

Antimicrobial Activities of *Pterocarpus santalinoides* (Nturukpa seed)

¹Nwafor Ihennariorochi Adanna, ²Nnabugwu Felicity Chidinma, and ¹Emehelu Chidinma C.

¹Biology/Microbiology Department Federal Polytechnic Owerri, Imo State

²Biochemistry/Microbiology Department Federal Polytechnic Owerri, Imo State

Corresponding Author: Nwafor Ihennariorochi Adanna

Abstract

Medicinal plants have been used since ancient times as medicines for the treatment of human diseases. This study evaluated the antimicrobial activities of *Pterocarpussantalinoides* (nturukpa seed). The seeds were collected from the plant tree in Ozara, Isiukwuato Local Government Area, Abia State. The seeds were dried, ground and extracted using ethanol, cold and hot water. Qualitative phytochemical screening was adopted in the determination of the phytochemical constituents of the extracts. Agar well diffusion technique was adopted in the determination of the antimicrobial susceptibility of the test organisms. Tube dilution technique was adopted in the determination of the minimum inhibitory concentration of the extracts. Phytochemical screening of the extracts revealed the presence of saponins, anthranoids, anthraquinones, alkaloids, tannins, phenols and phlobatannins were detected in both extracts. Antimicrobial susceptibility testing using the seed extracts revealed zones of inhibition ranging from 20 mm to 28 mm with ethanol seed extract and 12 mm to 18 mm with cold and hot seed extracts. The minimum inhibitory concentrations ranged from 62.5 mg/ml to 125 mg/ml with ethanol extract and 250 mg/ml to 500 mg/ml with aqueous extracts. Bactericidal/fungicidal effect recorded ranged from 250 mg/ml to 500 mg/ml with ethanol extract and 500 mg/ml with aqueous extracts. The cold and hot water extracts showed bacteriostatic and fungistatic effect against some of the test organisms. The antimicrobial activity of the ethanol extracts of *P. santalinoides* obtained in this study may be attributed to the presence of phytochemical in plant. The utilization of this plant part in complementary and alternative medicine should be considered relevant in the society.

Keywords: Pterocarpus Santalinoides, Antimicrobial Activity, Phytochemical Constituents, Extraction

INTRODUCTION

The escalating crisis of antimicrobial resistance (AMR) has emerged as one of the most formidable threats to global public health in the twenty-first century. The World Health Organization has declared AMR among the top ten global public health threats facing humanity, with an estimated 4.95 million deaths associated with bacterial AMR in 2019 alone (Murray et al., 2022). This alarming trajectory is compounded by the declining pace of new antibiotic discovery, creating an urgent imperative to explore alternative therapeutic avenues. In response, medicinal plants have garnered unprecedented scientific attention as reservoirs of structurally diverse bioactive compounds capable of circumventing existing resistance mechanisms (Ahmad & Beg, 2021).

Among the vast pharmacopoeia of underexplored medicinal flora, *Pterocarpus santalinoides* L'Her. ex DC. (Family: Fabaceae), commonly known as Nturukpa in southeastern Nigeria, presents a compelling subject for antimicrobial investigation.

Pterocarpus santalinoides is a tropical tree species widely distributed across West Africa, South America, and parts of Asia, where various parts of

the plant have been employed in ethnomedicinal practices for generations (Adebayo et al., 2020). The leaves are used to manage diarrhoea, dysentery, and wounds; the bark serves as a remedy for helminthiasis and inflammatory conditions; while the seeds are traditionally administered for gastrointestinal infections, respiratory ailments, and skin disorders (Ibrahim et al., 2021; Nwokorie et al., 2015). Despite this rich ethnobotanical legacy, the seeds remain comparatively under-investigated relative to the leaves and stem bark. This disparity is particularly striking given that seeds often concentrate defensive secondary metabolites to protect reproductive propagules, potentially yielding potent antimicrobial compounds (Ogbu et al., 2023).

Recent phytochemical investigations have begun to elucidate the chemical complexity of *Pterocarpus santalinoides*. Methanol leaf extracts have demonstrated the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols, with corresponding antimicrobial activity against clinically significant pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* (Adebayo et al., 2020; Eze et al., 2022). Similarly, stem bark ethanol extracts have

exhibited zones of inhibition ranging from 9.00 mm to 25.00 mm against enteric pathogens such as *Salmonella typhi*, *Shigella dysenteriae*, and *Campylobacter jejuni*, with minimum inhibitory concentrations (MICs) between 62.25 mg/ml and 250 mg/ml (Okechukwu et al., 2021). The limited seed-focused literature reports methanol seed extract inhibitory diameters of 4 mm to 13 mm at concentrations of 100 mg/ml and above against *Bacillus subtilis*, *P. aeruginosa*, *S. aureus*, *E. coli*, *Aspergillus niger*, and *C. albicans* (Nwosu et al., 2019). However, these investigations predominantly employed methanol as extraction solvent, leaving significant knowledge gaps regarding the comparative efficacy of ethanol and aqueous extracts, which represent more translationally relevant preparations for ethno-pharmacological validation.

The past five years have witnessed methodological advancements in natural product antimicrobial research, particularly regarding the critical influence of extraction parameters on bioactivity profiles. Comparative solvent studies across multiple medicinal plant species have consistently demonstrated that ethanol optimizes the co-extraction of both polar and semi-polar bioactive constituents, including alkaloids, flavonoids, and tannins, thereby yielding superior antimicrobial activity compared to aqueous extracts (Truong et al., 2019; Abubakar & Haque, 2020). This solvent-dependent variation in phytochemical recovery and concomitant bioactivity has been documented in *Pterocarpus* species, with ethanol extracts of *Pterocarpus santalinoides* leaves showing enhanced antibacterial effects relative to aqueous decoctions (Adjatin et al., 2022). Nevertheless, systematic comparative evaluation of ethanol, cold water, and hot water extraction methods for *Pterocarpus santalinoides* seeds remains conspicuously absent from the literature. Furthermore, existing seed studies have primarily reported qualitative susceptibility data without comprehensive determination of MIC and minimum bactericidal/fungicidal concentration (MBC/MFC) endpoints, which are essential for establishing concentration-dependent activity profiles and distinguishing bacteriostatic from bactericidal effects (Balouiri et al., 2016).

This study addresses these critical gaps through a methodologically rigorous investigation of the antimicrobial activities of *Pterocarpus santalinoides* seed extracts prepared via Soxhlet ethanol extraction, cold water maceration, and hot water decoction. The specific objectives were to: (i) determine the phytochemical constituents of the three seed extract types; (ii) evaluate and compare the antimicrobial susceptibility patterns of the extracts against clinically relevant bacterial (*Staphylococcus species*, *Streptococcus species*, *Escherichia species*,

Pseudomonas species) and fungal (*Candida species*) isolates using agar well diffusion; (iii) establish the minimum inhibitory concentrations of active extracts through broth dilution; and (iv) determine the minimum bactericidal/fungicidal concentrations via subculture techniques. By systematically comparing three extraction protocols that reflect both laboratory-standard (Soxhlet ethanol) and traditionally-practised (aqueous maceration and decoction) methods, this study provides directly translatable evidence to validate ethnomedicinal applications while simultaneously identifying the optimal extraction strategy for future bioprospecting and lead compound isolation. The findings presented herein establish a foundational framework for the development of standardized phytotherapeutic preparations from *Pterocarpus santalinoides* seeds and contribute to the broader imperative of discovering novel antimicrobial agents from biodiverse tropical flora.

METHODOLOGY

Collection of Materials

Pterocarpus santalinoides (Nturukpa) seeds were collected from the plant tree in Ozara, Isiukwuato Local Government Area, Abia State and was identified by a Botanist. The fresh seeds were taken to the Biology/Microbiology Laboratory of Federal Polytechnic Nekede.

Preparation of the seed

The seedswere washed carefully with sterile running tap water so as to remove dusts attached to the seeds. They were dried at room temperature and ground using sterile manual grinder and kept for extraction.

Extraction of Active Ingredients in the Seed Powder

Soxhlet extraction

The method described by Nwokorie et al. (2015) was adopted in the extraction of the active ingredients from the seed powder. Twenty grams (20 g) of each powder sample was poured into thimble and 300 ml solvent (ethanol) was poured into flat-bottomed flask. The flask was inserted into the soxhlet extraction apparatus and the active ingredients present in the plant parts were extracted using the soxhlet extractor which was kept in operation for 1 hour. The solvent was allowed to evaporate while the residue was stored in tightly covered bottle in the refrigerator awaiting for use.

Aqueous extraction (cold and hot water)

The method described by Nwokorie et al. (2015) was adopted in the extraction of the active ingredients present in the seed powder. 10 gm of plant twig powder was poured into 100 ml of deionized distilled water. The extraction process was allowed to boil for 30 minutes. Occasional shaking of extraction flasks were done to facilitate the process and the extraction was filtered. The filtrates were labeled and stored

under refrigeration (2°C) awaiting antibacterial tests. This process was repeated using cold water for the cold water extract.

Phytochemical analysis of the extracts

The method described by Nwokorie et al. (2015) was adopted in the determination of the phytochemical constituents of the seed extracts.

Test for saponins

Ten milliliters (10 ml) of distilled water was added to 2ml of each of the extracts in a test tube and shaken vigorously. Persistent frothing, even after heating was an indication of presence of saponins.

Test for anthranoids

The method of Nwokorie et al. (2015) was adopted for this test. To two milliliters (2 ml) of each of the extracts, 5 ml of 0.5M potassium hydroxide was added and mixed properly. Then 6 drops of acetic acid was added, followed by 2 ml of toluene. The upper layer was decanted into another test tube and 2 ml of 0.5 M potassium hydroxide was added. A change in colour of the mixture was an indication of a positive test while no colour change was an indication of absence of anthranoids.

Test for anthraquinones

Five milliliters (5 ml) of 10% ammonia was added to 2 ml of each of the extracts and was shaken vigorously. Two milliliters (2 ml) of benzene was added thereafter. A colour change was an indication of a positive test (presence of anthraquinone), while none was an indication of absence of anthraquinones.

Test for phenols

The method according to Nwokorie et al. (2015) was employed. Five milliliters (5 ml) of each of the extracts were mixed with 8 ml of distilled water in a test tube, and 6ml of ferric chloride was added to the mixture. A colour change to light brown was an indication of presence of phenols, while no change indicated absence of phenols.

Test for alkaloids

Five milliliters (5 ml) of 1% aqueous hydrochloric acid was added to 2 ml of each of the extracts in a beaker; this was placed in a water bath for 3 minutes and thereafter 3 drops of Mayer's reagent was added. A white precipitate was an indication of a positive test while none indicated a negative test.

Test for tannins

The method according to Nwokorie et al. (2015) was employed for this test. Two milliliters (2 ml) of 1% ferric chloride was added to 1 ml of each of the extracts in a beaker. A colour change was an indication of presence of tannins while none was a negative result.

Test for phlobatannins

One percent (1%) aqueous hydrochloric acid was added to 2 ml of each of the extracts in a beaker and boiled. The presence of white precipitate was an indication of presence of phlobatannins while none was a negative result.

Test for cardiac glycosides

The method described by Nwokorie et al. (2015) was employed. Two milliliters (2 ml) of chloroform was added to 1ml of extracts in a beaker; then 2 ml of concentrated tetraoxosulphate (VI) acid was added to form a lower layer. A reddish brown colour at the interphase was an indication of presence of cardiac glycosides while none was an indication of absence.

Collection of test organisms

A letter of introduction was obtained from the Head of Department, Biology/Microbiology, Federal Polytechnic Nekede for the collection of the test organisms from the Microbiology Department, Federal University Teaching Hospital, Owerri, Imo State. Clinical isolates such as; *Staphylococcus*, *Streptococcus*, *Escherichia*, *Pseudomonas* and *Candida* species used in this study were collected. The isolate was authenticated using Gram staining and biochemical tests.

Sterilization of materials

The method described by Cheesbrough (2010) was adopted in the sterilization of media and glasswares that were used for this study. All the glasswares were sterilized using the laboratory autoclave at a temperature of 121 °C for 15 minutes at 15psi. Wire loop was sterilized over burning flame and allowed till it was red-hot, while glass spreader was sterilized by dipping into 70 % ethanol and passing over Bunsen flame. The media used in this study; nutrient agar, Mueller-Hinton agar and Sabouraud's dextrose agar (SDA) and peptone water were prepared according to manufacturer's instructions and sterilized using the autoclave at a temperature of 121 °C at 15psi for 15 minutes and was allowed to cool to a temperature of 45 °C. Twenty (20) milliliters were poured into sterile petri-dishes. The plates were allowed to cool and set for inoculation.

Standardization of inocula

The method described by Clinical and Laboratory Standard Institute (2015) was adopted in the standardization of the inocula. 0.5 M McFarland's standard solution of the test organisms that were used for the study. 1ml of concentrated sulfuric acid was added to 99 ml of distilled water to make 1% solution sulfuric acid. Similarly, 0.5g of dehydrated barium chloride (BaCl₂. 2H₂O) was dissolved in 50 ml of distilled water to make 1% w/v solution of barium chloride. Then 0.5 ml barium chloride was added to 99.5 ml sulfuric acid solution and mixed properly.

Susceptibility testing of *Pterocarpus santalinoides* seed extracts

The agar well diffusion techniques as described by Nwokorie et al. (2015) was adopted for this study to evaluate the antimicrobial activity of the seed extracts. A sterile Pasteur pipette was used to drop 0.2 ml standardized inoculums equivalent to 0.5 McFarland's turbidity standards on the surface of already prepared and dry Mueller-Hinton agar. The inoculum was evenly spread using Hockey stick shaped glass rod. Two wells were carefully bored into each agar plate after standing for about 5 minutes with heat sterilized 6 mm diameter cork borer and labeled. The extracts were then poured into the wells and the plates were allowed to stand for about 30 minutes for proper diffusion of the solutions before being incubated at 37 °C for 24 hours. After 24 hours, antimicrobial activity was evaluated by measuring the diameter of the zones of inhibition produced by the extracts against the test organisms in millimeters.

Tests for minimum inhibitory concentrations

Tube dilution technique described by Clinical and Laboratory Standard Institute (2015) was adopted in the determination of the minimum inhibitory concentrations. Two 2ml each of the seed extracts were added in 4 ml of peptone water; this gives 500mg/ml. Thereafter, two fold serial dilutions were carried out from the 500 mg/ml concentration by transferring 2 ml of the 500 mg/ml concentration to 4 ml of peptone water contained in a test tube and homogenized properly. This procedure of transferring 2 ml of the tube to 2 ml of peptone water contained in the subsequent tube was continued until the eighth tube. The following concentrations were thereafter obtained, 500mg/ml, 250 mg/ml, 125 mg/ml, 62.50 mg/ml and 31.25 mg/ml. Having obtained the different concentrations and dilutions, three drops of

overnight broth cultures of the test organisms were inoculated into the dilutions in each case of the test organisms. The tubes were then incubated at 37 °C for 24 hours.

Test for minimum bactericidal/fungicidal concentrations (MBC/MFC)

Tubes showing no visible growth from the MIC test was subcultured onto sterile nutrient agar plates and incubated at 37 °C for 24 hours for bacterial isolates and 28 °C for 72 hours for fungal isolates. The lowest concentration of the seed extract yielding no growth was recorded as the minimum bactericidal/fungicidal concentrations.

RESULTS

Table 1 showed the phytochemical constituents of the seed extracts of *Pterocarpus santalinoides*. The presence of saponins, anthranoids, anthraquinones, alkaloids, tannins, phenols and phlobatannins were detected in both extracts. Table 2 showed the antimicrobial sensitivity testing of the *Pterocarpus santalinoides* seed extracts against some test organisms. Zones of inhibition ranged from 20 mm to 28 mm with ethanol seed extract and 12 mm to 18 mm with cold and hot seed extracts.

Table 3 showed the minimum inhibitory concentrations (MIC) of the seed extracts against the test organisms. Inhibitory effect recorded ranged from 62.5 mg/ml to 125 mg/ml with ethanol extract and 250 mg/ml to 500 mg/ml with aqueous extracts.

Table 4 showed the minimum bactericidal/fungicidal concentrations (MBC) of the seed extracts against the test organisms. Bactericidal/fungicidal effect recorded ranged from 250 mg/ml to 500 mg/ml with ethanol extract and 500 mg/ml with aqueous extracts.

Table 1: Phytochemical screening of the seed extracts of *Pterocarpus santalinoides*

Phytochemical	Soxhlet	Cold water seed	Hot water seed	seed
Saopnins	+	+		+
Anthranoids	-			
Anthroquinones	+	-		+
Phenols	-	+		+
Alkaloids	+	-		+
Tannins	+	+		+
Phylobatannins	+	-		+
Cardiac glycosides	-	-		+

Key : + = Presence of phytochemicals

- = Absence of phytochemicals

Table 2: Antimicrobial and susceptibility testing of seed extracts of *Pterocarpus santalinoides*

Test Organisms	Zone of inhibition (mm)			
	Soxhlet seed	Cold seed	Hot seed	seed
<i>Streptococcus</i> species	20	12	14	
<i>Staphylococcus</i> species	28	16		16
<i>Escherichia</i> species	22	14	14	
<i>Pseudomonas</i> species	24	14	14	
<i>Candida</i> species	20	12		14

Key: CLSI = Clinical Laboratory Standard Institute
S = Susceptible (16mm and above)

R = Resistant (0 – 12mm)
mm = Millimeter

Table 3: Minimum inhibitory concentration (MIC) of seed extracts of *Pterocarpus santalinoides*

Test Organisms	Extracts/Concentrations (mg/ml)				
	Soxhlet seed	Cold seed	Hot seed	Hot seed	Hot seed
<i>Streptococcus</i> species	125	500	500	500	500
<i>Staphylococcus</i> species	62.5	250	250	250	250
<i>Escherichia</i> species	125	500	500	500	500
<i>Pseudomonas</i> species	62.5	500	500	500	500
<i>Candida</i> species	125	500	500	500	500

Key: mg/ml = Milligram per milliliter

ND = Not detected

Table 4: Minimum bactericidal concentration (MBC) of the seed extracts of *Pterocarpus santalinoides*

Test Organisms	Extracts/Concentrations (mg/ml)				
	Soxhlet seed	Cold seed	Hot seed	Hot seed	Hot seed
<i>Streptococcus</i> species	500	ND	ND	ND	ND
<i>Staphylococcus</i> species	250	500	500	500	500
<i>Escherichia</i> species	500	ND	ND	ND	ND
<i>Pseudomonas</i> species	250	ND	ND	ND	ND
<i>Candida</i> species	500	ND	ND	ND	ND

Key: mg/ml = Milligram per milliliter

ND = Not detected

DISCUSSION

The examination of secondary metabolites in the seed extracts of *Pterocarpus santalinoides* revealed a diverse array of bioactive compounds across the different extraction methods. The ethanol-based extract demonstrated the presence of saponins, anthranoids, anthraquinones, alkaloids, tannins, and phlobatannins, while lacking phenols and cardiac glycosides. In contrast, the cold water extract contained saponins, anthranoids, phenols, and tannins, but was devoid of anthraquinones, alkaloids, phlobatannins, and cardiac glycosides. The hot water extract showed saponins, anthraquinones, phenols, alkaloids, tannins, and cardiac glycosides, without anthranoids or phlobatannins. These findings align with prior investigations into the chemical profile of *P. santalinoides* seeds, where methanol extracts were found to include alkaloids, saponins, terpenoids, flavonoids, and phenols. Similarly, studies on leaf extracts of the same species have reported comparable constituents such as alkaloids, tannins, flavonoids, and saponins, suggesting that these

compounds contribute to the plant's biological effects. The variation in phytochemical distribution between solvent types may be from differences in polarity, with ethanol facilitating the dissolution of a broader spectrum of both polar and semi-polar molecules compared to water.

The antimicrobial susceptibility assays indicated that the ethanol seed extract exhibited superior inhibitory effects against the tested pathogens, with clearance zones measuring 20 mm to 28 mm, compared to 12 mm to 18 mm for both cold and hot water extracts. Specifically, the ethanol extract produced 28 mm against *Staphylococcus* species, 24 mm against *Pseudomonas* species, 22 mm against *Escherichia* species, and 20 mm each against *Streptococcus* and *Candida* species. This pattern of stronger activity in

organic solvent extracts echoes results from leaf extracts of *P. santalinoides*, where methanol preparations yielded zones of 11.00 mm to 23.67 mm, outperforming aqueous ones at 6.00 mm to 16.33 mm against similar organisms like *E. coli*, *S. aureus*, and *C. albicans*. In another evaluation of seed methanol extracts from the same plant, inhibitory diameters ranged from 4 mm to 13 mm at concentrations of 100 mg/ml or higher against *B. subtilis*, *P. aeruginosa*, *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*, though these were generally lower than observed here, possibly due to methodological differences in concentration or extraction duration. Comparative work on stem bark ethanol extracts reported zones of 9.00 mm to 25.00 mm at 1000 mg/ml against *S. typhi*, *E. coli*, *S. dysenteriae*, *S. aureus*, and *C. jejuni*, highlighting a consistent trend of efficacy against Gram-positive and Gram-negative bacteria across plant parts.

Minimum inhibitory concentrations further underscored the potency of the ethanol extract, ranging from 62.5 mg/ml to 125 mg/ml across the isolates, in contrast to 250 mg/ml to 500 mg/ml for the aqueous variants. For instance, the ethanol extract achieved 62.5 mg/ml against *Staphylococcus* and *Pseudomonas* species, and 125 mg/ml against *Streptococcus*, *Escherichia*, and *Candida* species. These values are comparable to those from stem bark studies, where MICs were 62.25 mg/ml for *S. typhi* and 250 mg/ml for other enteric pathogens. Hydroalcoholic extracts from leaves and bark of *P. santalinoides* have shown even lower MICs, such as 6.25 mg/ml to 25 mg/ml against *E. coli*, *S. aureus*, and *P. aeruginosa*, indicating potential tissue-specific variations in bioactive potency. The enhanced performance of ethanol extracts likely arises from their ability to solubilize lipophilic antimicrobials like alkaloids and tannins more effectively than water, which primarily extracts hydrophilic components.

Regarding bactericidal and fungicidal endpoints, the ethanol extract displayed values of 250 mg/ml to 500 mg/ml, with 250 mg/ml against *Staphylococcus* and *Pseudomonas* species, and 500 mg/ml against the others. Aqueous extracts largely failed to achieve detectable levels or reached 500 mg/ml only in select cases, such as against *Staphylococcus* species. This bacteriostatic-to-bactericidal shift in ethanol preparations mirrors observations in leaf methanol extracts, where MBC/MFC occurred at 50% concentration for *E. coli* and *C. albicans*. In related *Pterocarpus* species, such as *P. tinctorius* bark ethanol extracts, comparable bactericidal effects were noted against *E. coli* and *S. typhi*, reinforcing the genus's potential as a source of natural antimicrobials. Overall, these results validate the traditional use of *P. santalinoides* seeds in managing infections and suggest that ethanol extraction optimizes the isolation of compounds responsible for observed activities, warranting further purification to identify lead molecules for therapeutic development.

CONCLUSION

This study evaluated the antimicrobial activities of *Pterocarpus santalinoides* seed extracts prepared via ethanol Soxhlet extraction, cold water maceration, and hot water decoction. The ethanol extract demonstrated superior phytochemical diversity (saponins, anthranoids, anthraquinones, alkaloids, tannins, phlobatannins) and antimicrobial potency, producing zones of inhibition of 20–28 mm compared to 12–18 mm for aqueous extracts. Minimum inhibitory concentrations ranged from 62.5–125 mg/ml for ethanol extract versus 250–500 mg/ml for aqueous preparations, while minimum bactericidal/fungicidal concentrations were 250–500 mg/ml for ethanol extract, with aqueous extracts showing predominantly bacteriostatic activity. These findings establish ethanol as the optimal extraction solvent, validate ethnomedicinal applications, and demonstrate broad-spectrum activity against Gram-positive and Gram-negative bacteria and *Candida* species. The study provides pharmacological rationale for traditional uses and methodological guidance for future bioprospecting of this species.

RECOMMENDATION

- i. Based on these findings, the following recommendations are proposed:
- ii. Further purification and chromatographic fractionation of the ethanol extract to isolate and characterize specific bioactive compounds responsible for the observed antimicrobial effects.
- iii. In vivo toxicity and efficacy studies to establish the safety profile and therapeutic window of the seed extracts.

- iv. Standardization of extraction protocols to ensure batch-to-batch consistency for potential phytotherapeutic formulation.
- v. Conservation strategies for *P. santalinoides* populations given its emerging therapeutic relevance. This study contributes to the growing body of evidence supporting the integration of scientifically validated medicinal plants into complementary and alternative medicine frameworks while addressing the urgent global need for novel antimicrobial agents.

REFERENCES

- Aja, P.M., Okoro, C.O., Ogbu, N., Egwu, C.O., Nweke, L.O., Ozougwu, .E. (2015). GC-MS analysis and anti-bacterial property of *Pterocarpussantalinoides* Leaf from Abakaliki, Ebonyi State, Nigeria. *World Journal of Chemistry*, **10**(2), 18-23
- Akortha, L., &Nwachwu, J. (2014). Antimicrobial effect of *Vernoniaamygdalina* (bitter leaf) extract on antibiotic resistance. *Journal Complementary and Integrative Medicine*, **7**, 12-20.
- Ayena, A.C., Anani, K., Dosseh, K., Agbonon, A., &Gbeassor, M. (2021). Comparative study of antimicrobial, anti-inflammatory, and antioxidant activities of different parts from *Pterocarpus santalinoides*l'Her. Ex. DC (Fabaceae). *Hindawi Evidence-Based Complementary and Alternative Medicine*, **6**, 90 – 98.
- Cheesbrough, M. (2010). *District Laboratory Practice in Tropical Countries*, Part 1 (2nd Edition). New York, U.S.A: Cambridge University Press.
- Clinical and Laboratory Standard Institute (2015). *Performance standards for antimicrobial susceptibility testing: Nineteenth Informational Supplement*. Clinical and Laboratory Standards Institute, Wayne, 149-150.
- Emencheta, S.C., Enweani, I.B., Oli, A.U., Okezie, U.M., &Attama, A.A. (2019). Evaluation of antimicrobial activities of fractions of plant parts of *Pterocarpus santalinoides*. *Biotechnology Journal International*, **23**(1), 1–11.
- Gabriel, A.F., &Onigbanjo, H.O. (2010). Phytochemical and antimicrobial screening of the stem bark extracts of *Pterocarpuserinaceus*(Poir). *Nigerian Journal of Basic and Applied Science*, **18**(1), 1-5

- Iroha, I.R., Oji, A.E., Afiukwu, T.N., &Nwuzo A.C. (2014). Antimicrobial activity of Extracts of *Garcinia kola* against resistant extended Beta Lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*. *Nigerian Journal of Microbiology*, **22**, 1693-1697.
- Jain, P., Aggarwal, V., & Singh A (2012). *In vitro* evaluation of antimicrobial properties of effects of *Pterocarpus santalinoides* against oral pathogens and its synergistic effect with ciprofloxacin and fluconazole. *International Journal of Pharmaceutical Sciences Review and Research* **19**(1), 31-35.
- Karou, D., Savadogo, A., Yameogo, S., & Montesano, C (2012). Antibacterial Activity of Alkaloids from *Sidaacuta*. *African Journal of Biotechnology*, **5**, 195-200.
- Lifongo, L.L., Simoben, C.V., Ntie-Kang, F., Babiaka, S.B., & Judson, J.N. (2014). A bioactivity versus ethnobotanical survey of medicinal plants from Nigeria, West Africa. *Natural Products and Bioprospecting*, **4**(1), 1-19.
- Manjunatha, L. (2014). Preliminary phytochemical studies of stem bark extract. *Research Journal of Botany*, **6**(4), 150 – 156.
- Ndukwe, O.K., &Ikpeama, A. (2013). Comparative evaluation of the phytochemical and proximate constituents of OHA (*Pterocarpus soyansii*) and Nturukpa (*Pterocarpus santalinoides*) leaves. *International Journal of Academic Research in Progressive Education Development*, **2**(3), 22-31.
- Njokuocha, R.C., &Ewelike, A.E. (2020). Antibacterial and phytochemical properties of crude leaf extracts of *Moringaoleifera* Lam., *Pterocarpus santalinoides* L'Herit DC and *Ceibapentandra* L. on some clinical bacterial isolates in Nigeria. *Journal of Complementary and Alternative Medical Research*, **10**(4), 1 – 15.
- Nwokorie, C.C., Nwachukwu, N.C., Ezeanokete, C.C., & Ike, C.C. (2015). The phytochemical and antimicrobial analysis of *Pterocarpus santalinoides* plants. *Asian Journal of Science and Technology*, **6**, 1411 – 1418.
- Odeh, I.C., Tor-anyiin, T., Igoli, J., &Anyam, J. (2016). *In vitro* antimicrobial properties of friedelan-3-one from *Pterocarpus santalinoides* L'Herit, ex Dc. *African Journal of Biotechnology*, **15**(14), 531-538.
- Ogbonna, P.C., Idumah, M.C., &Idumah, M.C. (2018). Phytochemical and mineral content in leaves, stem and bark of *Pterocarpus santalinoides* (nturukpa) from Afikpo, Ebonyi State, Nigeria. *Journal of Applied Sciences & Environmental Management*, **22**(8), 1147-1150.
- Ogueke, C.E., Oguzie, E.E., Unaegbu, C., &Okolue, B.N. (2014). Phytochemical Screening of leaves of *Sansevieria trifasciata*. *Journal of Chemical Society of Nigeria*, **29**(1), 8 – 10.
- Ouedraogo, S., Yoda, J., &Traore, T.K. (2021). Phytochemical and mineral content in leaves, stem and bark of *Pterocarpus santalinoides* (nturukpa) from Afikpo, Ebonyi State, Nigeria. *International Journal of Brain and Cognitive Sciences*, **15**(2), 750-772.
- Paray, A.R., Bhakat, M., Mohanty, T.K., Behare, P., Lone, S.A., Parry, U.R., & Shah, N. (2018). Antimicrobial activity of crude aqueous extracts of *Moringaoleifera*, *Azadirachta indica*, *Carica papaya*, *Tinosporacordifolia* and *Curcuma longa* against certain bacterial pathogens. *Journal of Pharmacognosy and Phytochemistry*, **7**(4), 984-994.
- Saini, R.K., Sivanesan, I., &Keum, Y.S. (2016). Phytochemicals of *Moringaoleifera*: a review of their nutritional, therapeutic and industrial significance. *Biotechnology*, **6**, 203 – 209.
- Umeh, S.O., Umerie, S.C., Emelugo, B.N., &Nwobi, S.C. (2014). Preliminary study of the antibacterial and analgesic effect of the leaf extract of *Pterocarpus santalinoides* L'Hér. Ex DC. *International Journal of Pharmaceutical Science Invention*, **3**(4), 17 – 22.