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Phytochemical and Antimicrobial Evaluation of Leaf-extracts of *Pterocarpus santalinoides*

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Authors' contributions

This piece is a collaborative work carried out by the authors. It is part of an on-going postgraduate research by author OIC under the supervision of author TTA. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: This study was undertaken to examine the phytochemical constituents and antimicrobial activity of leaf-extracts of *Pterocarpus santalinoides,* a plant with wide application in Igede people's traditional medicine against microbial infections.
Methodology: Successive extraction of leaves of this plant at room temperature using petroleum ether, ethyl acetate, butanol, ethanol and water was carried out. These extracts were phytochemically screened qualitatively for the presence of alkaloids, flavonoids, saponins, tannins and terpenoids using established literature procedures. Agar well diffusion technique was used to screen the extract for antimicrobial activity. MICs, MBCs and MFCs for the various extracts were determined by the tube dilution technique. Graded concentrations of the extract solutions in Mueller Hinton broth were used for the tests. MBCs and MFCs were done to establish the nature of antimicrobial activity of these extracts.
Results: Qualitative phytochemical screening of leaf-extracts of *P. santalinoides* revealed presence of alkaloids, flavonoids, terpenoids, saponins-glycosides and tannins (except ethanol extract that contained no tannins). These extracts inhibited growth of test organisms, and implies antimicrobial activity on *E. coli, P. mirabilis, S. typhi, S. aureus and C. albicans*. Zones of inhibition ranged from 17-24 mm. The MICs ranged from 5.0 mg/ml to 10 mg/ml while MBCs and MFCs ranged from 10 mg/ml to 20 mg/ml. Ethanol extracts showed the widest zone of inhibition followed by aqueous extracts (24 mm and 21 mm, respectively).
Conclusion: These results lend support to the ethnomedicinal applications of this plant

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by the Igede people of North Central Nigeria, in treating infections caused by these test organisms which are human pathogens. The ethanol extract in particular, may be exploited as a possible antimicrobial agent for the management of infectious pathogenic diseases caused by these microorganisms.

Keywords: Pterocarpus santalinoides; leaf-extracts; antimicrobial activity; Phytochemical constituents.

1. INTRODUCTION

Medicinal plants constitute an effective resource for both traditional and modern medicines, and herbal medicine has been shown to have genuine utility [1]. In Nigeria, many plants are used in traditional medicine as antimicrobial agents but only few are documented. Plants based system of traditional medicine has continued to play an essential role in health care in many cultures. The increased use of plant derived products as alternatives to orthodox or synthetic drugs and increasing awareness of beneficial effects of natural products has resulted in increased interest in alternative therapies. Extracts from plants have been utilised for their antifungal, antiviral and antibacterial activities globally [2].

Pterocarpus santalinoides (L'Herit. ex DC., family- Leguminosae: papilionoideae) is a plant believed to possess potent antibacterial properties in ethnomedicine [3]. It is a tree, 9 – 12 m tall with low straggling branches. Leaves compound, 5 – 9 leaflets, ovate-elliptic, abruptly acuminate, rounded at the base or slightly cuneate, glabrous, glossy, rather coriaceous with about 8 pairs of prominent main lateral nerves looping away from the margin, leaf-stalk slender, glabrous stalk 10-20cm long, leaflet stalk stout 2-5mm long [4]. It grows along riverine forests of Africa and tropical South America. It is a native of Brazil, Cameroon, Ghana, Nigeria and Senegal [4,5]. The leaves are eaten as vegetable, wood is termite resistant, the bark contains tannins and dyes used for dyeing; the bark is also used as stomach ache remedy. The plant is variously known locally as gunduru or gyadar kurmi (Hausa); maganchi (Nupe); ikyarakya or kereke (Tiv); gbengbe (Yoruba); okumeze (Edo); nturukpa (Igbo); nja (Efik) [5] and ugbam piegwu or uturukpa (Igede) [6]. The antidiarrheal activity of this plant has been investigated in an attempt to verify its traditional medical management of diarrhoea [3,7,8].

In south-east Nigeria, leaves of *P. santalinoides* is ethnomedicinally used against gastro-intestinal diseases, diabetic syndrome and is known to exhibit antipyretic property [7]. The leaves are also used for treatment of skin diseases [8,10]. Its stem-bark and leaf extracts are said to possess anti-enteropooling, antimalarial, anti-abortive and antibacterial properties [3,7,8,9]. The use of the leaves in veterinary medicine [8], fodder for livestock [10], analgesic [9] and together with leaves of *Solanum microcarpum* in management of high blood pressure [7,10] and many others have been reported.

Elsewhere in India, fruit extracts of *P. santalinoides* are used traditionally in treating headache, skin diseases, boils, fevers, etc. Its stem bark extracts have antibacterial, antidiabetic, antihyperglycaemic and hepatoprotective activities [11].

Other species of pterocarpus for instance *P. osun* and *P. soyaxuii* are reported to possess ethnomedicinal properties and are used for rheumatism, dysentery, gonorrhoea, malaria, etc. [10]; fever, headache, pains, convulsions, antimicrobial agents as well as in respiratory disorders [12]. Similar reports have been made for *P. milbraedii*, whose leaves are used for

soup making; plant is exploited for timber and wood for carving [13]. The antimicrobial activity of methanol stem extract of *P. marsupium*, which is believed to have potent antidiabetic, antifungal, hypoglycaemic, antimicrobial etc. values in nature is documented [14].

Based on the traditional medicinal use of this plant among the Igede people of North Central Nigeria in the treatment of inflammation of lower abdomen/lower abdominal pain, stomach ache and other infectious diseases [6], this study was undertaken to examine the phytochemical constituents and antimicrobial activity of leaves of *P. santalinoides*. Studies such as this will not only lend credence to traditional medical practice, but also are in line with advocacy by WHO that ethnomedicine should be exploited with a view to providing safe and effective remedies for ailments [1]. This becomes more imperative for developing countries where about 80% of local populace rely on traditional systems of medicine for treatment of a variety of diseases, as well as the emergence of uncommon infections, appearance of undesirable side effects of some antibiotics and increasing tendency of drug resistance in pathogenic microorganisms.

2. MATERIALS AND METHODS

Fresh leaves of *Pterocarpus santalinoides* were collected during flowering period of the plant from riverine village of Anyiwogbu-Ibila, near Oju (Igede speaking tribe) Benue State, Nigeria in March, 2011. The plant leaves were identified and authenticated by P. Ekwuno, Department of Forestry and Wildlife, University of Agriculture, Makurdi, Nigeria (Herbarium number: FUAM FD ed.1.1:97). The leaves were air-dried at room temperature in the laboratory for three weeks. These were pulverised using mortar and pestle.

2.1 Preparation of Extracts

Powdered plant material (150g) was exhaustively and successively (orsequentially) extracted at room temperature with petroleum ether (40-60°C), ethyl acetate, butanol, ethanol and water for 72 hours (300ml x 3 each), drying the marc before the next batch of extraction. Extracts were filtered using Whatman filter paper no. 2 and excess solvent in the different extracts obtained was removed by distillation. The combined water extract was however concentrated under reduced pressure using rotary evaporator. Concentrated extracts were dried at 40°C on a water bath. Dried extracts were collected and kept in sample bottles at ambient temperature. Their yields and other physical properties were noted and recorded.

2.2 Phytochemical Screening

The different extracts were subjected to various photochemical tests to identify the chemical constituents present using standard methods as described in the literature [15-22].

2.3 Antimicrobial Screening

2.3.1 Experimental microorganisms

The pathogenic micro-organisms used were: *Escherichia coli, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa* for Gram negative; Staphylococcus aureus, Streptococcus pyojenes for Gram positive and Candida albicans, Candida krusei for fungi.

The isolates were obtained from the Department of Medical Microbiology, Benue State University Teaching Hospital, Makurdi (BSUTH). An extract (0.2g) was weighted and dissolved in 10ml of dimethyl sulphoxide (DMSO) to obtain a concentration of 20 mg/ml. This was the initial concentration of the extract used to check the antimicrobial activities of the extracts from the plant. The agar well diffusion technique was used to screen the plant extracts for antimicrobial activities. An inoculums (0.1ml) of an overnight broth culture of test organism (1.5 x108 cfu/ml) was spread evenly on a Mueller-Hinton agar plate, and a 6 mm diameter well was cut at the center of the plate using a cork borer. A sample (0.1ml) of extract solution in DMSO (concentration 20 mg/ml) was introduced into the agar well. The agar plate was incubated (37ºC, 24h) and observed for zone of inhibition.

2.3.2 Determination of minimum inhibitory concentration

Extract solution (concentration 20 mg/ml) was serially diluted two-folds in Muller-Hinton broth to give decreasing concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml. An aliquot (0.1ml) of overnight broth culture of test microorganism (concentration 1.5 x 108 cfu/ml) in sterile normal saline was introduced into each extract dilution. The mixtures in sterile test tubes were incubated (37ºC, 24h) and observed for turbidity (signifying growth) or absence of it (signifying inhibition). Aprofloxacin (antibacterial drug) and fluconazole (antifungal drug) were used as positive controls, and sterile normal saline as negative control. The minimum inhibitory concentration was the lowest concentration of the extract solution that inhibited microbial growth.

2.3.3 Minimum bactericidal/fungicidal concentration

A loopful of the test mixture was removed from each MIC tube that showed no growth, inoculated onto antibiotic-free Mueller-Hinton agar plate, incubated (37ºC, 24h), and inspected for presence of colonies indicating growth. The minimal bactericidal or fungicidal concentration is the lowest concentration of extract that showed no bacterial or fungal growth.

3. RESULTS AND DISCUSSION

The profiles of various extracts of the plant are shown in Table 1. Highest yield of extract was with ethanol, whilst the least yield was obtained with butanol. This yield increased with increasing polarity of solvent used. Qualitative phytochemical analysis revealed presence of alkaloids, saponins, flavonoids, glycosides, and terpenoids in butanol, ethanol and aqueous extracts. Tannins were found in butanol and aqueous extracts only (Table 2).

Table 1. Yield and other physical properties of extracts of *P. santalinoides*

Extract	Yield (g)	Colour	Consistency
Pet. Ether	3.78	Deep green	Pasty solid
Ethyl acetate	4.74	Black	Gummy solid
Butanol	2.36	Dirty green	Pasty solid
Ethanol	7.67	Brown	Solid (hard)
Aqueous	7.17	Coffee/dark brown	Solid (very hard)

Table 2. Phytochemical screening of various extracts of *P. santalinoides*

Photochemical		Pet. Ether	Ethyl acetate	Butanol	Ethanol	Water
Alkaloids	Mayers' Test	-	-	++	++	-
	Dragandorff	-	-	++	++	++
	Wagner's	-	-	++	++	++
Tannins	Ferric chloride	-	-	++	-	++
	Lead ethanoate	-	-	++	-	++
Flavonoids	Shinoda's	-	++	++	++	-
	Ferric chloride	-	-	++	++	++
	Lead ethanoate	-	-	++	++	++
	Sodium hydroxide	-	-	++	++	++
Saponins	Frothing test	-	-	++	++	++
Saponins-glycosides	Fehling's test	-	-	++	++	++
Terpernoids	Salkowski	-	-	++	++	++

Key: ++ = Present, - = Absent.

Result for antibacterial activity of butanol, ethanol and aqueous extracts showed sensitivity to different strains of Gram-negative (*E. coli, S. typhi, P. mirabilis*) and Gram-positive (*S. aureus*) bacteria as well as the fungus (*C. albicans*) as shown inTable 3.

Table 3. Antimicrobial activities of leaf-extracts of *Pterocarpus santalinoides* against test microbes

Test organisms	Ethanol extract	Butanol extract	Aqueous extract	Apro-floxacin	Flucona-zole
Staphylococcus aureus	S	S	S	S	R
Staphylococcus pyogenes	R	R	R	S	R
Esherichia coli	S	S	S	S	R
Salmonella typhi	S	S	S	S	R
Proteus mirabilis	S	S	S	R	R
Pseudomonas aeruginosa	R	R	R	S	R
Candida albicans	S	S	S	R	S
Candida krusei	R	R	R	R	S

Key: S = Sensitive, R = Resistance

Table 4. Zones of inhibition (mm) of extracts (20 mg/ml) against test microorganisms

Test organisms	Ethanol extract	Butanol extract	Aqueous extract	Apro-floxacin	Flucona-zole
Staphylococcus aureus	24	18	21	32	0
Staphylococcus pyogenes	0	0	0	37	0
Esherichia coli	21	18	18	35	0
Salmonella typhi	20	18	20	30	0
Proteus mirabilis	20	17	19	0	0
Pseudomonas aeruginosa	0	0	0	32	0
Candida albicans	22	17	20	0	31
Candida krusei	0	0	0	0	41

Antibacterial activity was expressed as diameter zone of inhibition (Table 4). A zone of observable inhibition of growth of each micro-organism served as a criterion for declaring an extract sensitive and was indicated by a clear zone around the well. The diameter of inhibition zone of extract against test microorganisms (in mm) was highest for ethanol extract. In addition to having activity against bacteria and fungi, the ethanol extract exhibited strong activity against *S. aureus* with diameter zone of inhibition of 24 mm; *E. coli* 21 mm; *S. typhi* 20 mm; *P. mirabilis* 20 mm, and *C. albicans* 22 mm.

The MIC was 10 mg/ml for all the pathogens with aqueous and butanol extracts in general, but 5 mg/ml for ethanol extract, except *P. mirabilis* that was 10 mg/ml (Table 5). MBC/MFC tests were carried out to establish whether the extracts were bactericidal or bacteriostatic. MBC/MBF, in general was found to be 20 mg/ml for butanol and aqueous extracts. With ethanol extract, it was 10 mg/ml against *S. aureaus* and *C. albicans* but 20 mg/ml for the rest of the microbes tested (Table 6).

It was noted that *S. pyogenes* (Gram-positive) and *P. aeruginosa* (Gram-negative) were resistant against all the extracts (Tables 3-6). Similar result was obtained for the fungus C. krusei.

The ethanol extract demonstrated a moderate broad spectrum antibacterial activity including antifungal activity against the fungus *C. albicans*. This extract has exhibited strong activity against gram-negative strains of bacteria that are frequently reported to be less sensitive to plant extracts [23-25]. Nonetheless, it maintained the observation that Gram-positive strains are usually more sensitive, with *E. coli* having the widest zone of inhibition (24mm) at 20 mg/ml concentration. This ethanol extract generally has 2/3 times the activity of aprofloxacin (at 5 mg/ml) as standard used in the study. This observation is also identical with the fungus *C. albicans* compared to the standard used (fluconazole). Identical results have earlier been reported by Jain et al. [11], where in vitro anti-microbial properties of *P. santalinoides* extracts were investigated against some selected oral bacterial and fungal pathogens together with the synergistic effect of these extracts with ciprofloxacin and fluconazole, allowing for the possibility of concurrent use of both extracts and antibiotics together in therapy.

The antimicrobial activity of the ethanol extract of *P. santalinoides* may be attributed to the alkaloids, flavonoids, terpenoids and saponins present (Table 2). Similar results have been obtained elsewhere with this plant [7,10] and another specie, P. mildbraedii [13]. These phytocompounds, perhaps acting in synergy, may be the bioactive constituents responsible for the broad spectrum microbial efficacy demonstrated by the ethanol leaf extract of *P. sanalinoides* [10,11].

The phytoconstituents: alkaloids, tannins, flavonoids and saponins reported here and elsewhere [7,10,13] might be responsible for the ability of these extracts to inhibit the growth of these antimicrobials. For instance, tannins are linked with treatment of intestinal disorders, have anti-inflammatory and antibacterial properties and are used for the healing of wounds. Flavonoids are reported to have antibacterial, anti-inflammatory, anticancer, antifungal, antiallergic and diuretic properties. Alkaloids are medicinal agents for their analgesic antispasmodic and antibacterial effects [12,13]. Saponins also show anti-inflammatory, anticancer properties and exhibit adverse physiological response in animals [12].

Table 5. Minimum inhibition concentration (MIC) of leaf-extracts of *P. santalinoides* against test organisms

Test organisms	Ethanol extract			Butanol extract					Aqueous extract					
	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml
Staphylococcus aureus	-	-	-	+	++	-	-	-	++	+++	-	-	+	++	+++
Staphylococcus pyogenes															
Esherichia coli	-	-	-	+	++	-	-	+	++	+++	-	-	+	++	+++
Salmonella typhi	-	-	-	+	++	-	-	+	++	+++	-	-	+	++	+++
Profeus mirabilis	-	-	+	++	+++	-	-	+	++	+++	-	-	+	++	+++
Pseudomonas aeruginosa	-														
Candida albicans	-	-	-	+	++	-	-	+	++	+++	-	-	+	++	+++
Candida krusei															

Key: = - → No turbidity (no growth), + → Turbid (light growth), ++ → moderate growth +++ → high turbidity.

Table 6. Minimum bactericidal/fungicidal concentration (MBC/MBF) against test microbes

Test organisms	Ethanol extract					Butanol extract					Aqueous extract				
	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml
Staphylococcus aureus	-	-	+	++	+++	-	+	++	+++	++++	-	+	++	+++	++++
Staphylococcus pyogenes															
Esherichia coli	-	+	++	+++	++++	-	+	++	+++	++++	-	+	++	+++	++++
Salmonella typhi	-	+	++	+++	++++	-	+	++	+++	++++	-	+	++	+++	++++
Profeus mirabilis	-	+	++	+++	++++	-		++	+++	++++	-	+	++	+++	++++
Pseudomonas aeruginosa															
Candida albicans	-	-	+	++	+++	-	+	++	+++	++++	-	+	++	+++	++++
Candida krusei															

Key: = - → No colony growth, + → scanty colony growth, ++ → moderate colony growth +++ → heavy colony growth.

These metabolites are thus the agents inhibiting those pathogens with little toxicity to host cells [7,9] and are potential candidates for developing new antimicrobial agents [14].

The necessity in searching for novel and effective anti-infective agents especially from plants stems not only from the alarming reports of multiple drug resistance in medically important strains of bacteria, fungi and viruses but also the continuous evolution of resistant pathogens to currently available anti-infective agents and historical use of plants for the treatment of infectious and non-infectious diseases [2].

4. CONCLUSION

This study has demonstrated that *P. santalinoides* possess broad spectrum antimicrobial activity and supports the traditional use of the plant in ethnomedicine by the Igede people of North Central Nigeria. In particular, the ethanol extract has the potential to be exploited as a possible antimicrobial agent for the management of infectious pathogenic diseases. Further studies on identification of constituents of this ethanol extract is on-going in our laboratory.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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