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Full Length Research Paper

Bioactive compounds and antimicrobial efficacy of African myrrh (*Commiphora africana*) root extracts

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The crude ethanolic extract of *Commiphora africana* root was partitioned with *n*-hexane, chloroform; water and 10% aqueous methanol and screened for chemical constituents and antimicrobial activity. The *n*-hexane fraction, which was the most bio-active was chromatographed on a column silica gel to give a number of purified components. The components from the *n*-hexane were found to be active against *Staphylococcus aureus, Escherichia coli* and *Candida albicans. S. aureus* (ATCC 13709) was susceptible to fractions C_{1-11} giving zonal inhibition of between 1 - 3 mm. Standard antibiotics - amoxillin, chloramphenicol and tetracycline were compared with the extracts in antimicrobial activity.

Key words: Antimicrobial activity, *Commiphora africana,* phytochemical activity, root extracts.

INTRODUCTION

Commiphora africana (A. Rich) Engl. Syn. *Heudelotia africana* (Family Burseraceae) is a shrub or small tree which has a short lateral branches, sharply pointed at the apex, bearing leaves in small clusters below the tip (Arnold and Dewet, 1993). The plant which is well sui-ted to dry areas is often grown as a hedge in Northern Nigeria (Burkill, 1985). Parts of the plant are medicinally taken in several West African countries possibly because of the presence of phytochemicals such as methylisopropenyl furnace, sesquiterpenes and commiphoric acid (Abbiw, 1990).

A macerate of crushed leaves in oil is drunk in Cote d'Ivorie and in Burkina Faso as a sedative and sopariffic (Adebayo et al., 2006). Bark-extracts have been shown to have some insecticidal activity and to be termite repellant (Abbiw, 1990). The gum is widely used to prepare antiseptic washes and baths for skin infections, sores and leprosy. The seed contains tannin, dye stuff, a fixed oil, dihydroflavonol glucoside and Z-gugguls-terone (McGuffin et al., 2006). In Nigeria, a seed decoc-tion is held to be a very effective purgative and vermin-fuge. A dose of 6 g of powdered seed in a glass of wa-ter is certain to expel a tapeworm.

In a continued search for new antimicrobial agents from Nigerian higher plants for potential use in medicine and in crop protection, this work provides a report on the constituents of the biologically active *n* - hexane fraction of the ethanolic crude extract of the root of *C. africana* and was subsequently assessed for their anti-microbial efficacy.

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EXPERIMENTAL

Materials

The root sample of *C. africana* was collected from Giri village in the Federal Capital Territory, Abuja, Nigeria. This was dried and ground into a coarse powder. Extraction solvents used were ethanol (from BDH Chemicals), ethyl acetate (Rectapur) and *n*-hexane (Merek) and silica gel (Kiesel gel S. 0.2 - 0.5 mm) for column chromatography (from Riedel-Dettaen Ag. Seelze Han-nover). Rf values were obtained on pre-coated Merek grade TLC plates using a Camag Ultra Violet (U.V) lamp 366 - 254 mm and iodine vapour as detectors.

Extraction

In the Chemistry Laboratory of University of Abuja, Abuja, Nigeria, the powdered root 400 g was Soxhlet-extracted with 98% ethanol 2.5 L for about 12 h after which the extract was filtered and concentrated to dryness using a rotary evaporator to give 29.5 g of extract.

Fractions of the crude extract

The extract was dissolved in chloroform 200 ml and taken in a separatory funnel 1 L. The chloroform layer was partitioned with

water 100 ml x 2, upper aqueous layer separated and concen- trated to dryness to obtain dark-brown viscous syrup 13.33 g. The chloroform fraction was further fractionated by first evaporating to dryness, re-dissolved in 10% aqueous methanol 200 ml and extracted with *n*-hexane 200 ml. The two fractions were sepa-rated and evaporated to dryness to give *n*-hexane fraction 2.95 g and aqueous methanol fraction 5.20 g.

Phytochemical screening of crude extract

Ferric chloride test (Trease and Evans, 1989) on the crude extract gave a blue black coloration on TLC plate. The crude extract also gave positive Lieberman-Burchard test (Finar, 1988)

Isolation of constituents of *n*-hexane fraction

Vacuum liquid chromatography (VLC)

Vacuum liquid chromatography of the *n*-hexane fraction 2.20 g on TLC grade silica gel as the stationary phase and eluting with mix-tures of *n*-hexane and ethyl acetate gave a number of impure fractions (a) 0.10 g (b) 0.01 g (c) 0.23 g (d) 0.09 g (e) 0.05 g (f) 0.16 g and (g) 0.69 g. Fractions c and d were combined together because they contained common components.

Column chromatography

Combined fractions c and d 0.32 g were chromatographed over a column of silica gel and eluted with a mixture of *n*-hexane ethyl acetate. 100% *n*-hexane afforded component C_1 (0.02 g); Rf 0.81 (*n* - hexane ethyl acetate 9:1); *n* - hexane ethyl acetate (100:1) gave component C_2 (0.091 g); *n*-hexane ethyl acetate (20:1) afforded component C_3 (0.07 g), Rf 0.63 (*n*-hexane ethyl acetate 9:1). Similar process was carried out on impure fractions (e) to afford C_4 with 100% *n*-hexane 0.017 g, Rf 0.81 (*n*-hexane ethyl acetate 9:1).

The C_4 component was similar to component C_1 obtained from the combined impure fractions c and d and were therefore combined to obtain a total weight of 0.037g. *n* -hexane ethyl acetate 50:1 yielded component C_5 (0.02 g). Rf 0.41 (*n*-hexane*:* ethyl acetate 9:1). Ethyl acetate 5% in *n*- hexane yielded compo-nent C_6 (0.06 g), Rf 0.38 (*n*-hexane: ethyl acetate 9:1).

The impure fraction (g) yielded C_7 with 1% ethyl acetate in *n*-hexane (0.049 g) Rf 0.63 (*n*-hexane: ethyl acetate 1:1). Further elution with 6% ethyl acetate yielded C_8 (0.007 g), Rf 0.72 (*n*-hexane: ethyl acetate 1:1) 8% ethyl acetate in *n*-hexane yielded C_9 (0.00241 g) Rf 0.38 (*n*-hexane: ethyl acetate 1:1). Further elution with 9% ethyl acetate in *n*-hexane yielded C_{10} (0.0305 g) Rf 0.39 (*n*-hexane*:* ethyl acetate 1:1). 15 - 20% ethyl acetate in *n*-hexane yielded C_{11} (0.042 g) Rf 0.23 (*n*-hexane: ethyl acetate 1:1).

Antimicrobial screening of crude extract and fractions

Crude extract, the *n*-hexane, aqueous and 10% aqueous methanol fractions were screened for antimicrobial activity in the Micro- biology Laboratory of National Pharmaceutical Institute, Idu - Abuja, Nigeria, using Agar-Diffusion Technique (Murray, et al., 1995). The extract and fractions (0.01 g each) was added to 5 ml of different solvent to give a concentration of 2,000 μg/ml. One ml of diluted extract was mixed with 19 ml of sterile nutrient agar poured into a sterile Petri-dish and allowed to gel.

The procedure was repeated for each of the test organisms. The inoculated plates were dried and sterile cork borer (No. 4) was used to make four holes evenly distributed in the dried inoculated plates. These holes were filled with the diluted crude extract and fractions. Positive and negative controls were equally set up. The plates were then incubated at 37°C for 24 h and the sen-sitivity results obtained (Table 1).

Antimicrobial screening of purified components

The purified components C_{1-11} were all screened for antimicro-bial activity using a modified version of National Committee for Clinical Laboratory Standards (NCCLS) (Trease and Evans, 1989). Mueller-Hinton agar in molten state 19.8 cm^3 was aseptically incubated with 0.2 cm^3 of each of the test organisms *S. aureus* (ATTC 13709), *E. coli* (NITC 10418), *B. subtilis* (Phar-maceutical Microbiology Dept., ABU, Zaria), *P. aeruginosa* (ATCC 27853) and *C. albicans* (Diagnostic Unit NIPRD, Pharm Micro-biology., Department).

The well mixed media were each dispensed into sterile plates on a flat surface and allowed to gel. Each of the purified components 100 μgm was incorporated into sterile disc. Two discs of different components were placed evenly on the surface of each plate, at least 24 mm (centre to centre) between them with the aid of a forceps sterilized via a Bunsen burner. Three standard antibiotic discs (tetracycline, chloramphenicol and amoxicillin) were used as controls. Each of these antibiotic discs was inoculated onto fresh agar/organism surfaces similar to the purified components. The discs gently pressed down onto the Mueller-Hinton agar were left to stand for 15 min. Other controls equally set up were extract, me- dium and organism controls. The plates in duplicates were incuba- ted at 37°C for 24 h and the sensitivity results obtained are shown in table 2.

RESULTS AND DISCUSSION

The crude extract at 2000 μg/ml showed activities against *S. aureus*, *E. coli*, and higher degree of inhibition in *C. albicans* but no activity against *B. substilis* and *P. aeruginosa* (Table 1). Ten percent methanol aqueous extract showed no activity against *B. substilis* and *P. aeruginosa* but was active against *S. aureus* and *C. albicans;* and higher degree of inhibition in *E. coli*. The *n*-hexane fraction showed activities against *S. aureus*, *E. coli* and *C. albicans*. Water soluble extract showed activity only on *S. aureus and C. albicans.*

Table 2 shows the sensitivity results obtained from the purified components. The *S. aureus* was susceptible to fractions C_{1-11} giving zonal inhibition of between 1 - 3 mm. C_{1-7} and then $C_{11,}$ gave zonal inhibition of 1 mm, $C_{9,}$ and $_{10,}$ gave zonal inhibition of 2 mm while $C_{5,}$ and $_{8,}$ gave the maximum zonal inhibition of 3 mm. *E. coli* was susceptible to C_{1-11} giving 1 - 3 mm zonal inhibition. C_{2-9} and $C_{8-10,}$ gave 1 mm zonal inhibition, C_1 and $_7$ gave 2 mm zonal inhibition and C_6 and $_{11}$ gave 3 mm zonal inhi-bition. The *B. subtilis* was susceptible to C_{8-11} with 1 mm zonal inhibition. *C. albicans* was susceptible to C_6. *P. aeruginosa* was resistant to all C_{1-11}. The controls, OVC (Organism Viability Control) and ESC (Extract Sterility Control) was all positive while the MSC (Media Sterility Control) responded normally.

Standard antibiotics disc tested in parallel with this experiment gave the following results. Amoxillin showed activity against *C. subtilis*. Chloramphenicol displayed all round activity against all the organisms: 10, 20, 18, 12

Table 1. Antimicrobial screening of crude extract and fractions of *C. Africana.*

Extracts and Fractions	Micro organisms/Activity				
	S. aureus	***E. coli***	***B. subtilis***	***P. aeruginosa***	***C. albicans***
Ethanolic Crude extract(2000 μgml^{-1})	+	+	-	-	++
10% aqueous methanol	+	++	-	-	+
Water soluble	+	-	-	-	+
n-hexane	+	+	-	-	+

Key: + Activity
- No activity

Table 2. Antimicrobial screening of purified *C. africana* root extract components.

Organism	Component											OVC	S/C	MSC	Chloramphenicol	Amoxillin	Tetracycline
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}				(52 μg)	(25 μg)	(30 μg)
C. albicans	0	0	0	0	0	2	0	0	1	0	0	+	NA	N	10	8	8
S. aureus	1	1	1	1	3	1	1	3	2	2	1		NA	O	20	0	12
E. coli	2	1	1	2	1	3	2	1	1	1	3	+	NA	R	18	0	10
B. subtilis	0	0	0	0	0	0	0	1	1	1	1	+	NA	M	12	1	7
P. aeruginosa	0	0	0	0	0	0	0	0	0	0	0	+	NA	A	8	0	0
ESC	+	+	+	+	+	+	+	+	+	+	+	+	NA	L			

Key: O/NA = No Activity; S/C = Solvent control, that is, *n* –hexane; ESC = Extract Sterility Control; OVC = Organism Sterility Control; MSC = Medium Sterility Control; + = Normal response.

and 8 mm zonal inhibition against *C. albicans, S. aureus, E. coli, B. subtilis and P. aeruginosa* respectively. Tetracyclines also showed relativity all round activity against the five organisms tested for that is 8 mm zonal inhibition for *C. albicans,* 12 mm for *S. aureus,* 10 mm for *E. coli,* 7 mm for *B. subtilis* but no activity against *P. aeruginosa.*

From the above results, the zonal diameter obtained for the purified extracts was $\geq$ 3 mm. Earlier work (Okwute et al., 1989), on the biologically active *n*-hexane extract of the root of *C. africana* showed that the extract contained three triterpenes, α-amyrin, β-sitosterol and hydroxyl car- boxylic acid. The species or strains of the organism could affect the susceptibility or resistivity of the purified extract. This was indicated in the differential response of the tested species to the ethanolic extract components. The C_{1-11} fractions were mostly active against *S. aureus* and *E. coli* but least active against *P. aeruginosa* in Mueller-Hinton agar.

Paraskeva et al. (2008) reported from their studies of *in vitro* biological activity of selected South African *Commiphora* species that a greater selectivity was exhibited by the extracts against the Gram-positive bacteria 0.01 - 8.00 mg/ml and the yeasts 0.25 - 8.00 mg/ml than against the Gram-negative bacteria 1.00 - 8.00 mg/ml in an antimicrobial (MIC) assay. Rahman et al. (2008) confirmed the antibacterial efficacy of terpenes from the oleo-resin of *Commiphora molmol* (Engl.). It was found out that higher activity of terpenes 1 - 4 was determined against a multidrug resistant strain of *S. aureus* -SA1199B than other four strains.

Conclusion

It was confirmed from this experiment that *C. africana* root crude extracts, fractions and components have shown promising but differential *in-vitro* antimicrobial activity. It is possible that more potent components espe- cially against *S. aureus* and *C. albicans* might reside in the polar fractions which should be the subject of future investigation.

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