Original Article

In Vitro Antibacterial Activity of Ethanolic Extract of Neem Leave (Azadirachtaindica Linn) Against Clinical Isolates

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ABSTRACT

Background: Emerging multidrug resistance amongst pathogens is making the choice of antibiotics for the management of infections extremely difficult and threatens the return of the pre-antibiotic era in healthcare settings. To tackle this menace, there is a growing need for exploring bioactive compounds derived from herbal extracts, which could be incorporated as alternative therapeutic agents in the antimicrobial therapy of such infections.

Objectives: We evaluated the antibacterial activity of ethanolic extracts of Neem leaves against standard ATCC strains and the pathogens isolated from clinical specimens.

Methods: This cross-sectional study was undertaken to assess *in vitro* antibacterial activity of different concentrations of ethanolic Neem extract against three ATCC (American-type culture collection) strains and 63 clinical isolates using the disk diffusion method. The minimum inhibitory concentration (MIC) of the extract against test isolates was determined by the Broth dilution method.

Results: Neem extract exhibited the highest antimicrobial activity toward *Escherichia coli* ATCC-25922 followed by *Staphylococcus aureus* ATCC-25923 and *Pseudomonas aeruginosa* ATCC-27853 strains. Amongst the Gram-positive isolates, the extract exhibited significantly high antibacterial activity against *S. aureus* and *Enterococcus spp.* Amongst the Gram-negative isolates, high antibacterial activity was seen against *E. coli* followed by *Klebsiella pneumoniae* and *Proteus mirabilis.* In this study, the lowest MIC values were observed against *E. coli* followed by *S.aureus, P. mirabilis,* and *K. pneumoniae*. The highest MIC values of the extract were observed against non-fermenters, like *P. aeruginos*a and *Acinetobacter spp.* isolates.

Conclusion: This study strongly depicts that the ethanolic extract of Neem leaves exhibits significant antibacterial activity not only against the standard ATCC strains but also against various clinical isolates

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Introduction

Excessive usage of antibiotics is destructive to human health, the ecosystem, and the environment. The rampant irrelevant, irrational use of broad-spectrum antibiotics as empirical therapy, injudicious use of reserve drugs in the management of uncomplicated infections, and the misuse of antibiotics as over-the-counter drugs have led to the rapid emergence of drug-resistant bugs in the hospital as well as community settings. Multidrug resistance has been emerging rapidly and consistently in the commonly prevalent pathogens driven by selection pressure due to inappropriate irrational drug therapy. This has resulted in treatment failures leading to extended hospital stays, health complications, and a significant rise in morbidity and mortality. Emerging multidrug resistance amongst pathogens is making the choice of antibiotics for the management of infections extremely difficult and threatens the return of the pre-antibiotic era in Indian healthcare settings. A relatively slow pace of developing newer antibiotic molecules accompanied by the rapidity in developing drug resistance are major areas of concern [1, 2].

Most of the first- and second-line therapeutic agents are rendered ineffective against such strains and very few reserve drugs are left in the arsenal, which would meet the same fate in near future. The management of such infections is posing a serious challenge to the clinicians who are left helpless with very few alternatives left, which are also slipping off their hands. This is a big blow to our health care delivery system [3].

The World Health Organization (WHO) has recognized antimicrobial resistance as a global health security threat that requires action across government sectors and society as a whole [3].

To tackle this menace, there is a growing need for extensive research for exploring newer molecules derived from herbal extracts, which could be incorporated as an alternative or adjuvant therapeutic agent in the antimicrobial therapy of such infections. Botanical drugs prepared by processing crude herbal extracts are a mixture of several bioactive compounds and hence are harder to develop a resistance against [1, 2].

Numerous herbal products have antibacterial, antifungal, and antiprotozoal effects that could be used either systemically or locally. Plants have an amazing ability to produce a wide variety of bioactive secondary metabolites, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinines, coumarins, essential oils, lectin, polypeptides, polyacetylenes, catechins, catecholamines, thymol, and phenolic compounds. These biomolecules are the source of plant-derived antimicrobial substances (PDAMS) [4-7].

The plant extracts and phytochemicals, both with known antimicrobial properties, could be a potential alternative to antibiotics in the management of common infections [7]. The Neem tree (Azadirachtaindica) is an evergreen tree growing well in most tropical and semi-tropical countries, including India. This miraculous tree has multiple medicinal properties commonly used in Ayurvedic, Unani, and folklore traditional herbal medicine and is known as a potential source of many therapeutic agents [8, 9].

Almost every part of the Neem tree has some other medicinal value and has been used in household remedies to treat common ailments. The extract from bark, leaves, fruits, seeds, and roots has been used therapeutically for fever, skin diseases, leprosy, tuberculosis, intestinal helminthiasis, malaria, and respiratory infections in children. The use of Neem twigs (Datun) for brushing teeth is being practiced in traditional India as an effective form of dental care. Patients suffering from Chicken Pox are advised to sleep on Neem leaves in India due to their medicinal benefits. Amongst the innumerable plants explored as sources of antimicrobials, the Neem tree is one of the most promising, time-tested, and potential sources [10, 11].

Biological evaluation of herbal products, based on their use in traditional folklore practice is the key step in the development of new potential antibacterial agents from plants. Several studies paving the way for new drug discoveries have found that the therapeutic agents derived from plants could be used as an important surrogate, alternative, or complementary treatment of infectious diseases. Such concerted efforts are required for the research and development of new treatment strategies for combating these notorious bugs [12].

There is an urgent need to continue ethnobotanical research to find and document important medicinal plants endemic to the region and investigate their potential for antimicrobial drug discovery.

In light of the above facts, this cross-sectional analytical study was undertaken at a tertiary care teaching hospital in North India to evaluate the antibacterial activity of ethanolic extracts of Neem leaves against

standard ATCC strains and the pathogens isolated from clinical specimens.

We evaluated the antimicrobial activity of ethanolic extract of Neem tree leaves against standard ATCC strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* 25922, and *Pseudomonas aeruginosa* 27853) and clinical isolates.

Materials and Methods

This cross-sectional analytical study was conducted at the Department of Microbiology of a tertiary care teaching hospital in North India for two months after obtaining clearance from institutional ethics clearance. *In vitro* antibacterial activity of different dilutions of Neem extract was studied against:

Three standard American Type Culture Collection (ATCC) strains

1) *E. coli* (ATCC 25922)

2) *P. aeruginosa* (ATCC 27853)

3) *S. aureus* (ATCC 25923)

63 culture isolates obtained from clinical specimens sampled through convenience sampling [6]

Collection of plant materials

The leaves were harvested from Neem trees on the college campus and authenticated by a certified botanist with reference to the Voucher number: BSI-173100, Botanical Survey of India, Ministry of Forest, Environment and Climate Change, Western Regional Centre, Pune (BSI) [13].

Preparation of extracts by Cold extraction method

Leaves were cleaned, shade dried for one week, and pulverized to a coarse powder. About 25 g of powder was soaked in 100 mL of 95% ethanol and allowed to macerate at room temperature for seven days with intermittent shaking. This was followed by straining through sterile muslin cloth and filtering through sterile Whatman No. 1 filter paper. The filtrate was thereafter concentrated at 40°C. The solvent was completely evaporated to yield sticky black material and stored in sterile airtight containers at 4°C in the refrigerator. This material was reconstituted in 0.1% DMSO (dimethyl sulfoxide) prior to use to prepare five different concentrations of the extract: 200, 100, 50, 25, 12.5, and 6.25 mg/mL, which could be stored in separate sterile labeled aliquots in the refrigerator [2, 4, 14].

Standardization of extracts: Neem leaves collected from different localities were used to prepare different batches of the extracts. The antimicrobial efficacy of different batches was compared and the batch with better efficacy was selected. All the routine physicochemical and pharmacological parameters were checked in order to select the final finished product and also to validate the whole process [15-17].

Extract assay for Total Phenol Content: The total phenol content of the dried extract was assessed with the Folin- Ciocalteu's assay with the gallic acid as standard. Accordingly, 0.5 mL of extract solution was mixed with 1.5 mL Folin-Ciocalteu's reagent (FCR) diluted at 1:10 v/v and after 5 minutes, 1.5 mL of 7% sodium carbonate solution was added. The final volume of the tubes was made up of 10 mL using distilled water. The absorbance was recorded after 1.5 hour at 760 nm spectrometrically. The procedures were performed in triplicate. The values were expressed as Mean±SD in terms of phenol content (gallic acid equivalent, GAE) per gram of dry weight.

Extract assay for Total Flavonoid Content: The total flavonoid content was assessed by an aluminum chloride complex forming assay with quercetin as a standard. Accordingly,1 mL of the test sample was mixed with 4 mL of water in a volumetric flask (10 mL volume). Then, 0.3 mL of 5% sodium nitrite, and 10% aluminum chloride each were added after 5 minutes and left for 6 minutes in the dark at room temperature. Thereafter, 1 mL of 1 M sodium hydroxide was added to the reaction mixture. Immediately the distilled water was added to make the final volume of up to 10 mL. The absorbance was recorded at 510 nm spectrometrically. The values were expressed as Mean±SD in terms of flavonoid content (quercetin equivalent, QE) per gram of dry weight. The procedures were performed in triplicate [18].

Antibacterial activity test

The antibacterial activity of the ethanolic Neem extract was assessed by two methods:

1) Disc diffusion method

2) Broth dilution method

Disk diffusion antimicrobial tests

Modified disc diffusion assay (DDA) on Mueller Hinton Agar plate

Preparation of dried filter paper discs: Whatman filter paper No. 1 was used to prepare discs approximately 6 mm in diameter, which were placed in a Petri dish and sterilized by autoclaving.

Inoculum preparation: At least three to five well-isolated colonies of the same morphological type were selected from culture plates. The top of each colony was touched with a loop and the growth was transferred into a tube containing 4 to 5 mL of a suitable broth medium. The broth culture was incubated at 37°C until it achieved or exceeded the turbidity of the 0.5 McFarland standards (usually 2 to 6 hours).

The turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain turbidity optically comparable to the 0.5 McFarland standards. This results in a suspension containing approximately $1\text{-}2\times10^8$ CFU/mL bacteria.

A sterile cotton swab was inserted into the bacterial suspension and then rotated and compressed against the wall of the test tube to express the excess fluid. The surface of the Mueller-Hinton Agar plate was inoculated with the swab. A representative sample of each batch of the plate should be examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

Inoculation of test plates: Optimally within 15 minutes after adjusting the turbidity of the inoculum, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum. To obtain a uniform and confluent growth, the dried surface of a Mueller-Hinton Agar plate was inoculated by streaking the swab over the entire sterile agar surface three times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed. The lid was left ajar for 3 to 5 minutes, but no more than 15 min, to allow for any excess surface moisture to be absorbed before applying the disks.

Application of disk to inoculated agar: The six Whatman filter paper No. 1 paper disks approximately 6 mm in diameter were placed peripherally and pressed onto the surface of the inoculated agar plate. The markings corresponding to different concentrations, including negative control (NC) and positive control (PC) were made on the culture plate. A gentamicin (20 µg, Himedia) disk placed in the center was used as the PC. Disks were distributed eventually so that they were no closer than 24 mm from center to center. One drop of different concentrations of the extract (50 µL) comprising the moist weight of 7.08 mcg was placed on the corresponding five disks as per the markings on the culture plate. One drop of plain DMSO was added to the disk marked as NC. The plate was inverted and placed in an incubator set to 37°C within 15 minutes after the application of disks.

Reading plates and interpreting result: Plates were incubated at 37°C for 24 hours, and then the antibacterial activity was assessed based on the measurement of the diameter of the inhibition zone formed around the disk. The diameter of zones of inhibition was measured, including the diameter of the disc. The zones were measured to the nearest whole mm using a zone size measuring scale, which is held on the back of the inverted Petri plate [13, 19, 20, 23-25].

Dilution methods: Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobials in either agar or broth media.

Minimum inhibitory concentration (MIC)

Broth dilution method

The MIC of the extract for different test isolates was determined by the broth dilution method (Media used: Mueller-Hinton broth.

Five milliliters of Mueller-Hinton Broth were transferred to seven test tubes each. One test tube was labeled as PC (growth control tube), one as NC, and the remaining five test tubes were labeled as per the concentration of the Neem extract to be added (as 200, 100, 50, 25, and 12.5 mg/mL). Then, 100 µL of bacterial suspension equivalent to 0.5 McFarland standards was added in each test tube except NC. Then, 100 µL of different concentrations of the extract was added to the corresponding labeled test tubes except for controls, which were instead loaded with 100 µL of DMSO solvent. The broths were incubated at 37°C for 24 hours. After incubation, tubes were observed for any visible growth. MIC was expressed as the lowest concentration of the extract showing visibly complete clearance [6, 13, 19, 21, 22].

Results

This study was undertaken to evaluate the antibacterial activity of ethanolic extract of Neem tree leaves (Azadirachtaindica) against standard ATCC bacterial strains and clinical isolates. The assessment of the antibacterial activity of Neem extract in this study was based on a modified disk diffusion assay through the measurement of the diameter of zones of inhibition formed around the charged disks as depicted in Tables 1 and 2. Further, a better and more precise quantitative evaluation of the antibacterial activity of Neem extract was done by assessing MIC values against clinical isolates through the broth dilution method as depicted in Figure 1 and Table 3.

The Mean±SD values of total phenolic content and total flavonoid content in the neem extract turned out to be 68±0.46 and 114±2.7 mg QE/gm, respectively. Against standard bacterial strains, Neem extract exhibited the highest antimicrobial activity toward *E. coli* ATCC-25922 followed closely by *S. aureus* ATCC-25923 and *P. aeruginosa* ATCC-27853 strains. However, the *P. aeruginosa* strain was found to be resistant toward lower concentrations of extract (12.5 and 6.25 mg/mL). The marginal difference in the antibacterial activity of Neem extract against *S. aureus* and *E. coli* strains was evident

Table 1. Antibacterial activity of ethanolic Neem extract against standard ATCC strains

Bacterial Strains	Mean±SD (mm)							P**	F
	Inhibition Zone Diameter						Positive control Gentamicin (20 µgm)		
	200 (mg/mL)	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	6.25 (mg/mL)			
E. coli (ATCC 25922)	20.67±0.94	19±0.82	15.67±0.94	13.67±1.25	12±0.82	10.67±0.47	23.33±0.47	0	99.95
P. aeruginosa (ATCC 27853)	15.67±0.47	14.67±0.94	13±0.82	10.67±0.47	R	R	21.67±0.47	0	108.77
S. aureus (ATCC 25923)	19.33±0.47	16.67±0.47	14.67±1.25	13.67±0.47	11.67±0.94	10.33±0.47	19.67±0.94	0	116.93

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R: Resistant; Values are given as the mean of the triplicate experiment. Significance calculated using One-way ANOVA. *P<0.05: Significant; **P<0.001: Highly significant; P>0.05: Not significant.

Table 2. Antibacterial activity of Neem extract against laboratory isolates

Row	Clinical Isolates (No. of isolates)	Mean±SD (mm)						P**	F
		200 (mg/mL)	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	6.25 (mg/mL)		
1	*Staphylococcus aureus* (12)	18.5±1.45	15.9±1.16	13.8±1.47	13±1.41	10.8±1.27	8.92±1	0.00	123.32
2	*Enterococcus spp.* (5)	17±1.58	15.8±1.3	13.6±1.14	11±1	9.4±0.89	R	0.00	38.344301
3	*Escherichia coli* (15)	19.1±1.58	17.5±1.68	15.1±1.58	12.9±1.9	10.9±1.41	9.47±1.41	0.00	82.32
4	*Klebsiella pneumoniae* (10)	16.6±1.2	14.9±1.2	14.1±1.29	11.5±1.58	8.8±1.4	R	0.00	51.27
5	*Citrobacter spp.* (5)	14.2±1.64	13.8±1.3	11.8±1.3	11.2±0.84	9.8±1.3	R	0.000138	9.906
6	*Proteus mirabilis* (5)	15.6±1.14	14.8±1.3	13.6±1.14	11±1	9±1.58	R	0.00	24.17
7	*Pseudomonas aeruginosa* (7)	14±1.41	12.57±1.72	10.86±1.35	9.86±1.21	R	R	0.000075	11.44
8	*Acinetobacter spp.* (4)	9.5±1.29	R	R	R	R	R	-	-
Total: 63 isolates								-	-

PBR

R: Resistant; Significance calculated using One-way ANOVA. *P<0.05: Significant; **P<0.001: Highly significant; P>0.05: Not significant.

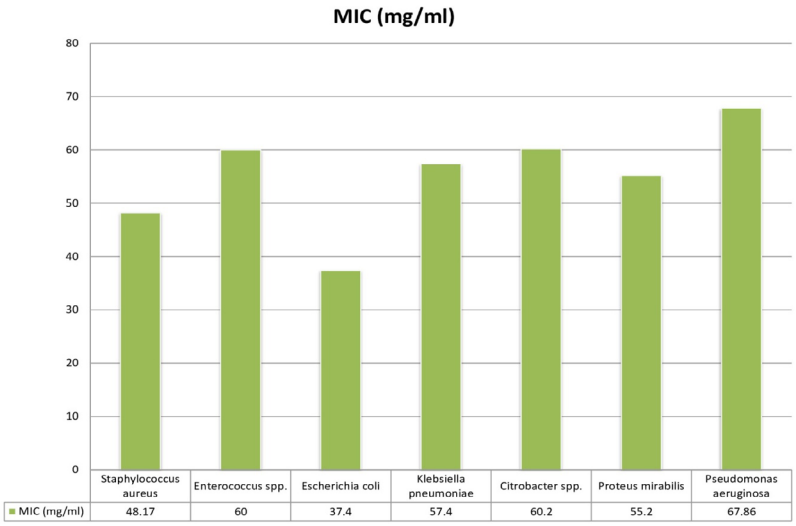

Figure 1. Average minimum inhibitory concentration (MIC) of ethanolic Neem extract against different clinical isolates

only at higher concentrations. At lower concentrations, the activity was almost the same for the two strains.

Amongst the Gram-positive isolates, the Neem tree extract exhibited significantly high antibacterial activity against *S. aureus* and *Enterococcus spp*. Amongst the Gram-negative isolates, high antibacterial activity was seen against *Enterobacteriaceae* group pathogens, like *E. coli* followed by *Klebsiella pneumoniae* and *Proteus mirabilis*.

Modest antibacterial activity was seen against *Citrobacter spp.* and *P. aeruginosa* only at high concentrations. At low concentrations of the extract, poor activity was seen against the two pathogens. Amongst all the test isolates, *Acinetobacter spp*. exhibited the least susceptibility toward the extract. Neem extract showed very weak antibacterial activity against *Acinetobacter spp.* at the highest tested concentration only (200 mg/mL). In general, the antibacterial activity of Neem extract exhibited a dose-dependent pattern against most of the tested strains, with a statistically significant increase in activity with increasing doses.

In this study, the lowest MIC values were observed against *E. coli* followed by *S. aureus, P. mirabilis,* and *K. pneumoniae*. The highest MIC values of the extract were observed against non-fermenters, like *P. aeruginosa* and *Acinetobacter spp*. isolates. In the case of *Acinetobacter* isolates, no visible clearance of growth was seen even at the highest tested concentration of extract.

Table 3. Mean±SD MIC of Neem extract against clinical isolates

No.	Organism	Mean±SD (mg/mL)	P (Z score)**
1.	*Staphylococcus aureus*	48.17±2.04	<0.00001
2.	*Enerococcus spp.*	60±3.08	<0.00001
3.	*Escherichoia coli*	37.4±2.26	<0.00001
4.	*Klebsiella pneumoniae*	57.4±2.12	<0.00001
5.	*Citrobacter spp.*	60.2±1.79	<0.00001
6.	*Proteus mirabilis*	55.2±2.17	<0.00001
7.	*Pseudomonas aeruginosa*	67.86±2.27	0.00008

MIC: Minimum inhibitory concentration; SD: Standard deviation; Significance calculated using Z-score, *P<0.05: Significant; **P<0.001:Highly significant; P>0.05: Not significant.

Discussion

The observations of this study strongly depict that the ethanolic extract of Neem tree leaves exhibits remarkably significant antibacterial activity not only against the standard ATCC strains but also against various clinical isolates. These findings are in accordance with a number of similar studies conducted recently [4, 9, 10, 20, 21, 24-27, 31, 34, 35].

A study by Abalaka et al. involving phytochemical screening of the Neem leaf extract assessed the antimicrobial activity of the extract by the agar cup plate technique. MIC and MBC values of the extract were also determined by broth dilution against *P. aeruginosa*, *K. ozanae*, *S. aureus*, and *E. coli* strains. Amongst all tested strains, the highest susceptibility toward the extract was exhibited by *P. aeruginosa* followed by *S. aureus*, *K. ozanae*, and *E. coli* [4].

Koona et al. reported the significant antimicrobial activity of methanolic Neem extract against *E. coli* and *Streptococcus faecalis*, but the lowest activity against *Bacillus subtilis. E. coli.* and *K. pneumoniae* strains were found to be highly susceptible to aqueous and ethanolic extracts [9].

Raja et al. compared the antimicrobial efficacy of aqueous extracts against various pathogenic bacteria (*S. aureus*, *Enterococcus faecalis, P. mirabilis*, and *P. aeruginosa*) and found that the leaf extract exhibited strong antimicrobial activity against these isolates at all the concentrations [10].

In a study by Rajasekaran et al., petroleum ether, dichloromethane, chloroform, ethanol, and aqueous extract of Neem leaves were tested against different bacterial strains and exhibited significant antibacterial activity against all strains. This activity was solvent-dependent and found to be higher for dichloromethane and ethanolic extracts compared to the others [24].

In a study by Rathod et al., the antimicrobial activity of aqueous and ethanol extracts of Neem leaves and bark was assessed by the disk diffusion method against standard bacterial strains. The ethanolic extracts of both Neem leaves and bark were found to exhibit remarkably higher antimicrobial activity compared to the aqueous extracts, against the standard strains. Like our study, the investigators reported high antimicrobial activity against *E. coli* and *S. aureus* followed by *K. pneumoniae* with the bark extract showing higher activity but exhibiting a similar spectrum [25].

Mohammad et al., Okemo et al., and Awasthi et al. found that the ethanolic extract of Neem leaves exhibited significant antimicrobial activity against a majority of test strains, especially *S. aureus* and *E. coli*. This finding is in accordance with our study. Maragathavalli further compared this activity with gentamycin and found that the ethanolic extract exhibited the strongest inhibitory effect on *S. aureus* followed by *P. aeruginosa* and *Bacillus pumillus* [20, 21, 26, 27].

The phytochemical composition of the A. indica has been explored and characterized in several ethnomedical studies and these include tannins, saponins, alkaloids, phenols, flavonoids, anthraquinones, cardiac glycosides, sterols, and resins [4, 28-31].

In many studies, the significant antibacterial activity of the Neem extracts was seen against *E. coli*, *Klebsiella pneumoniae*, and *Bacillus subtilis*, and needs further confirmation. Few investigators have reported the antimicrobial activity of methanolic Neem oil extract against *E. coli* and *K. pneumonia*. But similar activity was not seen with chloroform and hexane extracts as it is influenced by the pH of the final extract [32, 33].

In a study by Benisheikh et al., crude Neem leaf extracts were subjected to phytochemical screening and their antimicrobial activity against some pathogenic microbes was assessed by agar well diffusion technique. The investigators used chloroform, hexane, methanol, and ethyl acetate extracts of Neem leaves prepared by Soxhlet extraction. These extracts exhibited significant antimicrobial activity against the tested strains, the highest by chloroform extract followed by methanol extract toward *Streptococcus mutans*, *E. coli, S. aureus*, *P. aeruginosa*, and *Candida albicans*. The weak activity was recorded against *Aspergillus niger*, *Aspergillus flavus*, and *Streptococcus pyogenes* [34].

Subapriya and Nagini attributed the strong antibacterial and antifungal activity of Neem leaves to the presence of high concentrations of azadirachtins, quercetin, and *β-sitosterol* [35].

Neem tree is considered to be a rich source of diverse bioactive compounds. Several studies have linked these plant-derived bioactive components to antimicrobial activity. These active constituents can be further explored to derive bioactive lead compounds that could be partially utilized as the source of potent ingredients in developing pharmaceutically useful products. Hence, extensive investigation is needed to explore the various bioactive principles of Neem for their therapeutic utility [24, 28, 34, 35].

It is recommended that further research be done toward isolating, purifying, and standardizing the active antibacterial ingredients in *A. indica*. Also, more work should be carried out to determine the pharmacokinetics, pharmacodynamics, and possible toxicity of the active ingredients.

Conclusion

In conclusion, this study like many other *in vitro* studies conducted throughout the world regarding herbal antimicrobials proved, reconfirmed, and consolidated the fact that Neem extracts have significant antimicrobial activity at varying concentrations against different bacterial pathogens. The picture might be a bit different if the same study was conducted using individual bioactive compounds, like Azadirachtin rather than crude extracts. There is an immense scope of research in this field and such studies have paved the way for the exploration of a new group of herbal antimicrobials containing lead bioactive molecules, which could bring a new ray of hope in tackling the menace of emerging drug resistance. This could serve as an effective tool in hands of clinicians in the management of multidrug-resistant infections.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Institutional ethics committee of the Govt. Medical college, Datia (013/MIC/IECHP/DMC). In this study there was no direct involvement of human or animal participants. The antibacterial activity of the neem extract was tested against the stock strains preserved in the Lab.(the personal information regarding the source was kept confidential). So,as such there were no ethical issues to be considered in this research.

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Authors' contributions

Conceptualization and study designing: Abhishek Mehta; Literature search & data acquisition: Abhishek Mehta, Arti Jain; Data analysis and interpretation: Gaurav Saxena; Manuscript preparation: Abhishek Mehta, Manuscript editing & review: Arti Jain, Gaurav Saxena; Final version approval: All Authors; Agreement to be accountable for all aspects of the study: All Authors.

Conflict of interest

The authors declared no conflict of interest.

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