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Short Communication

Phytochemical and Antimicrobial Studies of *Commiphora africana* Root Extracts

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ABSTRACT

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, E. coli* and *C. albicans. S. aureus* (ATCC 13709) was susceptible to fractions C_1 - C_{11} giving zonal inhibition of between 1-3 mm. Ethanolic crude root extract (2000 μg mL^{-1}) proved highly active against *C. albicans*, while 10% aqueous methanol was the most active against *E. coli.* Though *C. africana* root crude extracts, fractions and components showed promising *in-vitro* antimicrobial activity, it was not as high as that of the standard antibiotics used for comparison.

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 with short lateral branches, sharply pointed at the apex, bearing leaves in small clusters below the tip (Arnold & Dewet, 1993). The plant is well suited to dry areas and often grown as a hedge in Northern Nigeria (Burkill, 1985). Parts of the plant are medicinally consumed in several West African countries possibly, because of the presence of phytochemicals such as methylisopropenyl furane, 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 are 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-guggulsterone (Yamini *et al.*, 1984; McGuffin *et al*., 2006). In Nigeria, a seed decoction is held to be a very effective purgative and vermifuge. A dose of 6 g of powdered seed in a glass of water is certain to expel intestinal 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 antimicrobial efficacy.

MATERIALS AND METHODS

Plant and chemical sources. 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 Hannover). Rf values were obtained on pre-coated Merek grade TLC plates using a Camag Ultra Violet (U.V) lamp 366-254 mm and iodine vapor 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 concentrated 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 separated 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 & Evans, 1989) on the crude extract gave a blue

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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 of the *n*-hexane fraction (2.20 g) was done on TLC grade silica gel as the stationary phase and eluting with mixtures 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.

For 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 0.037 g. *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 component 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 Microbiology 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^{-1}. 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 (4) 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 sensitivity results obtained (Table I).

Antimicrobial screening of purified components. The purified components C_1 - C_{11} were all screened for antimicrobial activity using a modified version of National Committee for Clinical Laboratory Standards (NCCLS) (Trease & 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-*Staphylococcus aureus* (ATTC 13709), *E. coli* (NITC 10418), *Bacillus subtilis* (Pharmaceutical Microbiology Dept., ABU, Zaria), *Pseudomonas aeruginosa* (ATCC 27853) and *C. albicans* (Diagnostic Unit NIPRD, Pharm Microbiology., Dept.). The well mixed media were each dispensed into sterile plates on a flat surface and allowed to gel. Each of the purified components (100 μg) 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 & 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, medium and organism controls. The plates in duplicates were incubated at 37°C for 24 h and the sensitivity results obtained (Table II.).

RESULTS AND DISCUSSION

The crude extract at 2000 μg mL^{-1} 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 I). A 10% 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 II shows the sensitivity results obtained from the purified components. The *S. aureus* was susceptible to fractions C_1 - C_{11} giving zonal inhibition of between 1 - 3 mm. C_1 - C_7 and then $C_{11,}$ gave zonal inhibition of 1 mm, C_9 and C_{10} gave zonal inhibition of 2 mm, while C_5 and C_8 gave the maximum zonal inhibition of 3 mm. *E. coli* was susceptible to C_1 - C_{11} giving 1-3 mm zonal inhibition. C_2-C_9 and C_8 - $_{10}$ gave 1 mm zonal inhibition, C_1 and C_7 gave 2 mm zonal inhibition and C_6 and C_{11} gave 3 mm zonal inhibition. The *B. subtilis* was susceptible to $C_8 – C_{11}$ with 1 mm zonal inhibition. *C. albicans* was susceptible to C_6. *P. aeruginosa* was resistant to all $C_1 – C_{11}$. 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 mm, 20 mm, 18 mm, 12 mm 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

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

Organism	Component											O.V.C.	S/C	M.S.C.	Chloramphenicol (52 μg)	Amoxillin (25 μg)	Tetra cycline (30 μg)
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}						
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 i.e. *n* –hexane; ESC = Extract Sterility Control; O.V.C. = Organism Sterility Control
M.S.C. = Medium Sterility Control; + = Normal response

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 carboxylic 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 - C_{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^{-1}) and the yeasts (0.25-8.00 mg mL^{-1}) than against the Gram-negative bacteria (1.00-8.00 mg mL^{-1}) 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. The *in vitro* and *in vivo* anthelmintic efficacy of *Terminalia arjuna* bark extract was reported by Bachaya *et al.* (2009). Also Imelouane *et al.* (2009) confirmed the antimicrobial activity of essential oil of thyme (*Thymus vulgaris*) from Eastern Morocco.

CONCLUSION

Results showed that *C. africana* root crude extracts, fractions and components have shown promising but differential *in-vitro* antimicrobial activity however, the efficacy was not as high as that of the three standard antibiotics used for comparison. It is possible that more potent components especially against *S. aureus* and *C. albicans* might reside in the polar fractions, which should be the subject of future investigation.

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