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Research Article

Biological and Chemical Evaluation of Leaf Extracts of *Dalbergia saxatilis* Hook F. (Fabaceae)

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Abstract: The air-dried powdered leaf of *Dalbergia saxatil is* was extracted with 95% ethanol to obtain the crude extract which was fractionated into acidic, basic, non-polar and polar neutral fractions. The crude ethanol extract exhibited a reasonably potent anti-oxidant activity in DPPH free- radical scavenving assay between 0.2 and 0.5 mg/ml. The crude extract and fractions were screened for antimicrobial activity against six pathogens. Among the fractions, the neutral hexane displayed appreciable antimicrobial activity against *S. aureus, E. coli, K. pneumoniae* and *C. albicans at* 200, 400, 400, and 600 µg/ ml, respectively. Column chromatographic purification of the non-polar neutral fraction followed by GC-MS analysis of the column fractions showed the presence of four phenolics and a pregnane derivative. Hydro-distillation of the fresh leaf gave essential oils which on GC-MS analysis revealed a number of volatile components including four terpenoids, and two fatty acid esters. These compounds may account for the observed bioactivities of the leaf extracts of *D. saxatilis*.
Keywords: *Dalbergia saxatil is*, leaf, antioxidant, antimicrobial, activities, chemical constituents.

INTRODUCTION

The plant species belonging to the genus *Dalbergia* (Fabaceae) are found generally in many tropical areas of the globe, particularly Africa, Asia, and central and southern America where they are used to manage a number of ailments [1-3]. The species *Dalbergia saxatilis* is found in both the northern and southern Nigeria. The powdered leaf is known to drive off flies from saws while the aqueous root extract is used to accelerate birth and expel the placenta in human subjects [4,5] Some *Dalbergia* species have been investigated and found to possess antimicrobial, antioxidant, anti-inflammatory and ani-diarrhoel activities [6,7,8,9]. The crop protectant activity of the powdered dry leaf, the insecticidal activity of the bark extract against adult mosquitoes, and the antimicrobial activity of both the leaf and bark have been previously reported [9].However, though a number of compounds have been isolated from other species of the genus *Dalbergia*, no secondary metabolite has been characterized from any part of *Dalbergia saxatilis.* This study traces the antimicrobial activity using bioassay-directed fractionation and assesses the anti-oxidant activity and the chemical constituents of the leaf of *Dalbergia saxatilis.*

MATERIALSANDMETHODS

Materials

The leaves of *Dalbergia saxatilis* were collected from the forest of Cukuku, Kwali Local Government Area Council, Federal Capital Territory, Abuja, Nigeria. The plant sample was authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, and a voucher specimen was deposited in the Herbarium.

All solvents were of standard grade and redistilled before use. Thin- layer chromatography (TLC) was run on pre-coated Merck Kieselgel 60F 254 with 0.25 mesh. The spots were visualized by exposure to UV light at 254/366 nm and to iodine vapour. Flash column chromatography was run using silica gel 300-400 mm mesh. The media for antimicrobial screening were the nutrient agar, using the agar-well diffusion method. The test organisms, *Esherichia coli, Staphyloccocus aureus, Klebsiella pneumoniae, Salmonella typhi, Proteus Spp.* and *Candida albicans* were clinical isolates were from the Microbiology Unit, Biotechnology Advanced Laboratory, Sheda Science and Technology Complex, Sheda, Abuja, Nigeria. IR spectra were obtained neat on Shmadzu Fourier Spectrophotometer and values are recorded in wavenumbers (cm^{-1}). GC-MS analyses were done using Thermo-Scientific Trace GC ULTRA system equipped with an AS300 auto sampler. The column was DB-(Optima-5), 30m x 0.25µm i.d., 0.25 µm d.f. The oven temperature was 250-280^{o}C while the injection temperature was 250^{o}C. The sample volume was 1.0µl and the carrier gas was helium at a flow rate of 1ml/min.

Extraction of plant material and fractionation

The powdered air-dried leaf (500g) was extracted with 95% ethanol (1.5L) using a Soxhlet

apparatus. The extract was filtered and evaporated to dryness with a Rotary evaporator to give the crude extract (88.5g).The crude extract was then subjected to a standard bioassay-guided fractionation protocol to give the acidic(2.1g), basic (1.1g), non-polar neutral(6.7g) and polar neutral(3.0g) residues[10].

Also, the fresh leaf (500g) was subjected to hydro-distillation, followed by extraction of the aqueous medium with diethyl ether. Evaporation of the ether extract to dryness gave a pale yellow oily residue (0.02g, 0.004%).

Phytochemical screening of crude ethanol extract

The crude extract was screened for the presence of some classes of natural products using standard procedures[11-13].

Biological screening of extractives

The crude 95% ethanol extract was screened for anti-oxidant activity using a standard procedure [14] in which 0.5 ml of 1Mm solution of DPPH was added to 3ml of various concentrations of the standard (ascorbic acid) and crude extract(o.5, 1.0, 2.0, and 5.0 mg/ml solutions in methanol). The reference was prepared by mixing 3 ml of methanol and 0.5ml of DPPH. The tests were done in triplicates and percentage inhibition (%I) values were then calculated.

The crude extract and fractions were screened for antimicrobial activity using a standard procedure [15]. Both sensitivity and minimum inhibitory concentrations (MIC) were determined.

Isolation and identification of components

The non-polar neutral fraction (5.0g) was subjected to flash column chromatography using mixtures of hexane and ethyl acetate as eluent.Pure hexane (fractions 1-8) gave a yellow viscous oil(KM1, 40mg, R_f-value =0.80, hexane). Further purification of combined column fractions 67-75(30% ethyl acetate in hexane) gave a gum(KM2, 70mg, R_f value=0.38, hexane/ethyl acetate, 3:2). The isolates KM1 and KM2 were subjected to GC-MS analysis.

The hydro-distillate was also subjected to GC-MS analysis to assess the identities of the major components of the essential oils of the leaf of *D. saxatilis.*

RESULTS AND DISCUSSION

Phytochemical screening of the crude 95% ethanol extract of the leaf of *D. saxatilis* revealed the presence of tannins, phenols, sterols, terpenoids and cardiac glycosides. The dry leaf extract did not show the presence of volatile oils, carbohydrates and resins. However, hydro-distillation of fresh leaves gave volatile oils (0.004%), suggesting that most of the volatile components of the leaf were probably lost during the period of drying.

The results of antimicrobial screening (Table 1) showed that the crude extract and the non-polar neutral fraction were both active against the six test organisms except *Candida albicans*, the polar neutral was inactive against *E. coli, K. pneumoniae*, and *Candida albicans*, but active against *P. spp., S. typhi and S.aureus*.However, the basic and acidic fractions did not show activity against any of the test organisms. These results are in slight contrast to the results for the woody root which showed that the basic fraction was active against *S. aureus*at 1000µg/ml[16]. Further screening of the antimicrobial non-polar neutral fraction of the leaves gave the following MICs (µg/ml) values: 200 for *S. aureus*, 400 for *E. coli*, 400 for *K. pneumoniae*, and 600 for *Candida albicans.* Previous screening of the leaves was only on the crude 95% ethanol extract and it was shown to be active against only *S. aureus* at MIC value of 1000µg/ml. No activity against *E. coli, K. pneumoniae*, and *S. typhi*was observed. It is obvious therefore that fractionation enhances activity as it increases the concentrations of the active components as we move from the crude extract to the fractions [10]. Tannins, phenols, sterols, cardiac glycosides and terpenoids recorded in this work have previously been reported to possess antimicrobial activity [17-19].

Free- radical scavenging (antioxidant) activity recorded for crude extract of the *D. saxatilis* leaf in 1,1-diphenyl-2-picrylhydrazyl (DPPH) using Vitamin C as standard (Table 2 and Fig. 1) indicated appreciable potency between 2.0 and 0.5mg/ml. The presence of phenols and tannins in the extracts probably played a major role in the free radical scavenging effects observed at lower concentrations as they are known to act as primary antioxidants [20, 21].

Column chromatography of the antimicrobial non-polar fraction of the leaf extract gave a yellow viscous oil KM1 and a gum, KM2, which were subjected to GC-MS analyses. The GC-MS of fraction KM1 showed it to be impure, containing two identifiable peaks with retention times/molecular ions peaks at 9.73 mins/m/z (M^+) 206 and 10.23 mins./m/z(M^+)192. Based on their fragmentation ions and direct comparison with MS computer library data the components have been assigned to thymol, 1 and phenol- 5- methyl- 2- (1- methylethyl)- acetate, 2, respecttively.

The GC-MS analysis of KM2 revealed the presence of the following compounds which were identified based on their retention times, MS characteristics and direct comparison with computer library data: 10.23 mins./m/z (M^+)164, 13.39 mins./m/z (M^+)164, and 12.26 mins./m/z (M^+)326, corresponding to eugenol 3, 2-methoxy-5-(-2-propenyl)-phenol 4, and pregn-5-en-20-one,3-(acetoxyl)-6, 16- dimethyl 3a,16a) 5,respectively.

Table1. Antimicrobial activity of extractives from *Dalbergiasaxatilis* leaf

Extractives	Ec	Kp	Ca	Pspp	St	Sa
Crude extract	-	-	+	-	-	-
Basic fraction	+	+	+	+	+	+
Acidic fraction	+	+	+	+	+	+
Polar neutral	+	+	+	-	-	-
Non-polar fraction	-	-	+	-	-	-

Key: (+) = Growth;(-)=No Growth; Ec= *Esherichia coli;* Kp=*Klebsceillapneumonia,* Ca= *Candida albicans;* Pspp=*Proteus species;* Sa =*Staphylococcus aureus*; St = *Salmonella typhi*

Table 2: Antioxidant activity of crude ethanol extracts of *D.saxatilis leaf* in DPPH compared to Vitamin C

Conc.(mg/	**% inhibition D.S**	**% inhibitionVit. C**	**% Vit. C-D.S**
5.0	41.75	99.994	58.244
2.0	68.82	99.995	31.175
1.0	75.03	99.995	24.966
0.5	79.80	99.996	20.196

Key: *D.S=Dalbergia saxatilis*; Vit C=Vitamin C (ascorbic acid)

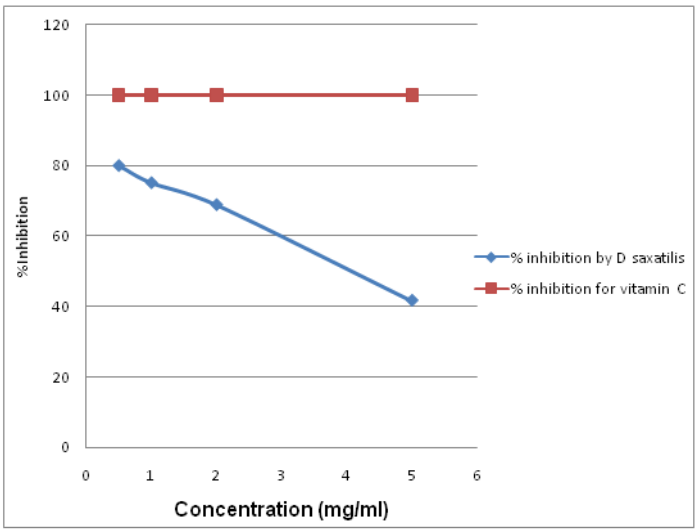
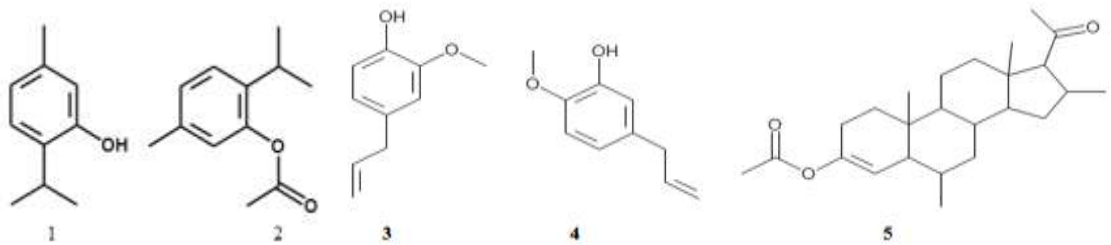


Fig. 1: Antioxidant activity of 95% ethanol crude extract of leaf of *D. saxatilis* in DPPH compared to Vitamin C

Steam distillation of fresh leaves of *D. saxatilis* gave an oily extract. The chemical constituents of the oil were assessed by subjecting it to GC-MS analysis. The GC (Fig. 2) showed a number of previously known volatile components which were differentiated and identified based on their retention times, MS characteristics and by direct comparison with computer MS library data (Table 3).The major compounds identified in the hydro-distillate were mainly the four monoterpenoids, 2, 6-octadienal-3,7-dimethyl ,(citral),6cubenol, 8caryophyll-ene oxide, 9isoaromadandrene epoxide, 10 and the two fatty acid esters, 7 and 11(Table 3). These compounds have been associated with various commercially important bioactivities and have been incorporated in perfumes, drugs and pesticides [22-28].



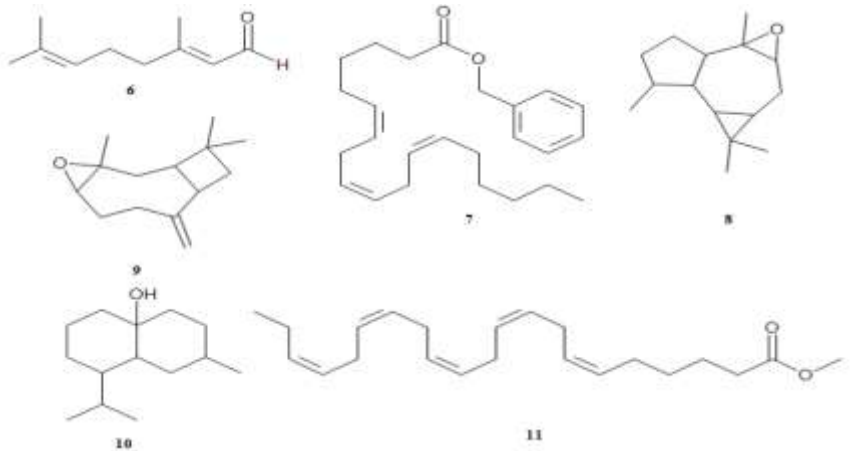


Table 3: Chemical constituents of the volatile oils of*D.saxatilis* leaf based on GC-MS analysis

Compounds	R.T(Mins)	% Yield	(M^+)BP
(6) 2, 6-octadienal, 3, 7- dimethyl (citral)	4.27	3.39	(152)69
(7) 6,9,12- octadecatrienoic acid phenyl methylester	6.00	7.19	(368)91
(8) Isoaromadendrene epoxide	7.95	3.40	(220) 91
(9) Caryophyllene oxide	8.16	19.44	(220)79
(10) Cubenol	8.81	8.27	(222)119
(11)Methyl-6,9,12,15,18-heneicosapentaenoate	10.41	10.18	(330)79

Key: R.T. =Retention Time; (M^+) = Molecular ion; BP = Base Peak

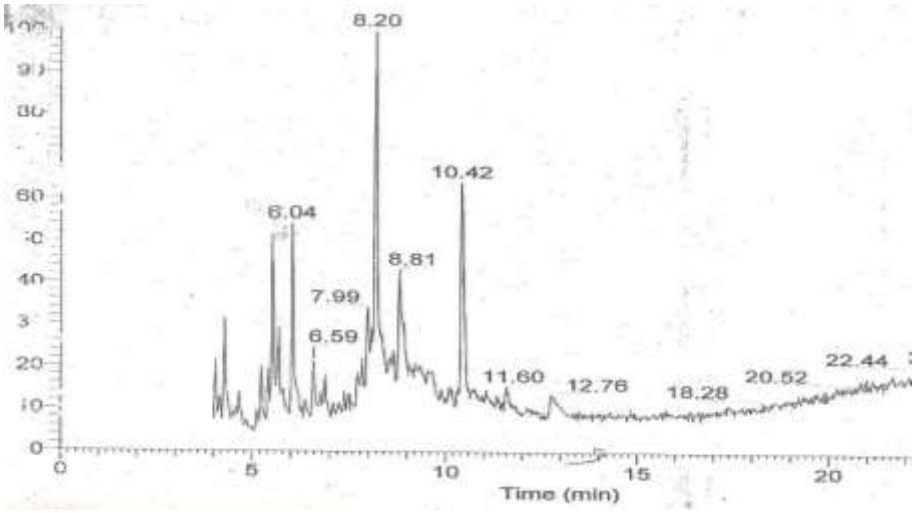


Fig. 2: Gas chromatogram of hydro-distillate from fresh leaf of *Dalbergia saxatilis*

CONCLUSION

This study has investigated the bioactivities and chemical constituents of the leaf of *Dalbergia saxatilis*. Using bioassay-guided fractionation, the antimicrobial activity of the crude extract has been shown to reside mainly in the neutral fractions (non-polar and polar). Phytochemical screening, chromatographic purification and GCMS have revealed the presence of compounds that are well known to possess these activities, particularly antimicrobial, pesticidal and anti-oxidant properties. Thus, the leaf of *D. saxatilis* can be harnessed for medicinal and agricultural purposes. This is the first report on the chemical constituents of the leaf of *D. saxatilis.*

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