ETHNO-MEDICINAL EVALUATION OF LEAF-EXTRACTS OF *PTEROCARPUS SANTALINNOIDES*

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Abstract

This research was conducted to evaluate and characterized active compound of antimicrobial activity of leaf-extracts of Pterocarpus santalinoides, a plant used as traditional medicine by Igede people of north-central Nigeria against microbial infections. Successive extraction of leaves of this plant by soxlhet using hexane, ethylacetate and methanol was carried out. These extracts were phytochemically screened for the presence of alkaloids terpenoids, flavonoids, glycosides among

others and antimicrobial activity was determined against S.aureus, S.dysenteriae, K.pneumonias among others using Agar well diffusion technique. MICs, MBCs and MFCs were determined by tube dilution technique. Zones of inhibition of 20-35mm, MICs ranged from 5 µg/mL to 10 µg/mL while MBCs and MFCs ranged from 10 µg /mL to 20 µg/mL. Spectral analysis of most active extract showed friedelan-3-one($C_{30}H_{50}O$). These results lent support to the ethno- medicinal applications of Pterocarpus santalinoides in treating infections caused by these test micro-organisms which are human pathogens.

Keywords: *Pterocaarpus santalinoides* ethno-medicinal applications.

Plant based system of traditional medicine has continued to play an essential role in the health care of many cultures. *Pterocarpus santalinoides* is a plant believed to possess potent antidiarrhoeal properties in folk medicinal practices (Nworu *et al;* 2009). The plant *Pterocarpus santalinoides* (L'Hert, ex Dc), family-*Fabaceae: papiloniodeae* 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 10-20 cm long leaflet stalk stout 2-5 mm long. It grows along riverine forests of Africa and tropical South America, and is a native of Brazil, Cameroon, Ghana, Nigeria and Senegal. *Pterocarpus santalinoides* is monoecious, flowering from December – March, fruit ripening between March –April. The Nigerian species are trees with light yellow flowers and usually have alternate leaflets. The fruit pod has an unusual irregular shape (Fig.1) (Keay, 1989; Agroforestry, 2011; Osuagwu and Akomas, 2013). Extracts from this plant have been utilized for their antifungal and antibacterial activities among the Igede people of Benue State, Nigeria in treatment of inflammation of lower abdomen, stomach ache and other infectious diseases (Igoli *et al*; 2003). This study was carried out to evaluate and characterize anti-microbial active compound from leaf extracts of the plant.

Figure 1: Stands, leaves, flowers, fruits and seeds of *Pterocarpus santalinoidess*

Materials and Methods

Plant Material

Leaves of *Pterocarpus santalinoides* were collected fresh in March, 2015 from Anyiwogbu- Ibila village, Oju L.G.A Benue state, and were identified and authenticated by Dr. S.A. Shomkegh of Department of Social Forestry and Environmental, University of Agriculture, Makurdi. The leaves were air-dried at room temperature in the chemistry laboratory, College of Education, Oju, then pulverized using mortar and pestle.

Preparation of Extracts

Pulverized plant material; 100 g was exhaustively extracted with hexane (500 mL), ethyl acetate (500 mL) and methanol (500 mL) for 8 hours each using soxhlet apparatus. The extracts were concentrated using rotary evaporator at 40 ^{o}C and then allowed to dry in air to give the crude extracts, coded: PSH, PSE and PSM for hexane, ethyl acetate and methanol, respectively. Their yields were recorded.

Phytochemical Screening:

These crude extracts were subjected to various phytochemical tests to identify the chemical constituents. Standard methods described in literature were used (Sofowora, 1984, Hassan *et al*;
2004: Edoga *et al*, 2005: Anowi *et al*, 2012 a,b).

Fractionation and Isolation

Ethyl acetate extract was purified by chromatography on silica gel column (Φ 5.0 x 40 cm) eluted with gradient mixtures of hexane- ethyl acetate (9:1), (6:4),(1:1),(9:1),(9:1),(6:4) ethyl acetate- methanol. Eluents were analyzed by Tlc, and similar fractions were combined after Tlc based on their Tlc behaviors and were coded PS vlc-1, PS vlc-2 upto PS vlc-6. PS vlc-1 gave a white non crystalline substance with MP (260-265 ^{O}C), $R_{f\,x100}$ (76).

Antimicrobial Activity of PS vlc-1

Susceptibility test was conducted using Disc and Broth Diffusion methods. The PS vlc-1 fraction was first dissolved in DMSO and diluted to give initial concentration of 40 μg/mL. The Mueller Hinton Agar was the medium used as the growth medium for the microbes. Two fold serial dilution of PS vlc-1 in the sterile broth gave final testing concentrations of 20 μg/mL, 10 μg/mL, 5 μg/mL and 2.5 μg/mL. Appropriate normal saline (negative control), growth and sterile controls were carried out with sparfloxacin, ciprofloxacin and fluconazole (vended drugs) as positive controls at concentrations of 5 μg/mL each. The inoculated culture medium was incubated at 37 ^{o}C for 6 hours for bacteria and 4 days for fungi. PS vlc-1 different concentrations were added into the broth, and incubation was made at 37 ^{o}C for 24 hours for bacteria and 7

days for fungi. After this incubation broth was observed for turbidity (signifying growth). The lowest concentration of the fraction in the broth that showed no turbidity was recorded as the Minimum Inhibitory Concentration (MIC). MBC/MFC was conducted to check whether the test microbes were killed or only their growth was inhibited.

Results

Extract	Yield(g)	% Yield	Colour
Hexane (PSH)	5	5	Green
Ethyl acetate(PSE)	7	7	Green
Methanol (PSM)	10	10	Green

Table 2: **Phytochemical Screening of Crude Extracts**

Phytochemical	Test	Observation	Extracts		
			Hexane	Ethyl acetate	Methanol
Alkaloids	Dragandorff's	Orange ppt	-	-	++
	Mayer's	Yellowish brown (amber) ppt	-	-	++
	Wagner's	Yellow ppt	-	-	++
Reducing sugar	Fehling's solution	Deep blue ppt	-	-	-
	Benedict's solution	No brown ppt	-	-	-
Glycosides	Fehling solution	Brick red ppt	-	-	++
Terpenoids	Liebermann	Yellow ring at inter phase	-	++	++
	Salkowski	Reddish brown at inter phase	-	++	++
Steroidal nucleus	Keller kiliani	Brown ring at inter phase	-	++	++
Saponnins	Frothing	Persistent Froth	-	-	++
Flavonoids	Shinoda	Orange colouration	-	++	++
	NaOH	Yellow ppt + dil HCl colourless	-	-	++
	$FeCl_3$	Pale yellow solution	-	-	++
	Lead ethanoate	Yellowish brown colouration	-	-	++
Tannins	$FeCl_3$	Greenish ppt	-	-	++
	Leadethanoate	White ppt	-	-	++
Free Anthraquinones	Borntragers	Brown ppt	-	-	-
Combined Anthraquinones		Colourless solution	-	-	-
Resins		Pink colouration changed to brown	-	-	++

Key: ++ = Present, - = Absent

Test micro-organism	Extracts		Vended drugs	
	Elhyl acetate	Methanol	Aprofloxacin	Fluconazol
Staphylococcus aureus	25	20	32	0
Streptococcus pyogenes	27	22	37	0
Escherichia coli	24	20	35	0
Proteus mirabilis	-	-	30	0
Salmonella typhi	0	0	30	0
Strigella dysenteriae	32	24	-	0
Pseudomonas aeruginosa	0	0	0	0
Klebsiella pneumonia	35	25	-	0
Candida albicans	27	21	0	32
Candida tropicalis	29	22	0	35
Candida stellatoidea	28	21	0	32
Candida krusei	0	0	0	41

Key - = not tested

Table 4: Minimum Inhibition Concentration (MIC) of PS-vlc-1 Against Test Micro organism

Test microorganism	40μg/ml	20μg/ml	10μg/ml	5μg/ml	2.5μg/ml
Methicillin rest. S. aureus	-	-	o*	+	++
Stapylococcus aureus	-	-	-	o*	+
Orgnebacterium ulcerans					
Streptococcus pneumoniae	-	-	-	o*	+
Campylobacter jejuni					
Helicobacter pylori	-	-	o*	+	++
Escherichia coli	-	-	o*	+	++
Strigella dysenteriae					
Candida tropicalis	-	-	-	o*	+
Candida krusei	-	-	-	o*	+

Key: - = no turbidity (no growth), o*= mic, +=turbidity (light growth) ++=moderate turbidity
MRSA = Methicillin resistant Staphylococcus aureus

Table 5: Minimum Bactericidal/Fungicidal Concentration(MBC/MFC) of PS vlc-1 Against Test Micro-organisms

Test microorganism	40 μg/ml	20 μg/ml	10 μg/ml	5 μg/ml	2.5 μg/ml
Methicillin rest. S. aureus	-	O*	+	++	+++
Stapylococcus aureus	-	-	O*	+	++
Orgnebacterium ulcerans					
Streptococcus pneumoniae	-	-	O*	+	++
Campylobacter jejum					
Helicobacter pylori	-	O*	+	++	+++
Escherichia coli	O*	+	++	+++	++++
Strigella dysenteriae					
Candida tropicalis	-	-	O*	+	++
Candida krusei	-	O*	+	++	+++

Key: - = no colony growth, o*=MBC/MFC, +=scanty colonies growth, ++=moderate colonies growth, +++=heavy colonies growth

Table 6: Spectral Data of PS vlc-1 in $CDCl_3$ Compared with Literature Reports.

Position of H/C	PS vlc-1		Abdullahi *et al* (2011)		Igoli & Gray (2008)	
	$^1H(\delta)$	$^{13}C(\delta)$	$^1H(\delta)$	$^{13}C(\delta)$	$^1H(\delta)$	$^{13}C(\delta)$
	δ H	δ C	δ H	δ C	δ H	δ C
1	1.96,1.67	22.3(t)	1.95, 1.71 (2H, add)	22.3(t)	1.97	22.51
2	2.37, 2.29	41.6(t)	2.37,2.27(2H,add)	41.5(t)	2.28, 2.40	41.76
3	-	213.3(s)	-	213.2(s)	-	213.00
4	2.25	58.3(d)	2.25(1Hq)	58.2(d)	2.28	58.48
5	-	42.2(s)	-	42.1(s)	-	42.70
6	*	41.3(t)	1.74,1.28 (2H,d)	41.3(t)	*	41.55
7	1.47	18.3(t)	1.74, 1.36 (2H,m)	18.2(t)	*	18.48
8	1.38	53.1(d)	1.38 (1H,dd)	53.1(d)	*	53.36
9	-	37.5(s)	-	37.4(s)	-	37.70
10	1.53	59.5(d)	1.53(1H,m)	59.5(d)	*	59.57
11	1.47	35.6(t)	1.45, 1.26 (2H,m)	35.6(t)	*	35.88
12	*	30.5(t)	1.33, 1.32(2H,m)	30.5(t)	*	30.75
13	-	39.7(s)	-	39.7(t)	-	39.95
14	-	38.3(s)	-	38.5(s)	-	38.55
15	*	32.5(t)	1.47,1.27 (2H,m)	32.6(t)	*	32.68
16	1.59	36.0(t)	1.58,1.35 (2H,m)	36.0(t)	*	36.26
17	-	30.0(s)	-	30.0(t)	-	29.93
18	*	42.8(d)	1.56(1H,m)	42.8(d)	*	43.06
19	*	35.4(t)	1.37,1.22(2H,m)	35.3	*	35.59
20	-	28.2(s)	-	28.2(s)	-	28.40
21	1.53,1.38	32.3(t)	1.50.1.31(2H,m)	32.8(t)	*	32.33
22	0.94(m)	39.3(t)	1.51.0.95(2H,m)	39.2(t)	*	39.49
23	0.87(d)	6.9(q)	0.88(3H,d)	7.0(q)	0.89(d) J=2.7	7.04
24	0.71(s)	14.2(q)	0.73(3H,s)	14.6(q)	0.74(s)	14.32
25	0.86(s)	18.1(q)	0.87(3H,s)	17.9(q)	0.88(s)	18.17
26	1.06(s)	20.3(q)	1.01(3H,s)	20.2(q)	1.01(s)	20.48
27	1.04(s)	18.7(q)	1.05(3H,s)	18.6(q)	1.02(s)	18.88
28	1.17(s)	32.1(q)	1.18(3H,s)	32.1(q)	1.06(s)	32.16
29	0.99(s)	35.1(q)	1.00(3H,s)	35.0(q)	1.19(s)	32.01
30	0.94(s)	31.8(q)	0.94(3H,s)	31.8(q)	0.96(s)1.26(s)	35.24

Key: * = overlapping signals; - = no hydrogen; δ= chemical shift

Discussion

The extraction of leaves of *P.santalinoides* yielded hexane(5 g ,pasty green); ethyl acetate(7 g ,green solid) methanol (10 g, green solid) respectively (Table 1). The extracts revealed the presence of alkaloids, glycosides, flavonoids, saponins, terpenoids, tannins and resins (Table 2). The antimicrobial activity results showed ethyl acetate to have the highest zone of inhibition (35 mm) at 20 mg /mL (Table 3). From the antimicrobial activity results ethyl acetate extract was selected for further purification via VLC to explore the bioactive constituent that could be responsible for the activity. The fractionation of the ethyl acetate extract (PS vlc-1) yielded 11 mg white non-crystalline substance. Previous investigations of the leaf- extracts of *Pterocarpus santalinoides* exhibited broad growth inhibition against microbes causing infectious diseases and in particular, it was found to be active against *E. coli*, *S.typhi. S.aureus,S. flexneri,A.faecalis,E.aerogenes*, *P.aeruginosa* (Manjunatha,2006; Osuagwu and Akomas,2013; Odeh and Tor-Anyiin,2014).

The spectral analysis showed a triterpenoid (C_{30} $H_{50}O$). The comparison of the NMR spectral features of existing literature (Igoli and Gray, 2008; Abdullahi *et al;* 2011) suggests that the isolated compound (PS vlc-1) is a pentacyclic triterpene(Table 6). Based on the chemical shifts obtained from 1D (^{1}H and ^{13}C) and 2-D (COSY, HMBC and HMQC) which were suggestive of triterpenoid of frieldelane skeleton and were consistent with reported values of frieldelan-3-one (Igoli and Gray, 2008;Abdullahi *et al;*2011) as shown in Table 6, Figure 2.

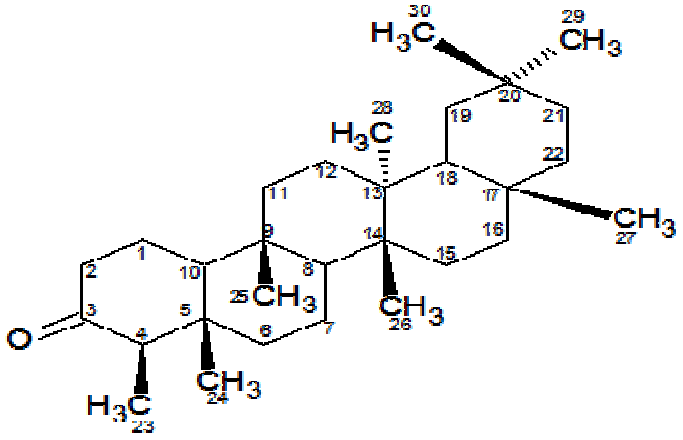

Figure 2: Structure of friedelan-3-one isolated from leaves of *Pterocarpus santalinoides*

The isolated compound (PS vlc-1) was investigated for antimicrobial activity against *MRSA , S.aureus, S.pneumoniae ,H.pylori , C.tropicalis* and *C.krusei*, and had

MIC value of 5 μg/mL for most pathogens *(*Table 4). MBC/MFC showed moderate antimicrobial activity against most of the pathogens tested (Table 5). From the phytochemical analysis, PS vlc-1 is a terpenoidal derivative suggesting that this group of terpenoidal moiety might be important for the observed activity. The isolated compound (friedelan-3-one) had earlier been reported to exhibit antifeedant and anti-inflammatory activities (Duke,1992). Friedelan-3-one had been found to show hepatoprotective activity (Dzubak*, et al; 2006)*. This study demonstrated that the leaves of *Pterocarpus santalinoides* could be good sources of compounds with antimicrobial activities.

Conclusion

Medicinal plants constitute an effective source of both traditional and modern medicines, and herbal medicine has been shown to have genuine utility. Most therapeutic attributes of medicinal plants are traced to the phyto-constituents, and the medicinal actions of these constituents are unique to a particular plant species or family. The results of this study lend support to the ethno-medicinal applications of *Pterocarpus santalinoides* in treating infectious diseases caused by these test micro-organisms which are human pathogens.

Recommendation

The advocacy by world health organization is that ethno-medicine should be exploited so that safe and effective remedies can be provided for ailments, thus more pharmaceutical investigations should be conducted on this plant. The plant can be source of new drug which can bring sustainable development or discovery in phyto-medicine in Nigeria.

References

Abdullahi**,** M.; Kolo,I.;Adebayo,O,O;Joseph,I.O (2011) .Antimycobacterial Friedelane – terpenoid from the Root-bark of *Terminalia avicennioides*. *American Journal of Chemistry,* 1(2):52-55.

Agroforestry Tree Database. A tree Species reference selection guide (2011). File//E:\speciesinformationP.santilinoides.htm.Retrieved on 2nd june 2011

Anowi. C.F., Okonkwo, C., Agbata, C.A. Ezeokafor, E. (2012a). Preliminary phytochemical screening, evaluation of Acute Toxicity and Antipyretic Activity of Methanol extract of *Pterocarpus santalinoides* (Fabaceae). *International Journal of Pharmaceutical Phytopharmacological Research,* 1(6):343-346.

Anowi. C.F., Umeokoli, B.O., Onyegbule, A.F., Okonkwo, C., Chibeze, I. (2012b) Analgesic, phytochemical and acute toxicity evaluation of the methanol extract of the leaves of *Pterocarpus santalinoides*- family Fabaceae *International Journal of Pharmaceutical Sciences and Research,* 3(7): 2018-2023.

Balaraju, K; Arokiyaj, S; Agastian, p; perinbam, K; Ignacimuthu, S. (2008). Antimicrobial Activity of Leaf Extracts of *Pterocarpus santalinus L*. (Fabaceae).*Journal of Pure and Applied Microbiology*,2(1): 161-164.

Duke, J.A (1992), *A Handbook of Biologically Active Phytochemicals and Their Activities,* CRC Press, Boca Raton, Ann Arbor Tokyo

Dzubak,P, Hajduch, M,Vydra,D, Hustova, A., Kvasnica, A., David, M., Biedermann, D., Markova, L., Urbanc, M., and Sarek, J., (2006), Pharmacological activities of natural triterpenoids and their therapeutic Implications. *Natural Product Report* 23: 394-411.

Edoga, H.O; Okwu, D.E and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology,* 4(7):685-688.

Hassan, M.M; Oyewale,A.O, Amupitan, J.O, Abdullahi, M.S, Okonkwo, E.M (2004). Preliminary Phytochemical and antibacterial investigations of crude extracts of the Root bark of *Detarium microcapum. Journal of chemical society of Nigeria,* 29 (1): 26-29

Igoli, J, O and Gray A, I (2008). Friedelanone and other triterpenoids from *Hymenocardia acida*. *International Journal of Physical Sciences* , 3(6): 156-158.

Igoli, J.O., Igwue, I.C., Igoli, N.P. (2003). Traditional Medicinal Practices among the Igede people of Nigeria. *Journal of Herbs, spices and Medicinal Plants,* 10(4):1-10.

Keay, RWJ.(1989). *Trees of Nigeria*. Claredon Press, Oxford, New York. 269pp

Manjunatha B. (2006). Antibacterial activity of *Pterocarpus santalinus. Indian Journal of Pharmacological Science,* 68(1): 115-116.

Nworu CS, Akah PA, Nwachukwu JO, and Asogwa C.(2009). Antidiarrheal Activity of *Pterocarpus santalinoides* L'Herit ex DC leaf extract. *Journal of Complementary and Integrative Medicine*, 6(1):8-11.

Odeh, I.C. and Tor-Anyiin, TA (2014). Phytochemical and Antimicrobial evaluation of leaf-extracts of *Pterocarpus santalinoides*. *European Journal of Medicinal plants*, 4(1):105-115.

Osuagwu, G.G.E. and Akomas, C.B. (2013). Antimicrobial activity of the leaves of three species of Nigerian *Pterocarpus* (Jacq.) *International Journal of Medicinal and Aromatic Plants*, 3(2):178-183.

Sofowora E.A. (1984). *Medicinal plants and Traditional Medicine in Africa.* New York,John Wiley and Sons Ltd. 57,128-233pp.