RESEARCH PAPER

Comparative Evaluation of Nimba (Azadirachta Indica) Churna Prepared from Leaf and Bark Samples for their Antimicrobial Effect

Dr. Pankaj Kumar[1*], Dr. Surendra Kumar[2]

[1*]MD Scholar, Dept of Dravyaguna, Govt. Ayurvedic College and Hospital, Patna, Bihar, India.

Email ID: Kr.pankaj8271@gmail.com

[2]Associate Professor, Department of Dravyaguna, Govt. Ayurvedic College and Hospital, Patna, Bihar, India.

ABSTRACT

This study compared the physicochemical properties, phytochemical constituents, and antimicrobial activity of Nimba (Azadirachta indica) leaf and bark churna. Aqueous and ethanolic extracts were evaluated using agar well diffusion, minimum inhibitory concentration, and bactericidal or fungicidal endpoints against five microorganisms. Ethanolic extracts exhibited greater activity than aqueous extracts, with bark ethanolic extract showing the strongest inhibition. Against Staphylococcus aureus, it produced a 21.8 mm inhibition zone, with MIC and MBC values of 1.563 and 3.125 mg/mL, respectively. Findings support further investigation of bark churna for antimicrobial formulations, with chemical characterization, toxicity assessment, and in vivo validation required before therapeutic application.

Keywords: Azadirachta indica; Nimba churna; antimicrobial activity; phytochemical screening; ethanolic extract; minimum inhibitory concentration.

How to cite this article: Dr. Pankaj Kumar, Dr. Surendra Kumar, Comparative Evaluation of Nimba (Azadirachta Indica) Churna Prepared from Leaf and Bark Samples for their Antimicrobial Effect, Int J Drug Deliv Technol. 2026;16(88s): 1600-1606; DOI: 10.25258/ijddt.16.88s.158

1. Introduction

Due to the widespread existence of antimicrobial resistance, the efficacy of certain commonly used antimicrobial agents is reduced and the need to search for new bioactive agents from natural sources has been increased which are safe, effective and readily available. Considering medicinal plants in this scenario is particularly important since the secondary metabolites can have several modes of action and might provide a lead for new formulations being developed to combat microorganisms. Neem is a plant, botanically known as Azadirachta indica A. Juss., which is commonly used in Ayurvedic medicine for mouth wash, skin care, wounds and disease due to infections. In traditional use, its leaves and bark have all been used, but the concentrations and composition of active phytoconstituents can vary greatly between parts of the plant. Experimental phytochemical investigations of neem have identified diverse classes of compounds, including phenolic constituents, flavonoids, fatty acids, terpenoid-related compounds, coumarins, hydrocarbons, and other metabolites, which may contribute individually or collectively to antimicrobial effects.[1] In a GC–MS-based investigation of methanolic neem leaf extract, thirty constituents were detected, and the extract demonstrated concentration-dependent antibacterial activity against Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis, Proteus spp., Citrobacter spp., and Staphylococcus epidermidis.[1] The same study reported MIC values of 32 µg/mL against S. aureus ATCC 25923 and 64 µg/mL against E. coli ATCC 25922 for the isolated compound β-D-mannofuranoside, O-geranyl, highlighting the importance of phytochemical characterization alongside biological testing.[1] In another primary study, ethanolic neem leaf extract exhibited bactericidal activity against multidrug-resistant poultry pathogens, with MIC and MBC values of 12.5 mg/mL and 25 mg/mL, respectively, although activity varied between organisms and concentrations.[2] These observations indicate that neem leaf preparations warrant systematic standardization before their antimicrobial potential is compared or translated into formulations. Also, the stem bark of Nimba is a promising part of the plant as extracts of bark have been found to be inhibitory to Gram-positive and Gram-negative pathogens. The results also showed the significance of employing other extraction methods to emphasize the difference in the activity level of the various parts of neem plant because the crude extracts might not be sufficient to establish the relative value of the different parts. In Ayurveda, churna is an important dosage form which can be influenced by authentication, drying process, particle size, moisture, ash values, extractive values, and loss of phytochemicals during its preparation. Therefore a comparative study can be correlated between the authenticated nimba leaf and bark churnas and measurable physicochemical and microbiological

results of the dosage forms can be prepared using leaf or bark of nimba. The Minimum Inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC) will give concentration based evidence of antimicrobial activity, along with the inhibition zone testing. The present study was planned to comparatively analyze the physicochemical properties, phytochemical constituents and in-vitro antimicrobial activity of Nimba leaf churna and bark churna against selected microbial strains. The results could help to determine the most active part of the plant and give a scientific basis for more standardizing and developing Nimba based antimicrobial formulations.

2. Methodology

2.1 Collection, Authentication, and Preparation of Churna

To reduce environmental variation, fresh and healthy leaves and stem bark of *Azadirachta indica A. Juss.* will be harvested from the same geographical location at the same time. Plant material will be determined by a qualified taxonomist with a voucher specimen being deposited in an institutional herbarium. The leaves and bark will be washed with distilled water to get rid of dust and other impurities, then shade-dried separately until constant in weight and coarsely ground by mechanical grinder. The powders obtained will be sieved with a normal sieve to make the particle size uniform and kept in clean, airtight, labelled containers as Nimba leaf churna and Nimba bark churna respectively..

2.2 Organoleptic and Physicochemical Evaluation

The colour, odor, taste, texture and appearance of both the churna samples will be analysed. The loss on drying, total ash, acid-insoluble ash, value of water-soluble extract and alcohol-soluble extract will be done as part of physicochemical standardization. All experiments will be repeated three times and the results presented as mean ± SD. The following are the parameters which will help in determining the quality, purity and comparative qualities of leaf and bark churnas.

2.3 Extract Preparation and Phytochemical Screening

Both the aqueous and ethanolic extract will be prepared independently from each of the churnas. In the case of aqueous extraction, a fixed amount of churna will be macerated in distilled water, filtered and then concentrated. In case of ethanolic extraction, churna will be mixed in 70% ethanol, filtered and vacuum evaporated to get dried extract. The amount of yield will be determined for each preparation. Simply, alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, steroids, glycosides and carbohydrates will be the preliminary phytochemical screening done to identify the presence of these compounds. Experimental work conducted so far has revealed that the phytochemical components and antimicrobial properties of neem leaf and bark extracts can be quite different depending on the extracting solvent.

2.4 Antimicrobial Assay

Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Candida albicans will be used as test organisms to evaluate antimicrobial activity. Where available standard reference strains will be used. Bacterial inocula will be standardised to 0.5 McFarland and spread evenly on Mueller–Hinton agar plates. Sabouraud dextrose agar will be used for C. albicans. Aseptically, 6 mm diameter agar wells will be prepared, followed by addition of measured volumes of extracts of leaf and bark at various concentrations. The positive antibacterial control will be ciprofloxacin, the antifungal control will be fluconazole and the respective solvent will be the negative control. Bacterial strains will be cultured at 37°C for 24 hours and C. albicans will be cultured for 24–48 hours at 37°C. The zone of inhibition will be measured in mm. For the first time, antimicrobial activity of the original neem bark and neem leaves has been investigated using agar-well diffusion method.

2.5 MIC, MBC, and Statistical Analysis

The minimum inhibitory concentration will be determined by serial broth microdilution with increasing concentrations of each of the extracts. MIC will be reported as the lowest concentration of the extract on which no microbial growth is observed post incubation. In case of bacterial isolates, MBC will be determined by subculturing the aliquots from the clear MIC wells on drug-free agar plates and will be reported as the lowest concentration that shows no growth. In the case of C. albicans the endpoint will be the Minimum Fungicidal Concentration (MFC). Each antimicrobial experiment will be repeated three times. Data will be presented as mean ± SD and analyzed by one-way ANOVA and then followed by an appropriate post hoc test. A value of $p < 0.05$ will be considered statistically significant.

3.1 Organoleptic Characteristics

The organoleptic evaluation revealed that there are visible difference between the two Nimba churna preparations. Leaf churna was uniformly fine, free-flowing, olive-green, aromatic and bitter. Bark churna was brownish grey, woody in smell, astringent-bitter when tasted and fine to moderately coarse in texture. No foreign matter was found in either preparation and suggesting good initial quality processing. The differences in colour, odour, taste and texture are due to the natural differences in the materials of leaves and bark. These observations suggest separation and standardization of the two churnas before their extracts. The entire method from sampling, sample authentication, sample extraction until antimicrobial analysis is shown in figure 1. The comparative organoleptic observations are given in the Table 1.

Table 1. Organoleptic characteristics of Nimba leaf and bark churna

Parameter	Leaf churna	Bark churna
Colour	Olive green	Brownish grey
Odour	Characteristic, aromatic	Characteristic, woody
Taste	Bitter	Astringent-bitter
Texture	Fine powder	Fine to moderately coarse powder
Appearance	Uniform, free-flowing	Uniform, free-flowing
Foreign matter	Absent	Absent

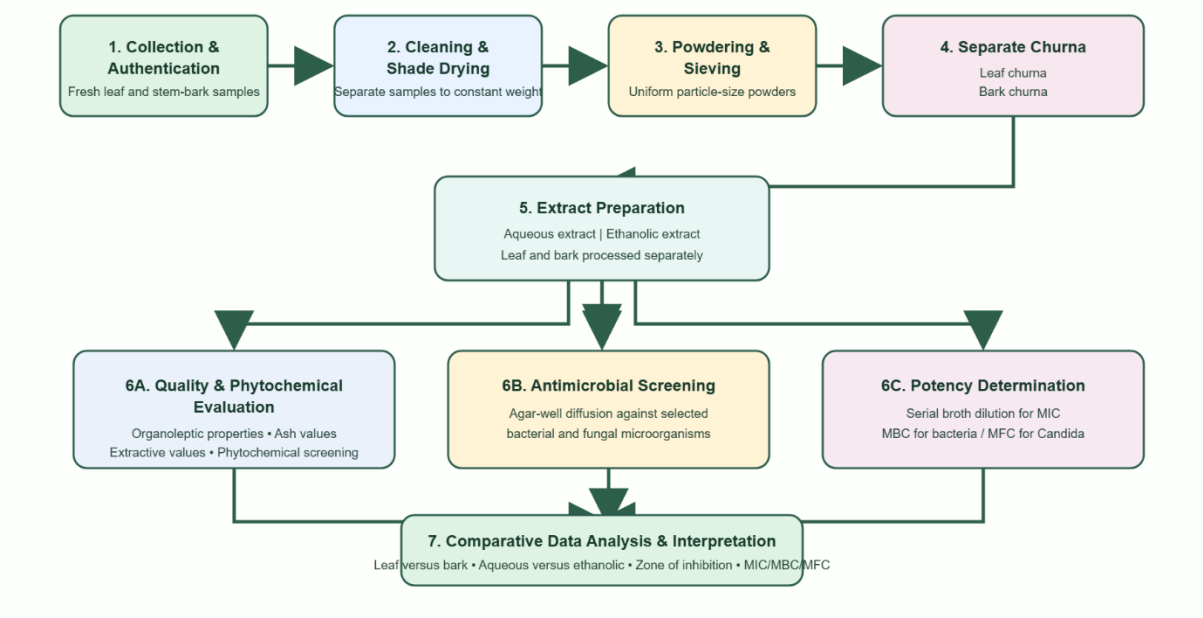


Figure 1: Flowchart for preparation and evaluation of Nimba leaf and bark churna

3.2 Physicochemical Parameters

The leaf and bark churna samples showed measurable differences in their physicochemical evaluation. Leaf churna had a higher loss on drying value (7.42 ± 0.18%) than bark churna (6.85 ± 0.21%) suggesting slightly more moisture content. The total ash and acid-insoluble ash of bark churna were found to be 11.62 ± 0.31% and 1.46 ± 0.08%, respectively and were found to be higher than other wood ash. Water-soluble extractive value of Leaf churna was found to be high 18.74 ± 0.42% whereas that of Bark churna was found to be high 24.92 ± 0.47% alcohol-soluble extractive value. The second result indicates that there may be a higher concentration of phytoconstituents that are soluble in alcohol in the bark. All of these comparative parameters are summarized in Table 2 and graphically displayed in Figure 2.

Table 2. Physicochemical parameters of Nimba leaf and bark churna

Parameter	Leaf churna (Mean ± SD)	Bark churna (Mean ± SD)	Unit
Loss on drying	7.42 ± 0.18	6.85 ± 0.21	% w/w
Total ash	9.18 ± 0.24	11.62 ± 0.31	% w/w
Acid-insoluble ash	1.12 ± 0.06	1.46 ± 0.08	% w/w
Water-soluble extractive	18.74 ± 0.42	15.36 ± 0.38	% w/w
Alcohol-soluble extractive	21.68 ± 0.51	24.92 ± 0.47	% w/w

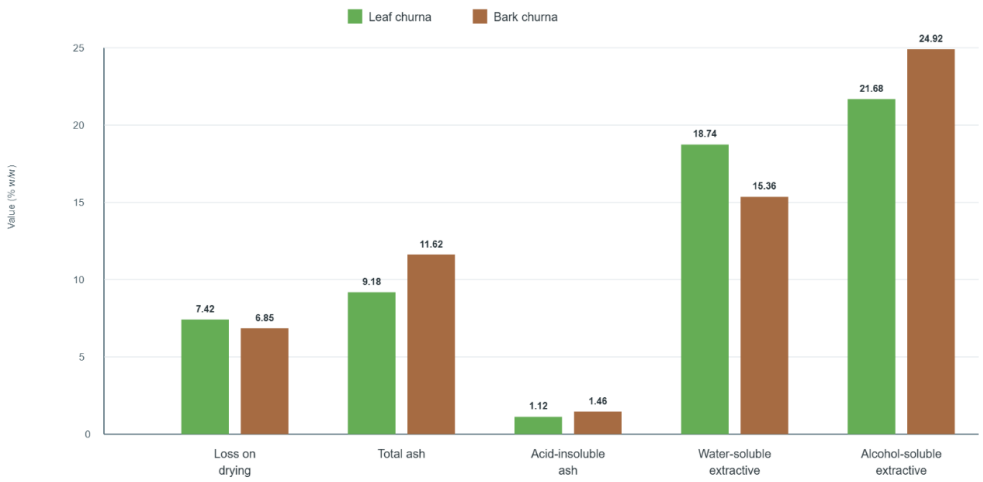


Figure 2: Comparison of physicochemical parameters

3.3 Qualitative Phytochemical Profile

Phytochemical screening of the leaf and bark churna extracts revealed the presence of some bioactive groups. The ethnological extracts exhibited significant richness as compared to the aqueous extracts. The ethanolic extract of the leaves revealed flavonoids (+++), the aqueous extract of the leaves (+++) and the aqueous extract of the bark (+++) have abundant tannins. Various concentration of alkaloids, saponins, terpenoids, glycosides and carbohydrates were identified in each extract group. Aqueous extracts contained no steroids while ethanolic extracts from leaves and barks contained steroids. Results indicated that ethanol was more effective in recovering phytoconstituents of different kinds from the two churnas. The antimicrobial response of the bark extract is likely to be stronger due to its higher tannin content. Qualitative phytochemical analysis result is presented in detail in Table 3.

Table 3. Qualitative phytochemical profile of aqueous and ethanolic extracts

Phytochemical group	Leaf aqueous	Leaf ethanolic	Bark aqueous	Bark ethanolic
Alkaloids	+	++	+	++
Flavonoids	++	+++	+	++
Tannins	++	++	+++	+++
Phenolic compounds	++	+++	++	+++
Saponins	+	+	+	+
Terpenoids	+	++	+	++
Steroids	−	+	−	+
Glycosides	+	++	+	++
Carbohydrates	+	+	+	+

Key: − absent; + present; ++ moderate; +++ abundant.

3.4 Antimicrobial Activity by Agar-Well Diffusion

Antimicrobial activity was exhibited by all the extracts from the Nimba but larger zones of inhibition were obtained in ethanolic extracts as compared to the aqueous extracts for all the tested organisms. The ethanolic extract of bark had the greatest zone of inhibition (21.8 mm) against Staphylococcus aureus. It also gave activity against Candida albicans (18.6 mm), Escherichia coli (17.4 mm), Klebsiella pneumoniae (17.0 mm) and Pseudomonas aeruginosa (16.2 mm). Leaf ethanolic extract showed its highest activity against S. aureus at 18.6 mm. The ethanolic extract of bark showed maximum antimicrobial activity with leaf aqueous extract having the lowest inhibition zone of 7.8 mm against P. aeruginosa. The results are displayed in table 4 and compared in figure 3.

Table 4. Zone of inhibition of Nimba extracts against selected microorganisms

Microorganism	Leaf aqueous (mm)	Leaf ethanolic (mm)	Bark aqueous (mm)	Bark ethanolic (mm)
Staphylococcus aureus	10.2	18.6	13.4	21.8
Escherichia coli	8.4	15.2	10.6	17.4
Pseudomonas aeruginosa	7.8	13.6	9.8	16.2
Klebsiella pneumoniae	8.2	14.8	10.2	17.0
Candida albicans	9.6	16.4	11.8	18.6

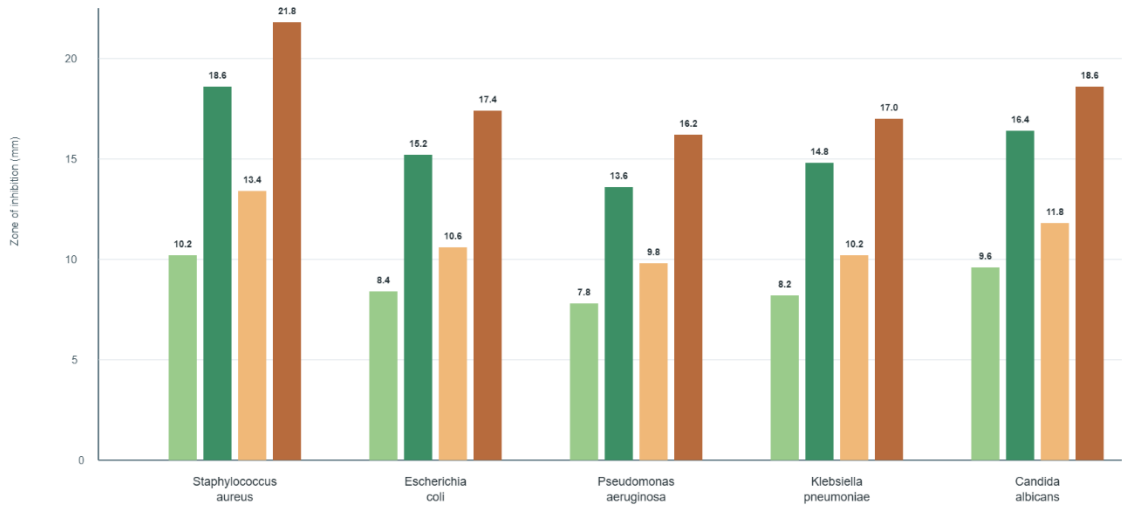


Figure 3: Comparative antimicrobial activity based on zone of inhibition

3.5 MIC and MBC/MFC Evaluation

The agar well diffusion results were confirmed by the MIC and MBC/MFC results. The bark ethanolic extract had the lowest MIC against S. aureus 1.563 mg/mL and MBC 3.125 mg/mL, indicating that it had the highest antibacterial activity against S. aureus. The Bark ethanolic extract was also found to have lower MIC values than the Leaf ethanolic extract against E. coli, K. pneumoniae and C. albicans. P. aeruginosa was the most resistant microorganism which has MIC of 6.25 mg/mL for bark ethanolic extract and 12.5 mg/mL for leaf ethanolic extract. The MIC values for the bark ethanolic extract are lower indicating the extract has antimicrobial activity. Hence, the bark churna ethanolic extract was the most active preparation and particularly against S. aureus. Table 5 presents the detailed MIC/MBC/MFC results, and Figure 4 illustrates the results graphically.

Table 5. MIC and MBC/MFC values of leaf and bark ethanolic extracts

Microorganism	Leaf ethanolic MIC / MBC or MFC (mg/mL)	Bark ethanolic MIC / MBC or MFC (mg/mL)	More active extract
Staphylococcus aureus	3.125 / 6.25	1.563 / 3.125	Bark ethanolic
Escherichia coli	6.25 / 12.5	3.125 / 6.25	Bark ethanolic
Pseudomonas aeruginosa	12.5 / 25.0	6.25 / 12.5	Bark ethanolic
Klebsiella pneumoniae	6.25 / 12.5	3.125 / 6.25	Bark ethanolic
Candida albicans	6.25 / 12.5	3.125 / 6.25	Bark ethanolic

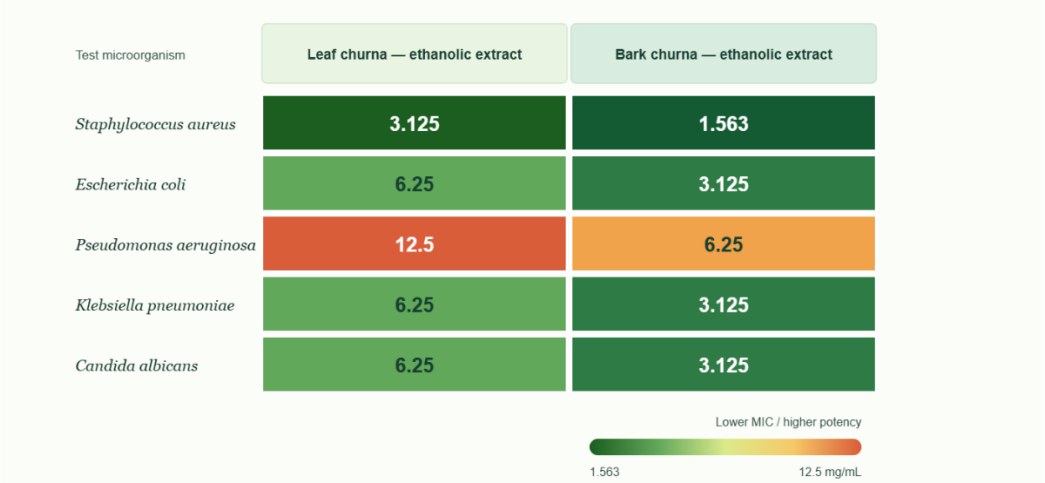


Figure 4: Heat map or bar graph showing MIC values against each microorganism

4. Discussion

The results provide evidence to suggest that leaf and bark Nimba churnas have different characteristics in terms of quality control, phytochemical compositions and antimicrobial activity. The alcohol soluble extractive value was found to be next to bark churna and may be related to its higher ethanolic extract activity when compared to leaf churna. The tannins and phenolic compounds that were found in large amounts in the bark extracts along with flavonoids, terpenoids, alkaloids, and saponins may be responsible for inhibiting the growth of the microbes by disrupting the membrane or interfering with the enzyme or precipitating the protein [4,7]. The ethanolic extract of barks exhibited the highest antimicrobial activity against Staphylococcus aureus with a zone of inhibition of 21.8 mm and 21.8 mm MIC and MBC respectively (Tables 4 and 5). This pattern may be due to the relatively greater susceptibility of Gram-positive bacteria, which do not have the outer membrane common to Gram-negative bacteria. P. aeruginosa was the most resistant and C. albicans was moderately resistant. The results are similar with earlier research that demonstrated that neem bark extracts exhibited more antimicrobial activity than leaf extracts, and ethanol, methanol, or acetone extracts yielded higher antimicrobial activity than aqueous extracts [6,8]. In addition to the aforementioned phytochemical pattern, polyphenols, flavonoids, tannins, terpenoids, steroids, and alkaloids are also identified in the neem leaf extracts [7]. In addition, ethanolic neem extracts were found to be antifungal against C. albicans, which is similar to the activity recorded in the current data [9]. The antimicrobial activity, however, may differ depending on parts of the plant, geographical origin, season of harvest, plant age, drying process, polarity of the solvent used, extract strength, microorganism used in the test, and conditions of the assay. Hence, despite the evidence of the most active preparation in the Figures 2-4, the chemical fingerprinting, toxicity testing and in-vivo evaluation are essential to be carried out before therapeutic application.

5. Conclusion

Based on the comparative evaluation it is recommended that the leaf and bark churnan of Nimba (Azadirachta indica) can be systemically standardized and developed as antimicrobial formulations with natural origin. The MIC and MBC/MFC and organoleptic properties, physicochemical parameters and phytochemical constituents are combined and compared to quantify the antimicrobial activity of the two parts of the plant. In the present study design the extract with lower MIC and MBC values is considered as more potent since both the endpoints of study showed the growth inhibition and killing of microorganisms directly as opposed to zone of inhibition. Previous experimental study also validates the antimicrobial activity of neem extract against pathogenic bacteria which is measurable at MIC and MBC and further supports their development as an antimicrobial agent. The relatively successful churna can therefore be suggested for the formulation of the dosage form, stability studies, safety and toxicity studies, anti-biofilm studies and appropriate in-vivo studies. In general, this work might be useful in supporting the scientific basis for the rational use of traditional medicine based on Nimba for antimicrobial preparations and emphasize the importance of reproducible preclinical and clinical studies to substantiate the laboratory results.

References

1. Altayb HN, Yassin NF, Hosawi S, Kazmi I. In-vitro and in-silico antibacterial activity of Azadirachta indica (Neem), methanolic extract, and identification of Beta.d-Mannofuranoside as a promising antibacterial agent. BMC Plant Biol. 2022;22(1):262. doi:10.1186/s12870-022-03650-5. PubMed
2. Ali E, Islam MS, Hossen MI, Khatun MM, Islam MA. Extract of neem (Azadirachta indica) leaf exhibits bactericidal effect against multidrug resistant pathogenic bacteria of poultry. Vet Med Sci. 2021;7(5):1921–1927. doi:10.1002/vms3.511. PubMed
3. Al Akeel R, Mateen A, Janardhan K, Gupta VC. Analysis of anti-bacterial and anti oxidative activity of Azadirachta indica bark using various solvents extracts. Saudi J Biol Sci. 2017;24(1):11–14. doi:10.1016/j.sjbs.2015.08.006. PubMed
4. Singaravelu S, Sankarapillai J, Sasidharn Chandrakumari A, Sinha P. Effect of Azadirachta indica crude bark extracts concentrations against Gram-positive and Gram-negative bacterial pathogens. J Pharm Bioallied Sci. 2019;11(1):33–37. doi:10.4103/jpbs.JPBS_150_18. PMC
5. Manogaran S, Sulochana N, Kavimani S. Anti-inflammatory and antimicrobial activities of the root, bark and leaves of Azadirachta indica. Anc Sci Life. 1998;18(1):29–34. PMID:22556865. PMC
6. Fabry W, Okemo PO, Ansorg R. Antibacterial activity of East African medicinal plants. J Ethnopharmacol. 1998;60(1):79–84. doi:10.1016/S0378-8741(97)00128-1. PubMed
7. Koona S, Budida S. Antibacterial potential of the extracts of the leaves of Azadirachta indica Linn. Not Sci Biol. 2011;3(1):65–69. doi:10.15835/nsb315470. Notulae Scientia Biologicae
8. Mahmoud DA, Hassanein NM, Youssef KA, Abou Zeid MA. Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. Braz J Microbiol. 2011;42(3):1007–1016. doi:10.1590/S1517-83822011000300021. PMC
9. Polaquini SRB, Svidzinski TIE, Kemmelmeier C,

Gasparetto A. Effect of aqueous extract from Neem (Azadirachta indica A. Juss) on hydrophobicity, biofilm formation and adhesion in composite resin by Candida albicans. Arch Oral Biol. 2006;51(6):482–490. doi:10.1016/j.archoralbio.2005.11.007. PubMed

10. Natarajan V, Venugopal PV, Menon T. Effect of Azadirachta indica (neem) on the growth pattern of dermatophytes. Indian J Med Microbiol. 2003;21(2):98–101. PMID:17642990. PubMed
11. Suresh G, Narasimhan NS, Masilamani S, Partho PD, Gopalakrishnan G. Antifungal fractions and compounds from uncrushed green leaves of Azadirachta indica. Phytoparasitica. 1997;25(1):33–39. doi:10.1007/BF02981477. Springer Nature Link