international. 2021 Jun 1;146:105023.

16. Dineshkumar B, Vigneshkumar P, Bhuvaneshwaran SP, Mitra A. Phyto-pharmacology of Acalypha indica: a review. International Journal of BioSciences, Alternative and Holistic Medicine. 2010 Apr 1;1(2):27.

17. Okoh SO, Okoh OO, Okoh AI. Inhibitory effects of Azadirachta indica secondary metabolites formulated cosmetics on some infectious pathogens and oxidative stress radicals. BMC complementary and alternative medicine. 2019 Dec;19:1-3.

18. Moyo B, Masika PJ, Muchenje V. Antimicrobial activities of Moringa oleifera Lam leaf extracts. African Journal of Biotechnology. 2012;11(11):2797-802.

19. El-Mahmood AM, Ogbonna OB, Raji M. The antibacterial activity of Azadarichta indica (neem) seeds extracts against bacterial pathogens associated with eye and ear infections. J. Med. Plants Res. 2010 Jul 18;4(14):1414-21.

20. Faisal UM, Saifi MS, Kaish M, Ibrahim M, Kwakuri SS, Arif M. Azadirachta indica (neem): An important medicinal plant: A literature review of its chemistry, biological activities, role in COVID-19 management and economic importance. Journal of Pharmacognosy and Phytochemistry. 2023;12(6):59-65.

21. Pullangott G, Kannan U, Gayathri S, Kiran DV, Maliyekkal SM. A comprehensive review on antimicrobial face masks: an emerging weapon in fighting pandemics. RSC advances. 2021;11(12):6544-76.

22. Sharma P, Nandave M, Nandave D, Yadav S, Vargas-De-La-Cruz C, Singh S, Tandon R, Ramniwas S, Behl T. Reactive oxygen species (ROS)-mediated oxidative stress in chronic liver diseases and its mitigation by medicinal plants. American Journal of Translational Research. 2023;15(11):6321.

23. Wylie MR, Merrell DS. The antimicrobial potential of the neem tree Azadirachta indica. Frontiers in pharmacology. 2022 May 30;13:891535.

24. Jubair N, Rajagopal M, Chinnappan S, Abdullah NB, Fatima A.

Review on the antibacterial mechanism of plant-derived compounds against multidrug-resistant bacteria (MDR). Evidence-Based Complementary and Alternative Medicine. 2021;2021(1):3663315.

25. Singh V, Roy M, Garg N, Kumar A, Arora S, Malik DS. An insight into the dermatological applications of neem: a review on traditional and modern aspect. Recent Advances in Anti-Infective Drug Discovery Formerly Recent Patents on Anti-Infective Drug Discovery. 2021 Aug 1;16(2):94-121.

26. Jindal A, Kumar A. Physical characterization of clove oil based self Nano-emulsifying formulations of cefpodoxime proxetil: Assessment of dissolution rate, antioxidant & antibacterial activity. OpenNano. 2022 Nov 1;8:100087.

27. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan NJ. Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids and Surfaces B: Biointerfaces. 2010 Mar 1;76(1):50-6.

28. Georgiou G, Kotzé A. Eradication of Antibiotic-Resistant E. coli, S. aureus, K. pneumoniae, S. pneumoniae, A. baumannii, and P. aeruginosa with Chlorine Dioxide In Vitro. Medical Research Archives. 2023 Jul 29;11(7.2).

29. Almowallad SJ, Alqahtani LS. Synergistic antimicrobial action of chitosan-neem extracts nanoformulation as a promising strategy for overcoming multi-drug resistant bacteria. International Journal of Biological Macromolecules. 2024 May 24:132337.

30. Khairy T, Amin DH, Salama HM, Elkholy IM, Elnakib M, Gebreel HM, Sayed HA. Pioneering study of Egyptian Neem and Jojoba extracts with molecular docking combat hospital multidrug resistant bacteria. Brazilian Journal of Microbiology. 2025 Jan 8:1-21.

31. Ramesh SP, Karajgi S, Singh R, Naik P, Sahoo U, Sharma MC, Nainwal P, Wal A. Herbal Antibiotics: A Review of Traditional Medicinal Plants with Antibacterial Properties and Their Potential for Future Treatment. Anti-Infective Agents. 2025 Apr;23(2):E080724231696.

32. Adeyemi OO, Alabi AS, Adeyemi OA, Talabi OT, Abidakun OM, Joel IY, Stonehouse NJ. Acute gastroenteritis and the usage pattern of antibiotics and traditional herbal medications for its management in a Nigerian community. PLoS One. 2021 Oct 4;16(10):e0257837.

33. Banna QR, Umar BU, Rahman SN, Alam T, Anwar KS, Shirin L. In Vitro comparison in the antimicrobial effect between Ciprofloxacin and Neem leaf extract (Azadirachta indica) on Eschericia coli Growth. Bangladesh Journal of Medical Science. 2016 Apr 1;15(2).

34. Khare T, Anand U, Dey A, Assaraf YG, Chen ZS, Liu Z, Kumar V. Exploring phytochemicals for combating antibiotic resistance in microbial pathogens. Frontiers in pharmacology. 2021 Jul 21;12:720726.

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35. Oladunjoye IO, Tajudeen YA, Oladipo HJ, El-Sherbini MS. Planetary health and traditional medicine: a potential synergistic approach to tackle antimicrobial resistance. Challenges. 2022 Jun 1;13(1):24.

36. Asif M. Antimicrobial potential of Azadirachta indica against pathogenic bacteria and fungi. Journal of Pharmacognosy and phytochemistry. 2012;1(4):78-83.

37. Benelli G, Canale A, Toniolo C, Higuchi A, Murugan K, Pavela R, Nicoletti M. Neem (Azadirachta indica): towards the ideal insecticide?. Natural product research. 2017 Feb 16;31(4):369-86.

38. Eze MO, Ejike CE, Ifeonu P, Udeinya IJ, Udenigwe CC, Uzoegwu PN. Anti-COVID-19 potential of Azadirachta indica (Neem) leaf extract. Scientific African. 2022 Jul 1;16:e01184.

39. Boeke SJ, Boersma MG, Alink GM, van Loon JJ, van Huis A, Dicke M, Rietjens IM. Safety evaluation of neem (Azadirachta indica) derived pesticides. Journal of ethnopharmacology. 2004 Sep 1;94(1):25-41.

40. Sagar SS, Rani S, Pushpa Sadanandan S. Combined Effect of Isolated Bacteriophage and Neem Extract on Isolated Multiple Drug-Resistant Pathogenic Escherichia coli E1 From Well Water. Environmental Health Insights. 2023 May;17:11786302231166818.

41. Mallik S, Nayak S, Panda A, Behera S, Swain K, Patra A, Mishra R, Nath Mohanty J. Does manipulation of phyto-based nanoparticles is a promising solution against multi-drug resistant (MDR) pathogens? A critical opinion towards tackling MDR pathogens. Novel Research in Microbiology Journal. 2024 Sep 20;8(5):2604-31.

42. Torwane NA, Hongal S, Goel P, Chandrashekar BR. Role of Ayurveda in management of oral health. Pharmacognosy reviews. 2014 Jan;8(15):16.

43. Adeneye AA. Subchronic and chronic toxicities of African medicinal plants. InToxicological survey of african medicinal plants 2014 Jan 1 (pp. 99-133). Elsevier.

44. Brahmachari G. Neem—an omnipotent plant: a retrospection. Chembiochem. 2004 Apr 2;5(4):408-21.

45. Turkez H, Arslan ME, Ozdemir O. Genotoxicity testing: progress and prospects for the next decade. Expert opinion on drug metabolism & toxicology. 2017 Oct 3;13(10):1089-98.

46. Kumar VS, Navaratnam V. Neem (Azadirachta indica): Prehistory to contemporary medicinal uses to humankind. Asian Pacific journal of tropical biomedicine. 2013 Jul 1;3(7):505-14.

47. Kilani-Morakchi S, Morakchi-Goudjil H, Sifi K. Azadirachtin-based insecticide: Overview, risk assessments, and future directions. Frontiers in agronomy. 2021 Jul 20;3:676208.

48. Dimetry NZ. Toxic Effect of Different Neem Formulations against Pests and Mammals. Journal of Botanical Research. 2020 Aug

4;2(3):6-19.

49. Wen H, Jung H, Li X. Drug delivery approaches in addressing clinical pharmacology-related issues: opportunities and challenges. The AAPS journal. 2015 Nov;17:1327-40.

50. Braga TM, Rocha L, Chung TY, Oliveira RF, Pinho C, Oliveira AI, Morgado J, Cruz A. Azadirachta indica A. Juss. In vivo toxicity—an updated review. Molecules. 2021 Jan 6;26(2):252.

51. Atawodi SE, Atawodi JC. Azadirachta indica (neem): a plant of multiple biological and pharmacological activities. Phytochemistry reviews. 2009 Oct;8:601-20.

52. Kirkland DJ, Aardema M, Banduhn N, Carmichael P, Fautz R, Meunier JR, Pfuhler S. In vitro approaches to develop weight of evidence (WoE) and mode of action (MoA) discussions with positive in vitro genotoxicity results. Mutagenesis. 2007 May 1;22(3):161-75.

53. Mužinić V, Želježić D. Neciljana toksičnost novih insekticida. Arhiv za higijenu rada i toksikologiju. 2018 Jun 27;69(2):86-101.

54. Chaudhary S, Kanwar RK, Sehgal A, Cahill DM, Barrow CJ, Sehgal R, Kanwar JR. Progress on Azadirachta indica based biopesticides in replacing synthetic toxic pesticides. Frontiers in plant science. 2017 May 8;8:610.

55. Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine. 2006 Feb 1;27(1):1-93.

56. Nicoletti M. New solutions using natural products. Insect-Borne Diseases in the 21st Century. 2020:263.

57. Shishupala S. Ethnomedicinal and Current Applications of Neem (Azadirachta indica). Ethnic Knowledge and Perspectives of Medicinal Plants. 2024:287-309.

58. Abebe W. Review of herbal medications with the potential to cause bleeding: dental implications, and risk prediction and prevention avenues. EPMA Journal. 2019 Mar 1;10:51-64.

59. Shlaes DM, Gerding DN, John Jr JF, Craig WA, Bornstein DL, Duncan RA, Eckman MR, Farrer WE, Greene WH, Lorian V, Levy S. Society for Healthcare Epidemiology of America and Infectious Diseases Society of America Joint Committee on the Prevention of Antimicrobial Resistance guidelines for the prevention of antimicrobial resistance in hospitals. Infection Control & Hospital Epidemiology. 1997 Apr;18(4):275-91.

60. Saini A, Singh B, Suttee A, Verma S, Garg LK, Thakur S. A review on phytoconstituents: the potent synergistic approach in antimicrobial formulations. J Pharm Res. 2018;12(2):151-9.

61. Warnke PH, Lott AJ, Sherry E, Wiltfang J, Podschun R. The ongoing battle against multi-resistant strains: in-vitro inhibition of hospital-acquired MRSA, VRE, Pseudomonas, ESBL E. coli and Klebsiella

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species in the presence of plant-derived antiseptic oils. Journal of Cranio-Maxillofacial Surgery. 2013 Jun 1;41(4):321-6.

62. Al-Samarrai G, Singh H, Syarhabil M. Evaluating eco-friendly botanicals (natural plant extracts) as alternatives to synthetic fungicides. Annals of Agricultural and Environmental Medicine. 2012;19(4).

63. Fernandes JB. Secondary metabolism as a measurement of efficacy of botanical extracts: The use of Azadirachta indica (Neem) as a model. Insecticides: Advances in Integrated Pest Management. 2012 Jan 5;367.

64. Mukherjee PK, Bahadur S, Chaudhary SK, Kar A, Mukherjee K. Quality related safety issue-evidence-based validation of herbal medicine farm to pharma. InEvidence-based validation of herbal medicine 2015 Jan 1 (pp. 1-28). Elsevier.

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