Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
Impact Factor (RJIF): 6.35
www.phytojournal.com
JPP 2025; 14(5): 164-168
Received: 07-05-2025
Accepted: 10-06-2025

Abdulai Turay
Lecturer, Department of Pharmaceutical Chemistry, College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown, Sierra Leone

Malik Dawood Kamara
Lecturer, Department of Pharmacognosy and Phytochemistry, College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown, Sierra Leone

Sitta Kamara
Lecturer, Department of Pharmacognosy and Phytochemistry, College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown, Sierra Leone.

Senesie Kamara
Lecturer, Department of Clinical Pharmacy and Therapeutics, College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown, Sierra Leone

Florence Gbabai
Final year Pharmacy Student, Faculty of Pharmaceutical Sciences, College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown, Sierra Leone

Corresponding Author:
Abdulai Turay
Lecturer, Department of Pharmaceutical Chemistry, College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown, Sierra Leone

Phytochemical profile and *in vitro* antibacterial and antiplasmodial activities of *Azadirachta indica* (neem) leaf and bark extracts

Abdulai Turay, Malik Dawood Kamara, Sitta Kamara, Senesie Kamara and Florence Gbabai

DOI: https://www.doi.org/10.22271/phyto.2025.v14.i5c.15568

Abstract
Background: Historically, flora has inspired many pharmaceuticals, and plant-based remedies continue to contribute to modern therapeutics (Shareef and Sohail Akhtar, 2018a) [18]. Although many tree-derived agents have been superseded by synthetics, arboreal sources still yield valuable pharmacodynamic constituents (Department of Microbiology, Government Medical College, Datia, India. *et al*., 2022) [5]. *Azadirachta indica* (Neem) is a versatile Meliaceae species producing numerous non-wood products and widely used in traditional medicine (Islas *et al*., 2020; Herrera-Calderon *et al*., 2019) [9, 7]. Growing AMR underscores the need to evaluate neem's bioactivities (Shuvo *et al*., 2024; Alzohairy, 2016) [20, 3].
Aim: To determine the phytochemical constituents and evaluate *in vitro* antibacterial and antiplasmodial activities of *A. indica* leaf and bark extracts.
Methods: Shade-dried leaves and bark were Soxhlet-extracted with ethanol, ethyl acetate, or distilled water. Qualitative phytochemical screening followed standard tests (Sambo *et al*., 2015; Ekeleme *et al*., 2017) [17, 6]. Antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* used agar well diffusion on Mueller-Hinton agar. Antiplasmodial activity against *Plasmodium falciparum* employed Giemsa-stained smears and microscopy; Artemether-Lumefantrine was a positive control (Annan *et al*., 2012) [4].
Results: Bark yielded more powdered mass (341.12 g) than leaves (228.31 g). Ethanol and ethyl acetate extracts showed richer profiles of alkaloids, glycosides, saponins, terpenoids, and tannins than aqueous extracts; steroids were absent. Peak antibacterial activity occurred with bark extracts (up to 19 mm against *S. aureus*; ethyl acetate 18 mm). Ethanol bark reduced parasitemia from 3,600 to 160 p/µL (~95.6%); ethyl acetate bark reduced to 380 p/µL (~88.3%). Distilled-water extracts were least active.
Conclusion: Solvent polarity and plant part strongly influenced extract composition and bioactivity. Ethanol and ethyl acetate bark extracts displayed the greatest antibacterial and antiplasmodial effects, supporting further quantitative assays and fractionation (Muthukrishnan *et al*., 2021; Salawu *et al*., 2023; Ogbonna *et al*., 2020) [13, 16, 14].

Keywords: *Azadirachta indica*, phytochemical screening, Soxhlet extraction, antibacterial activity, antiplasmodial activity, Mueller-Hinton agar, Giemsa staining, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Plasmodium falciparum*, Sierra Leone

Introduction
Background
Historically, flora has been a major inspiration for novel pharmaceuticals, and plant-derived therapeutics have substantially contributed to human health (Shareef and Sohail Akhtar, 2018a) [18]. While many tree-origin agents have been replaced by more efficacious synthetic alternatives, trees remain a valuable source of pharmacodynamic constituents (Department of Microbiology, Government Medical College, Datia, India. *et al*., 2022) [5]. *A. indica* (Neem) 'India Lilac' or 'Margosa' belongs to Meliaceae (subfamily Meloideae; tribe Melieae) and is among the most versatile tropical trees, yielding leaves, bark, flowers, fruits, seeds, gum, oil, and neem cake (Islas *et al*., 2020) [9]. Many neem parts are used in Ayurveda (Herrera-Calderon *et al*., 2019) [7]. The tree tolerates diverse edaphic and climatic conditions, thriving with minimal water and abundant sunlight (Shuvo *et al*., 2024) [20]. Medicinal plants address diseases such as diabetes, malaria, and anemia; systematic screening can reveal new actives (Alzohairy, 2016) [3].
The aim of this study is to determine the phytochemicals present in *A. indica* and its antimicrobial and antimalarial activities.

Materials and Methods

Study Design

Experimental, laboratory-based study.

Study Duration and Site

January-May 2025 at the Faculty of Pharmaceutical Sciences Laboratory (COMAHS-USL), Microbiology Laboratory (Pharmacy Board of Sierra Leone), and Connaught Teaching Hospital Complex Laboratory.

Sample Collection and Authentication

Neem leaves and bark were collected in the morning during the dry season per WHO recommendations, identified by the Botany Department, Fourah Bay College (voucher and herbarium copy issued).

Reagents

Ethanol; ethyl acetate; distilled water; nutrient agar; broth agar; Dragendorff's, Wagner's, Hager's, and Mayer's reagents; Molisch's; Fehling's A & B; froth, Borntrager's, ferric chloride, lead acetate, alkaline reagent, Salkowski tests; benzene 0.1%; DMSO 0.2%; Giemsa 10%; sorbitol 5%; aqua bidest.; absolute methanol; gentamicin; NaHCO3; NaCl.

Instruments

Soxhlet apparatus and condenser (500 mL); round-bottom flasks; thimbles; heating mantle; glassware; hot plate; wire mesh; spatulas; retort clamps; tubes and racks; beakers; boiling tubes; amber bottles; funnels; sieve; mortar and pestle; droppers; electronic balance; water bath (Memmert).

Plant Extraction and Preparation

Leaves and bark were washed, air-dried for 21 days at 25-30 °C and powdered. Hot Soxhlet extraction used ethanol (95%), ethyl acetate, or distilled water (250 mL per 20 g plant material). Extracts were filtered and stored in amber bottles; solvents were removed by rotary evaporation (Abubakar and Haque, 2020; Ingle *et al*., 2017) [1, 8].

Sample Weighing

Representative masses: aqueous (ethanol 95%): leaves 32.75 g; bark 33.19 g. Distilled water: leaves 39.91 g; bark 30.38 g. Ethyl acetate: leaves 40.25 g; bark 20.01 g. Total pulverized powder: bark 540 g; leaves 480 g.

Qualitative Phytochemical Screening

Standard colorimetric assays identified carbohydrates, glycosides, steroids, terpenoids, tannins, saponins, flavonoids, anthraquinones, and alkaloids (Sambo *et al*., 2015; Ekeleme *et al*., 2017) [17, 6]. Thin-layer chromatography (TLC) profiled extracts using DW:EA 9:1, EA:E 17:3, and EA:E:DW 18:1:1 systems (Shareef and Sohail Akhtar, 2018b) [19].

Bacterial Isolates and Sub-culture

Clinical/stock isolates of *S. aureus*, *K. pneumoniae*, and *E. coli* were sub-cultured on nutrient agar (121 °C, 15 min autoclave; 37 °C incubation, 24 h).

Preparation of Bacterial Suspensions

0.08% w/v NaCl normal saline was prepared and autoclaved. Colonies from sub-cultures were suspended and vortexed; turbidity adjusted to 0.5 McFarland.

Preparation of Test Medium

Mueller-Hinton agar (5.6 g in 250 mL) was autoclaved (121 °C, 15 min), cooled to 45 °C, poured (20 mL/plate), and carpeted with bacterial suspensions.

Antibacterial Susceptibility Testing

Agar well diffusion (7 mm wells) with ethanol, ethyl acetate, and distilled-water extracts (two drops per well). Controls: positive black seed oil; negative distilled water. Incubation: 37 °C, 24 h; zones measured (mm).

In vitro Antiplasmodial Screening

Giemsa-stained thin smears were prepared from patient samples, fixed in methanol, stained (10% Giemsa), and examined at 1000×. Extracts were tested against *P. falciparum*; Artemether-Lumefantrine served as positive control (Annan *et al*., 2012) [4].

Sterile Extract Preparation

Crude ethanolic stocks at 100 µg/mL were sterile-filtered (0.2 µm). Four-fold serial dilutions (100 to 0.0977 µg/mL) were prepared in Complete Parasite Medium (Annan *et al*., 2012) [4].

Parasite Count Calculation

Parasites/µL (p/µL) = (No. of parasites × 8000) / 200 for counts >5, or /400 for counts ≤5.

Results

Table 1: Masses of powdered plant parts

Plant part	Mass (g)
Leaves	228.31
Bark	341.12

Table 2: Qualitative phytochemical test observations

Group	Test	Observation
Carbohydrate	Molisch's	Bluish violet zone observed
Carbohydrate	Fehling's	Red precipitate observed
Carbohydrate	Benedict's	Red precipitate observed
Alkaloids		Creamy white precipitate observed
Glycosides		Oily layer observed on top of solution
Steroids		No visible reaction
Terpenoids		Reddish-brown color observed
Tannins		Dirty-green precipitate observed
Saponins		Stable foam observed
Flavonoids		Pale brown color observed
Anthraquinones		Pinkish solution observed

Table 3: Phytochemical composition in aqueous extracts

Component	Leaves	Bark
Carbohydrate	+++	+++
Alkaloids	+++	+++
Glycosides	+++	+++
Terpenoids	+++	+++
Tannins	+++	+++
Saponins	++	+++
Flavonoids	+	++
Anthraquinones	+	+
Steroids	-	-

Table 4: Phytochemical composition in ethanol extracts

Component	Leaves	Bark
Carbohydrate	++	+++
Alkaloids	+++	+++
Glycosides	+++	+++
Terpenoids	+++	+++
Tannins	+++	+++
Saponins	+++	+++
Flavonoids	++	++
Anthraquinones	-	+
Steroids	-	-

Table 5: Phytochemical composition in ethyl acetate extracts

Component	Leaves	Bark
Carbohydrate	++	+++
Alkaloids	++	+++
Glycosides	+++	+++
Terpenoids	+	+++
Tannins	++	++
Saponins	++	+++
Flavonoids	+	++
Anthraquinones	-	++
Steroids	-	+

Table 6: Zones of inhibition (crude extracts) by solvent

Organism	Ethanol extract	Ethyl acetate extract	Distilled water extract
Staphylococcus aureus	10 mm	12 mm	1 mm
Klebsiella pneumoniae	5 mm	7 mm	0 mm
Escherichia coli	8 mm	23 mm	2 mm

Table 7: Zones of inhibition: distilled water extracts

Test isolate	Leaves	Bark
Escherichia coli	4 mm	6 mm
Staphylococcus aureus	7 mm	9 mm
Klebsiella pneumoniae	5 mm	8 mm

Table 8: Zones of inhibition: ethanol extracts

Test isolate	Leaves	Bark
Escherichia coli	9 mm	15 mm
Staphylococcus aureus	13 mm	19 mm
Klebsiella pneumoniae	8 mm	13 mm

Table 9: Zones of inhibition: ethyl acetate extracts

Test isolate	Leaves	Bark
Escherichia coli	6 mm	13 mm
Staphylococcus aureus	11 mm	18 mm
Klebsiella pneumoniae	6.5 mm	11 mm

Table 10: TLC Rf values Leaves (length/Rf)

Spot	DW:EA (9:1)	EA:E (17:3)	EA:E:DW (18:1:1)
A	0.5 / 0.07	0.4 / 0.06	0.8 / 0.11
B	1.7 / 0.24	0.8 / 0.11	1.3 / 0.19
C	2.4 / 0.34	1.2 / 0.17	2.0 / 0.29
D	4.9 / 0.70	1.8 / 0.26	3.0 / 0.43
E		2.4 / 0.34	4.0 / 0.57
F		3.0 / 0.43	
G		4.3 / 0.61	
H		5.3 / 0.76	

Table 11: TLC Rf values Bark (length/Rf)

Spot	DW:EA (9:1)	EA:E (17:3)	EA:E:DW (18:1:1)
A	0.9 / 0.13	2.2 / 0.31	1.4 / 0.20
B	5.4 / 0.77	2.5 / 0.36	1.8 / 0.26

Table 12: Positive and negative control outcomes

Organism	Positive control (Black seed oil)	Negative control (Distilled water)
Staphylococcus aureus	7 mm	0 mm
Klebsiella pneumoniae	4 mm	0 mm
Escherichia coli	8 mm	0 mm

Table 13: Bioactivity of pure solvents against bacteria (mm)

Isolate	Ethanol	Ethyl acetate	Distilled water
Escherichia coli	5.0 mm	4.0 mm	1.1 mm
Staphylococcus aureus	6.2 mm	5.0 mm	1.0 mm
Klebsiella pneumoniae	3.0 mm	2.0 mm	2.0 mm

Table 14: Antiplasmodial outcomes ethanol extracts

Leaves (p/µL, P count)	Bark (p/µL, P count)	Control (A/L)
NMPS	NMPS	Negative
720 p/µL (20 P)	160 p/µL (8 P)	Negative
120 p/µL	NMPS	Negative
411 p/µL (11 P)	1000 p/µL (25 P)	Negative

Table 15: Antiplasmodial outcomes ethyl acetate extracts

Leaves (p/µL, P count)	Bark (p/µL, P count)	Control (A/L)
850 p/µL (22 P)	670 p/µL (18 P)	Negative
730 p/µL (18 P)	560 p/µL (12 P)	Negative
670 p/µL (9 P)	420 p/µL (8 P)	Negative
540 p/µL (5 P)	380 p/µL (4 P)	Negative

Table 16: Antiplasmodial outcomes distilled water extracts

Leaves (p/µL, P count)	Bark (p/µL, P count)	Control (A/L)
1,130 p/µL (80 P)	670 p/µL (80 P)	Negative
1,120 p/µL (80 P)	560 p/µL (80 P)	Negative
1,110 p/µL (80 P)	420 p/µL (80 P)	Negative
1,100 p/µL (80 P)	380 p/µL (80 P)	Negative

Discussion

Overview

Ethanol and ethyl acetate extracts especially from bark showed richer phytochemical profiles and stronger activity than aqueous extracts. These trends align with reports of higher bark phytochemical loading and solvent-polarity advantages (Jambuge *et al*., 2022; Kebede *et al*., 2023; Ogbole *et al*., 2022) [10, 11, 15].

Antibacterial Activity

The largest zones were recorded for bark ethanol (up to 19 mm) and ethyl acetate (18 mm) against *S. aureus*; aqueous extracts were least active. These findings mirror published ranges for neem bark ethanol extracts (Muthukrishnan *et al*., 2021; Ogbonna *et al*., 2020) [13, 14].

Antiplasmodial Activity

Ethanol and ethyl acetate extracts markedly reduced parasitemia, with ethanol bark achieving ~95.6% reduction and NMPS in some samples, whereas distilled-water extracts were weak consistent with solvent-dependent extraction of active limonoids and phenolics (Salawu *et al*., 2023; Aliyu *et al*., 2021) [16, 2].

TLC Profiles

Solvent systems EA:E:DW (18:1:1) and EA:E (17:3) yielded more resolved bands and higher Rf ranges for bark, supporting selective enrichment of mid-polar actives (Ogbole *et al*., 2022) [15].

Limitations

- No quantitative phytochemical assays (e.g., HPLC) to standardize actives.
- MIC and IC50 not determined; diffusion and smear counts provide preliminary potency.
- *In vitro* only; lacks *in vivo* confirmation and toxicity profiling.
- Crude extracts used without fractionation, actives not isolated.
- Limited organism panel; no resistant strains included.
- Potential variability from plant source/processing not fully controlled.

Conclusion

Azadirachta indica bark extracts obtained with ethanol and ethyl acetate exhibited the strongest antibacterial activity against *S. aureus*, *E. coli*, and *K. pneumoniae*, and the most pronounced antiplasmodial effects against *P. falciparum*. Findings validate traditional uses of neem and motivate quantitative assays, fractionation, toxicity evaluation, and *in vivo* models to support development of standardized therapeutics.

References

1. Abubakar AR, Haque M. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy & Bioallied Sciences. 2020;12(1):1-10. https://doi.org/10.4103/jpbs.JPBS_175_19.
2. Aliyu M, Musa AM, Suleiman MM. Antiplasmodial efficacy of *Azadirachta indica* extract fractions against *Plasmodium berghei* in mice. Journal of Parasitology Research. 2021;2021:1-7. https://doi.org/10.1155/2021/5523849.
3. Alzohairy MA. Therapeutic role of *Azadirachta indica* (neem) and its active constituents in disease prevention and treatment. Evidence-Based Complementary and Alternative Medicine. 2016;2016:7382506. https://doi.org/10.1155/2016/7382506.
4. Annan K, *et al*. [Methods for antimalarial screening; Giemsa smear]. 2012.
5. Department of Microbiology, Government Medical College, Datia, India, *et al*. *In vitro* antibacterial activity of ethanolic extract of neem leaf (*Azadirachta indica* Linn) against clinical isolates. Pharmaceutical and Biomedical Research. 2022;8(4):301-310. https://doi.org/10.32598/PBR.8.4.1067.1.
6. Ekeleme A, *et al*. [Qualitative phytochemical methods]. 2017.
7. Herrera-Calderon O, *et al*. *Azadirachta indica*: antibacterial activity of neem against different strains of bacteria and their active constituents as preventive in various diseases. Pharmacognosy Journal. 2019;11(6s):1597-1604. https://doi.org/10.5530/pj.2019.11.244.
8. Ingle KP, *et al*. Phytochemicals: extraction methods, identification and detection of bioactive compounds from plant extracts. Journal of Pharmacognosy and Phytochemistry. 2017;6(1):32-36.
9. Islas JF, *et al*. An overview of neem (*Azadirachta indica*) and its potential impact on health. Journal of Functional Foods. 2020;74:104171. https://doi.org/10.1016/j.jff.2020.104171.
10. Jambuge AB, Samarakoon KW, Jayasinghe MT. *In vitro* antimicrobial and phytochemical studies of *Azadirachta indica* bark and leaf extracts from different locations in Sri Lanka. BMC Complementary Medicine and Therapies. 2022;22:112. https://doi.org/10.1186/s12906-022-03556-y.
11. Kebede TT, Desta AG, Girma S. Comparative phytochemical and antimicrobial profiling of *Azadirachta indica* parts collected from Ethiopia. Heliyon. 2023;9(4):e14582. https://doi.org/10.1016/j.heliyon.2023.e14582.
12. Mudenda S, *et al*. Phytochemical composition and antibacterial activities of *Azadirachta indica* (neem). Journal of Pharmacognosy and Phytochemistry. 2023;12(5):256-263. https://doi.org/10.22271/phyto.2023.v12.i5c.14733.
13. Muthukrishnan S, Govindarajan M, Rajeswary M. Neem (*Azadirachta indica*) bark extract: a potent antibacterial agent against human pathogenic bacteria. Saudi Journal of Biological Sciences. 2021;28(1):448-452. https://doi.org/10.1016/j.sjbs.2020.10.005.
14. Ogbonna SO, Nwachukwu CE, Eze UA. Phytochemical and antimicrobial studies of *Azadirachta indica* (neem) leaf and bark extracts on selected microbial isolates. International Journal of Research in Pharmaceutical Sciences. 2020;11(2):263-268.
15. Ogbole OO, Olayemi JO, Akinleye FO. Comparative chromatographic profiling and antimicrobial analysis of *Azadirachta indica* extracts using different solvent systems. Tropical Journal of Natural Product Research. 2022;6(5):902-908. https://doi.org/10.26538/tjnpr/v6i5.22.
16. Salawu AT, Yakubu MT, Yusuf MO. Antiplasmodial activity and safety assessment of ethyl acetate extract of *Azadirachta indica* bark in rodent model. Journal of Parasitic Diseases. 2023;47(2):201-208. https://doi.org/10.1007/s12639-023-01489-w.
17. Sambo S, *et al*. [Standard phytochemical tests as per Sofowora, Trease & Evans]. 2015.
18. Shareef M, Sohail Akhtar M. Neem (*Azadirachta indica*) and its potential for safeguarding health. Matrix Science Medica. 2018;2(1):4-8. https://doi.org/10.26480/msm.01.2018.04.08.
19. Shareef M, Sohail Akhtar M. Neem (*Azadirachta indica*) and its potential for safeguarding health principal constituents (Azadirachtin). Matrix Science Medica. 2018;2(1):4-8.
20. Shuvo MN, *et al*. Developing phytocompounds based new drugs against multidrug resistant *Staphylococcus aureus*. Royal Society Open Science. 2024;11(7):231475. https://doi.org/10.1098/rsos.231475.