World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Evaluation of African Neem Plant (Dogon Yaro) antibacterial activity against certain pathogenic organisms

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World Journal of Advanced Research and Reviews, 2024, 24(01), 2208–2214

Publication history: Received on 13 September 2024; revised on 19 October 2024; accepted on 22 October 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.24.1.3232

Abstract

The neem plant is a tropical evergreen plant native to the Indian sub-continent. It has been used in ayurvedic medicine for more than 4000 years due to its medicinal properties. The phytochemical screening carried out on the extract of Neem plant revealed the presence of some active ingredients such as alkaloids, tannins, saponins, and phenols also glycosides, steroids, Terpenoids, flavonoids, and phlobatanins are present in this extract. It was observed that alkanoids has a concentration of 0.52g/ml, tannins has a concentration of 9.00g/ml, saponins has a concentration of 1.99g/ml, flavonoid has a concentration of 0.62g/ml and phenol has a concentration of 0.024g/ml. The antimicrobial activity of the Leaf, Stem, Stem-bark and Root extract of Neem plant *(Azadirachta indica)* against some of the pathogenic bacteria such as *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Staphylococcus epidermidis,* was determined. This was done by using alcoholic and water extracts of Neem leaves, stem, stem bark and roots. Varying concentration of each extracts 200mg/ml, 150mg/ml and 100mg/ml were prepared and tested. When compared with Gentamycin 10mg, the Ethanol Extract of Neem Root shows maximum inhibition on *Pseudomonas aeruginosa, Staphylococcus epidermidis, Staphylococcus aureus* and *E.* coli in an ascending order, followed by the ethanol extract of Neem Leaf which shows maximum inhibition on *Pseudomonas aeruginosa, Staphylococcus aureus, E. coli* and *Staphylococcus epidermidis* in an ascending order. Conclusively, this can explain the rationale for the use of the plant in treating infections in traditional medicine. The plant could be a veritable and cheaper substitute for conventional antibiotics since it is easily obtainable and the extract can easily be made via a simple process of maceration or infusion. However, further research is necessary to determine and purify the active components against micro-organisms.

Keywords: Antibacterial; Neem; Plant; Dogon Yaro; Medicinal plant

1. Introduction

The neem is a tropical evergreen plant native to the Indian sub-continent (Roxburgh, 1874). It has been used in ayurvedic medicine for more than 4000 years due to its medicinal properties. Neem is a natural source of eco-friendly insecticides, pesticides and agrochemicals (Usharani *et al*., 2019). Neem is considered to be part of India's genetic diversity (Sateesh, 1998). Neem is used in traditional medicine as a source of many therapeutic agents in the Indian

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culture and grows well in the tropical countries. Its twigs provide a chewing stick and are widely used in the Indian sub-continent (Subhashini *et al*., 2023), earlier studies on Neem have shown that it contains active substances with multiple medicinal properties (Prabagar *et al*., 2020). *Azadirachta indica* in folklore medicine for the treatment of Diabetes and show the potential role of anti-diabetic activity (Moaty *et al*., 2022). Aqueous extract of Neem leaf as a good therapeutic potential as anti-hyperglycaemic agent in insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) (Chi *et al*., 2022).

Verma et al. (2024) suggests that anti-inflammatory effect of Neem extract is less than that produced by dexamethasone. Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise (Benisheikh *et al*., 2019; Mudenda *et al*., 2024). Neem seeds are used in traditional medicine to treat infections especially those involving the eye and ear. Administration of alcoholic extract of Neem flower disrupts the estrous cycle in Sprague Dawley rats and causes a partial block in ovulation and as the potential of an ideal antifertility agent (Al-awadhi *et al*., 2024). The Neem plant aqueous extract also has powerful chemotherapeutic and viral agent (Guchhait *et al*., 2022; Iyevhobu *et al.*, 2022). The purpose of the present study was to investigate the antimicrobial activity of Neem leaves, stem and root against human pathogenic bacteria, including *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis.*

The tree has adaptability to a wide range of climatic, topographic and edaphic factors. It thrives well in dry, stony shallow soils, and even on soils having hard clay pan, at a shallow depth. Neem tree requires little water and plenty of sunlight (Sateesh, 1998). Neem trees have the ability to neutralize acidic soils, thus, this is a unique property of calcium mining (Hedge,1995).

Biologically active principles isolated from different parts of the plant include: azadirachtin, meliacin, gedunin, salanin, nimbin, valassin, and many other derivatives of neem seed oil (Iyevhobu *et al.*, 2022). The seed also contain tignic acid (5-methyl-2-butanic acid) responsible for the distinctive odour of the oil (Schmutterer, 1990; Uko and Kamalu, 2001; and Lale, 2002). These compounds belong to natural products called triterpenoids (limonoids). The active principles are slightly hydrophilic, freely lipophilic and highly soluble in organic solvents like hydrocarbon, alcohols, ketones and esters (Schmiatterer and Singh, 1995).

The role and use of neem plant in the health sector cannot be over emphasized. During the past five decades, intensive investigations on the diverse properties of neem plants have been carried out and a large umber of researched articles and books have been published and documented (Iyevhobu *et al.*, 2022). The neem plant has been discussed in many conferences both at national and international levels. Hundreds of active compounds that have been isolated from various parts of the neem plants find their applications in pesticides, health care and cosmetic industries all over the world (Iyevhobu *et al.*, 2022). The role of the neem plant in the treatment of various disease conditions has already been established. It is important, however, that most common bacterial isolates within rural communities are tested against extracts of the neem plant to know which are more sensitive to he plants. This will supplement antibacterial agents where they are not affordable or available.

2. Materials and Methods

2.1. Materials

- **Reagents/Culture Media:** These includes: Gram staining reagents, Kovac's reagent for indole test, 1% tetra methyl paraphenylene diamine dihydrochloride in water stored in a dark bottle (oxidase test), Glucose peptone water, Simon's citrate agar for citrate utilization test, Christensen's medium for urease test, Deoxy cholate agar (DCA), Blood agar, Chocolate agar and Nutrient agar.
- **Glassware/Equipment:** These include: Glass slides/cover slips, Pasteur pipettes, Applicator sticks, Wire loops, Hot plate/Bunsen burner, Beakers, Test tubes, Petri dishes, Filter papers, Incubator, Bucket centrifuge, Cotton wool, Forceps and Microscope.

2.2. Plant Selection

The leaves, stem, stem-bark and root of the Neem plant were collected from Emaudo Campus of Ambrose Alli University, Emaudo District, Ekpoma, Edo State and were identified and authenticated by botanists in the Department of Botany, Faculty of Life Sciences, Ambrose Alli University, Ekpoma. It was then processed at the Diagnostic and Research Laboratory of the Department of Medical Microbiology, Faculty of Medical Laboratory Science, College of Medical Sciences, Ambrose Alli University, Ekpoma, Edo State.

2.3. Preparation of Extract (Neem leaf, Stem, Stem-Bark and Root)

The leaves, stem, stem-bark and root were washed in sterile distilled water and weighed in a sterile disposable cup. The completely shade-dried neem plant was coarsely powdered, mixed and allowed to soak for successive extraction with ethanol, cold water and hot water. The obtained liquid extracts were subjected to Rotary evaporated to dryness and stored at 4^0C in air tight bottle.

- **Ethanol Extract:** Similarly, 10g each of dried leaves, stems, stem-barks and roots powder of *Azadirachta indica* were taken in separate containers and 20ml of ethanol was added and kept for 24hrs with periodic shaking and filtered. The procedure was repeated three times and the filtrates were pooled. Extract was ready and stored in an airtight amber colour container.
- **Cold and Hot Water Extract:** The same procedure as in ethanol was applied to both cold and hot water extract except that Hot water was used for Hot water method and the filtrates were pooled and stored in airtight amber colour container separately.

2.4. Source of Bacteria Isolates

Reference strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29123 and *Staphylococcus epidermidis* ATCC 12228 were used which were gotten from Irrua Specialist Teaching Hospital (ISTH) Irrua, Edo State. The colony growth from overnight culture was Gram stained and the various biochemical tests were carried out to identify the organisms. The isolates were all subjected to sensitivity testing.

2.5. Sterilization of Glass Wares

All the glass wares for the research work were thoroughly washed with detergent and rinsed with clean water. The glass wares such as test tubes, Petri dishes, beakers, conical flasks, pipettes, Durham's tubes and McCartney bottles were sterilized using the hot air oven at 180 ^{0}C for 30minutes and wire loops were flamed red-hot before and after use.

2.6. Antimicrobial Screening

- **Agar disc diffusion method**: This method (Kirby *et al.*, 1966) is suitable for organisms that grow rapidly over night at 35-37 ^{0}C. The antibiotic impregnated disc absorbs moisture from the agar and antibiotic diffuses out into the agar medium. The rate of release of the antibiotic from the disc is greater than the rate of diffusion. As the distance from the disc increases, there is a logarithmic reduction in the antibiotic concentration. Zone of inhibition of bacterial growth around each disc is measured and the susceptibility is determined.
- **Inoculums:** The microorganisms were inoculated in 1% peptone water medium, incubated at 37 ^{0}C for 3 -4 hours and used as inoculums.

2.7. Determination of Minimum inhibitory concentration

The minimum inhibitory concentration was defined as the lowest concentration of the compound to inhibit the growth of microorganisms (Kumar *et al.,* 2007). The minimum inhibitory concentration values were determined by broth dilution assay of micro-dilution assay. Varying concentrations of the extracts (200mg/ml, 150mg/ml and 100mg/ml) were prepared. 0.1ml of standardized test organism of Controls was equally set up by using solvents and test organisms without extract. The be with the least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration (MIC).

3. Results

Table 1 shows the *In-vitro* activities of the various leaf extract by the Neem plant on selected bacteria such as *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Staphylococcus epidermidis.* When compared with Gentamycin 10mg/ml, the Ethanol Extracts shows maximum inhibition *Pseudomonas aeruginosa, Staphylococcus aureus, E. coli* and *Staphylococcus epidermidis* in an ascending order.

Table 2 shows the *In-vitro* activities of the various stem extract by the neem plant on selected bacteria such as E. *coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis* and when compared with Gentamycin 10mg/ml, the Ethanol Extracts also shows maximum inhibition on *Pseudomonas aeruginosa, Staphylococcus aureus, E. coli* and *Staphylococcus epidermidis* in an ascending order.

Table 3 shows the *In-vitro* activities of the various stem-bark extract the neem plant on selected bacteria such as *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis* and when compared to the Gentamycin

10mg/ml, the Ethanol Extracts shows maximum inhibition on *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli* and *Staphylococcus epidermidis* in an ascending order.

Table 4 shows the *In-vitro* activities of the various root extract by the neem plant on selected bacteria such as *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis* and when compared with Gentamycin 10mg/ml, the Ethanol Extracts shows maximum inhibition on *Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *E. coli* in an ascending order.

Table 5 shows the *In-vitro* activities of various ethanol extracts of Leaf, Stem, Stem-bark and Root of neem plant, tested on some selected bacteria such as *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis* and When compared with Gentamycin 10mg, the Ethanol Extract of Neem Root shows maximum inhibition on *Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *E. coli* in an ascending order, followed by the ethanol extract of Neem Leaf which shows maximum inhibition on *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis* and *E. coli* in an ascending order.

Table 1 *In-vitro* activities of the various leaf extracts of the neem plant tested on selected bacteria

Tested Organisms	**Zones of inhibition at 200 mg/ml of the extract**			**GEN (10 mg/ml)**
	EL	**CL**	**HL**	
Escherichia coli	10 mm	6 mm	7 mm	15 mm
Staphylococcus aureus	8 mm	6 mm	6.5 mm	16 mm
Pseudomonas aeruginosa	66.5 mm	6 mm	6 mm	13 mm
Staphylococcus epidermidis	11 mm	6 mm	7 mm	17 mm

Key: EL = Ethanol Leaf Extract; CL = Cold water Leaf Extract; HL = Hot water Leaf Extract; GEN = Gentamycin

Table 2 *In-vitro* activities of the various stem extracts of Neem plant tested on selected bacteria

Tested Organisms	**Zones of inhibition at 200 mg/ml of the extract**			**GEN (10 mg/ml)**
	ES	**CS**	**HS**	
Escherichia coli	9 mm	6 mm	7 mm	15 mm
Staphylococcus aureus	7.5 mm	6 mm	7 mm	16 mm
Pseudomonas aeruginosa	7 mm	6 mm	6 mm	13 mm
Staphylococcus epidermidis	10 mm	6 mm	8 mm	17 mm

Key: EL = Ethanol Leaf Extract; CL = Cold water Leaf Extract; HL = Hot water Leaf Extract; GEN = Gentamycin

Table 3 *In-vitro* activities of various stem-bark extracts tested on selected bacteria

Tested Organisms	**Zones of inhibition at 200 mg/ml of the extract**			**GEN (10 mg/ml)**
	ES-b	**CS-b**	**HS-b**	
Escherichia coli	9 mm	6 mm	6 mm	15 mm
Staphylococcus aureus	7 mm	6 mm	6 mm	16 mm
Pseudomonas aeruginosa	7 mm	6 mm	6 mm	13 mm
Staphylococcus epidermidis	10 mm	6 mm	7 mm	17 mm

Key: EL = Ethanol Leaf Extract; CL = Cold water Leaf Extract; HL = Hot water Leaf Extract; GEN = Gentamycin

Table 4 *In-vitro* activities of root extract from ethanol, cold water and hot water

Tested Organisms	**Zones of inhibition at 200 mg/ml of the extract**			**GEN (10 mg/ml)**
	ER	**CR**	**HR**	
Escherichia coli	11 mm	6 mm	6 mm	15 mm
Staphylococcus aureus	7 mm	6 mm	6 mm	16 mm
Pseudomonas aeruginosa	9 mm	6 mm	7 mm	13 mm
Staphylococcus epidermidis	11 mm	7.5 mm	8 mm	17 mm

Key: EL = Ethanol Leaf Extract; CL = Cold water Leaf Extract; HL = Hot water Leaf Extract; GEN = Gentamycin

Table 5 *In-vitro* activities of the various ethanol extract of Leaf, Stem, Stem-bark and Root

Tested Organisms	Zones of inhibition at 200 mg/ml of the extract				GEN (10 mg/ml)
	EL	**ES**	**ES-b**	**ER**	
Escherichia coli	10 mm	9 mm	9 mm	11 mm	15 mm
Staphylococcus aureus	8 mm	7.5 mm	7 mm	7 mm	16 mm
Pseudomonas aeruginosa	6.5 mm	7 mm	7 mm	9 mm	13 mm
Staphylococcus epidermidis	11 mm	10 mm	10 mm	11 mm	17 mm

Key: EL = Ethanol Leaf Extract; CL = Cold water Leaf Extract; HL = Hot water Leaf Extract; GEN = Gentamycin

4. Discussion

All test strains of bacteria were found to be sensitive to Gentamycin. All extracts were investigated using agar diffusion (disc) method, against *Escherichia Coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis*. All the examined extract showed varying degrees of antibacterial activities against the pathogens. According to previous work, the Phytochemical test were done to find the presence of active chemical constituents such as glycosides, alkaloids, tannins, flavonoids, terpenoids, saponin, reducing sugar and volatile oil (Emencheta *et al*., 2019; Baker *et al*., 2022; Basnayake & Gunatilake, 2024).

Many of the existing synthetic drugs cause various side effects which are more severe than drugs from plant-based compounds. Hence, drug development from plant-based compounds could be useful in meeting the demand for newer drugs with minimal side effects (Vaou *et al*., 2021). *Azadirachta indica* leaves possessed good anti-bacterial activity, confirming the great potentials of its bioactive compounds and for rationalizing the use of this plant in primary health care (Wylie & Merrell, 2022). The extracts of Neem plant when used as medicinal plant, could be useful for the growth inhibition of the carcinogenic bacterium; *Streptococcus sobrinus* (Prabagar *et al*., 2020). The phyto-constituents: alkaloids, glycosides, flavonoids and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens (Herrera-Calderon *et al*., 2019; Browne *et al*., 2020).

5. Conclusion

Azadirachta indica leaf extract has antibacterial activity against human pathogens. It is expected that using natural products as therapeutic agents will probably not elicit much resistance by microorganisms. This can explain the rationale for the use of the plant in treating infections in traditional medicine. The plant could be a veritable and cheaper substitute for conventional drugs since the plant is easily obtainable and the extract can easily be made via a simple process of maceration or infusion in alcohol (ethanol).

The Neem plant should continue to be used in traditional medicine to treat infectious conditions especially those infected by the enterobacteriaceae. For better extractions, alcoholic extract (ethanol) should be used compared to water extraction. Administration of alcoholic extract of Neem flower disrupts the estrous cycle in Sprague Dawley rats and causes a partial block in ovulation and has the potential of an ideal antifertility agent (contraceptive for rats). More

studies/researches should be carried out to unravel the importance of these plants and continue to isolate and purify the active components of this natural herb against other pathogenic organisms.

Compliance with ethical standards

Acknowledgements

The authors would like to acknowledge the management of Ambrose Alli University (AAU), Ekpoma, Edo State, Nigeria for creating the enabling environment for this study. Thanks to all the Laboratory and technical staff of St Kenny Research Consult, Ekpoma, Edo State, Nigeria for their excellent assistance and for providing medical writing support/editorial support in accordance with Good Publication Practice (GPP3) guidelines.

Disclosure of conflict of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Availability of Data and Materials

The authors declare consent for all available data present in this study.

Authors' Contributions

The entire study procedure was conducted with the involvement of all writers.

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