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Chemical Constituents, Antimicrobial and Termite Repellent Activities of *Commiphora Africana* (Caesalpiniaceae) Root Extracts

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ABSTRACT

Commiphora africana is very important in traditional medicine among the natives of Northern Nigeria as a remedy for several ailments and the resin, root, bark, leaves and wood are usually burnt to keep off mosquitoes and as an incense to ward off evil spirits. The powdered air-dried root was successively extracted with benzene, chloroform and methanol. Phytochemical analysis of the extracts showed the presence of tannins, steroids, alkaloids, saponins, phenols, flavonoids and glycosides. Column chromatographic fractionation followed by GC-MS analysis led to the identification of two triterpenoids, α-amyrin and stigmasterol acetate. Also, the powdered air-dried root was directly extracted with methanol and the crude extract as well as the fractions were tested for activity against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Candida albicans and Salmonella spp*. The fractions were found to be more active than the crude extract, including against those organisms that were not sensitive to the crude extract, underscoring the importance of fractionation in the study of bioactive plants. However, repellent activity screening against *Thoracotermes macrothorax* (common soldier ant), using Filter Paper Disc method showed that the crude methanol extract was more active than the fractions. The phytochemicals contained in the root, including the two triterpenoids identified in the extracts may account for the biological activities observed in this study and support the traditional claims on the plant.

Keywords: Antimicrobial, *Commiphora africana*, Repellent, Triterpenoids

INTRODUCTION

The Burseraceae family is composed of trees and shrubs which are widely distributed in tropical and sub-tropical regions (Watson & Dallwitz, 1992). It consists of approximately 700 species from 18 genera (Weeks *et al.*, 2005). *Commiphora africana* (A. Rich.) Endl., commonly called African myrrh, is a small deciduous tree belonging to the family and occurs widely in sub-Saharan Africa. Various parts of the plant are traditionally used to treat a wide range of ailments (Hadissa & Jean-Pierre, 2005; Kokwaro, 2009). It is widely used in many parts of northern Nigeria as incense, insecticidal and antiseptic fumigant. It is also commonly found in Angola, Botswana, Burkina Faso, Chad, Eritrea, Ethiopia, Kenya, Mali, Mauritania, Mozambique, Namibia and Niger, among many other countries (Dalziel, 1937). Some pharmacological investigations on the plant included the study on its anti-inflammatory and analgesic effects of the hydro-ethanolic extract of the stem bark on rodents (Ezekiel, 2010), the anti-oxidant activity of the essential oil of the species from the West African country, Benin (Ayeodoun *et. al.*, 1998; Ma *et. al.*, 2004, Alvessi *et. al.*, 2005) and the antimicrobial and cytotoxic properties of the leaves and root extracts from the Nigerian species(Okwute *et al.*, 1989, Akor and Anjorin, 2009, Isyaka and Okwute, 2013, Okwute and Ochi, 2017). Earlier, chemical investigations of the species of the genus, *Commiphora* have been on the resins which yielded various classes of terpenoids and a lignin (Carl and Noble,1980; Carl and Noble, 1983; Waterman and Amopofo, 1985; Provan and Waterman,1985). More recently, the leaves and bark were investigated for phytochemicals and in addition to the terpenes commonly found in many species of the genus *Commiphora* alkaloids were also detected (Ezekiel *et al.*, 2010; Isyaka and Okwute, 2013). Also, some triterpenoids including α-amyrin and β-sitostenone have been reported for the first time from the antimicrobial hexane fraction of the root (Okwute *et al.*, 1989).

This work intends to evaluate the biological characteristics and chemical constituents of the *Commiphora africana* (Caesalpiniaceae) root extracts.

MATERIALS AND METHODS

General

All solvents used were of Analar grade or purified appropriately. The GC-MS was carried out at the National Research Institute for Chemical Technology (NARICT), Zaria, on Shimadzu GC/MS-QP2010 Plus (Japan). The samples were

run at column oven temperature of 60 ^{o}C and injection temperature of 250°C using hydrogen as carrier gas at 46.3 cm^3/sec linear velocity. Silica gel for column chromatography was Silica 60 G. TLC was carried out on pre-coated silica gel plates, using H_2SO_4-MeOH spray as chromogenic reagent, as well as iodine vapour and UV Lamp(366/254 nm) for detection of spots.

Collection and Identification of Plant Material

The *Commiphora Africana* root was collected from Gumau, Toro L.G.A., Bauchi State, Nigeria and was authenticated at the National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria(Voucher specimen number: NIPRD 6613). It was air-dried, powdered and stored in a polythene bag until used.

Sourcing of Organisms

The organisms for antimicrobial screening, *Candida pseudotropicalsis, Staphylococcus aureus, Bacillus substillis, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans* were obtained from the National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria. The termites, *Thoracotermes macrothora,*, were collected from Gwagwalada, Abuja and stored in the laboratory overnight for acclimatization.

Extraction and Fractionation

For chemical investigations, including phytochemical analysis and isolation, the powdered dried root (613.0 g) was successively extracted with 2.5L of benzene, chloroform and methanol each. Each extract was evaporated to dryness using a rotary evaporator to give 6.5g, 7.3g and 15.8g, respectively.

The extract used for the bioassays was obtained by extracting the powdered dried root (1 kg) by percolation with a total of 5.0 L of methanol (2x 2.5L) at room temperature. Evaporation of the combined yellowish extract gave a viscous brown residue (23.74 g). A portion of the crude extract (21.0g) was dissolved in 200 ml of 30% aqueous methanol. It was then partitioned with petroleum ether (150 ml) using a separatory funnel to give a brownish viscous petroleum ether residue(8.31g) and a brownish methanol residue(10.6g) on evaporation to dryness *in vacuo.* This procedure was adopted because fractionation has been shown in some studies to enhance bioactivity of plant extractives by pooling structurally close constituents based on solvent polarity or acidic, basic and neutral characteristics (Mitscher *et al.*, 1987).

Phytochemical Screening of Extracts

The three extracts, benzene, chloroform and methanol from *Commiphora africana* root were subjected to phytochemical screening using standard procedures (Trease and Evans, 2002).

Column chromatography of benzene extract

The benzene extract (5.0g) was subjected to column chromatography (36.5 cm length and 2.8 cm diameter) on silica gel (25.0g). It was eluted with hexane (200 cm^3) and then with mixtures of hexane and ethyl acetate and finally with ethyl acetate (200 cm^3) collecting 20 cm^3 fractions. Evaporation of fractions eluted with 5% ethyl acetate in hexane gave a fraction coded CAB (150 mg).

Column chromatography of chloroform extract

The chloroform extract (5.0 g) was chromatographed on a silica gel column as above by eluting with mixtures of hexane and ethyl acetate, starting with 10% ethyl acetate in hexane to 100% ethyl acetate, collecting 20 cm^3 fractions. Monitoring the column fractions with TLC showed that 7% ethyl acetate in hexane eluted a compound coded CAC (62 mg).

Column chromatography of petroleum ether fraction

The petroleum ether residue (6.0 g) was subjected to vacuum liquid chromatography (VLC) using TLC grade silica gel GF254 and eluting with a mixture of diethyl ether and chloroform to give two major fractions coded X(0.41g) and Y(3.5g). Column chromatography(2.0g of Florisil, burette-size glass column) of fraction X with mixtures of chloroform and methanol(1-2%), collecting 2 cm^3 fractions, gave fractions BCA1, BCA2 and BCA3(20 mg each). Fraction Y(2.0g) on column chromatography(10.0g Florisil, 20 cm length and 1.0 cm diameter column) and elution with mixtures of diethyl ether in hexane (1-3%), collecting 5cm^3 fractions gave petroleum ether sub-fractions BCA4(80mg), BCA5(20mg) and BCA6(20mg).

Biological Evaluation of Extracts of Root of *Commiphora africana*

The crude methanol extract, the petroleum ether and aqueous methanol fractions from solvent-solvent partitioning and the 6 chromatographic sub-fractions from petroleum ether fraction were subjected to antimicrobial and termite repellent tests.

Antimicrobial Screening of Extracts

The extractives were screened for antimicrobial screening using agar-streak dilution technique (Murray *et al.*, 1995). Various concentrations of each extractive were prepared in purified hexane as solvent and subjected to screening against the test organisms. Ciprofloxacin at 10 µg/ml was used as the control.

Each concentration (Table 2) of each of the extracts (1ml) prepared by dilution according to a standard procedure(Mitscher *et al.*,1987) was mixed with 19 ml of warm sterile nutrient agar in a petri-dish and allowed to cool to solidify. The microorganisms were inoculated into the mixture

of agar and extract by streaking. The positive control (10µg/ml of ciprofloxacin) was also subjected to the above procedure. These were incubated at 37^{o}C for 24 hrs and observed for any growth.

Termite Repellent Activity of Extracts

The repellent activity screening was based on filter paper disc method according to a previously described procedure (Alam *et al.*, 2014, Chindo *et al.*, 1997) using *Thoracotermes macrothorax.*

The crude methanol extract and the 6 chromatographic sub-fractions of the petroleum ether fraction each was taken in hexane to prepare solutions of 0.050, 0.025 and 0.010 g/ml and 5 drops of each solution were dropped using a micropipette at the centre of the filter paper in the petridish. The insects (14) were placed at about 2 cm away from the sample spot and the rate at which the insects are repelled determined after 2 minutes in terms of number of insects that moved away from centre. The control experiment had only the solvent, hexane.

RESULTS AND DISCUSSION

Phytochemical screening of benzene, chloroform and methanol extracts. The results of phytochemical screening are given in Table 1.

Table 1: Phytochemical screening of root extracts of *Commiphora africana*

	Extracts		
Phytochemicals	**Benzene**	**Chloroform**	**Methanol**
Tannins	+	+	+
Alkaloids	+	+	+
Saponins	+	+	+
Phenols	+	+	+
Sterols	+	+	+
Glycosides	+	+	+

Key: (+)=Present

Phytochemical screening of benzene, chloroform and methanol extracts of the root of *Commiphora africana* obtained by successive extractions revealed the presence of tannins, alkaloids, saponins, phenos, sterols and glycosides in the three extracts (Table 1). This suggested that the phytochemicals were all soluble in the three solvents, but to varying degrees depending on polarity of both solvent and phytochemicals. Simple alkaloids and phenols may be soluble in benzene, but glycosides may only be sparingly soluble in benzene. However, between chloroform and aqueous methanol, the former is much less polar and this is reflected in the yields of the extracts which were 6.5, 7.3 and 15.8 g, respectively. Previous phytochemical analysis was on the crude 95% ethanol extract which showed that the root contained the above phytochemicals along with others (Okwute and Ochi, 2018). Of particular interest is the consistent detection of alkaloids in the extracts of the plant, including the leaves (Isyaka and Okwute, 2013). However, it appears alkaloids are yet to be isolated from the genus *Commiphora*. These phytochemicals have been suggested and even established by various workers as responsible for the pharmacological activities of many plant extracts and therefore account for their ethno-medicinal uses (Ezikiel *et al.*, 2010, Valsaraj *et al.*, 1997, Mahato and Sen, 1997, Lin *et al.*,2004, Akiyama *et al.*, 2001,Waller and Yamasak,1995, Balandrin,1996).

The crude methanol extract, the petroleum ether and aqueous methanol fractions and the petroleum ether chromatographic sub-fractions, BCA1-BCA6 were screened against some selected pathogens. The results are recorded in Table 2.

Table 2: Antimicrobial Activity of *Commiphora africana* Root Extracts

Extractive	Microorganisms						
	1	2	3	4	5	6	MIC(µg/mL)
Crude methanol	-	+	-	+	-	-	1075
Petroleum ether	-	+	-	+	+	-	1000
Aqueous methanol	-	+	-	+	+	P	1000
BCA1	-	+	+	+	+	p	1000
BCA2	-	+	+	+	-	-	2000
BCA3	-	+	+	+	+	p	2000
BCA4	-	+	+	+	+	p	1000
BCA5	-	+	+	+	+	P	2000
BCA6	-	+	+	+	-	-	1000
Control	-	+	+	+	+	+	10

Key:(+)=Active,(-)=Inactive, P=Partially active,1=*Candida pseudotropicalsis,* 2=*Staphylococcus aureus*,3=*Bacillus subtillis,* 4=*Escherichia coli,* 5=*Pseudomonas aeruginosa*, 6=*Candida albicans,* Control=Ciprofloxacin

The crude methanol extract, the petroleum ether and aqueous methanol extracts, and the petroleum ether chromatographic fractions were screened for antimicrobial activity against some selected pathogens. The results (Table 2) showed that the crude methanol extract was only active against *S. aureus* and *E. coli* among the test organisms, while the two fractions, petroleum ether and aqueous methanol displayed broader activity than the crude, with the aqueous methanol fraction possessing partial activity against *P. aeruginosa and C. albicans* at 1000µg/mL MIC.

Similarly, the petroleum ether chromatographic sub-fractions generally displayed broader activity than the crude and fractions at 1000-2000µg/mL, supporting the observation by some workers that fractionation increases antimicrobial activity by increasing the concentration of active components (Mitscher *et al.*, 1987). Previous studies on *Commiphora africana* were on the hexane fraction of the crude 95% extract (Okwute *et al*, 1989, Akor and Anjorin,2009) and on the acidic, basic, polar and non-polar neutral fractions(Okwute and Ochi, 2018), but recorded activity against very few organisms at much higher MIC values ranging from 2.0 to 25.0 mg/mL except Okwute *et al.* (1989) who recorded 1000µg for crude extract against *S. aureus* and *Mycobacterium smegmatis*, while the hexane and ether fractions were surprisingly and selectively active only against *S. aureus* and at a much lower and very significant MIC value of 100µg/mL. Thus, the results of that work and this present work underline the important role of fractionation in enriching activity of plant extracts to the extent that hitherto inactive extracts of plants have been found to yield active components when subjected to fractionation (Mitscher *et al.*, 1987).

The crude methanol extract and the petroleum ether chromatographic sub-fractions were screened for insect repellent activity using *Thoracotermes macrothorax*. The results of the behavioural bio-assay are presented in Table 3.

Table 3: R**epellent activity of *Commiphora africana* Root Extracts against *Thoracotermes macrothorax***

Extracts	Concentration(g/ml)/Number of Insects Repelled/14		
	0.050	0.025	0.010
Crude methanol extract	9	6	6
BCA1	5	3	1
BCA2	7	6	5
BCA3	6	5	4
BCA4	6	4	3
BCA5	4	1	1
BCA6	3	2	1
Control(Solvent)	2	1	1

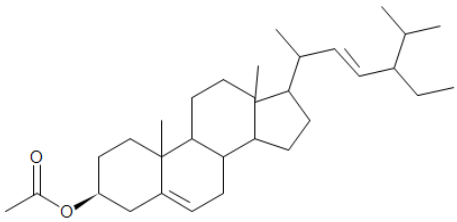
The results (Table 3) showed that the extracts exhibited relatively low to moderate to high repellent activity, with the crude extract possessing the highest potency, and that the repellent activity of the extracts is generally concentration-dependent when measured against the blank (solvent). Thus, while the crude extract at concentration of 0.05g/ml repelled 9 out of 14 insects, at 0.01g it repelled 6 insects. While the chemical constituents of the extracts were not individually investigated with respect to their repellent activity, it may be reasonable to suggest that the varying potencies of the extracts were due to varying chemical constitutions and concentrations of the active components and that the crude extract had a pool of these components acting individually and possibly synergistically. The insect repellent activity of the extracts may not be unrelated to the presence of steroids in the root reported in this work and by previous workers (Akor and Anjorin, 2009, Okwute and Ochi, 2018). Also, a triterpenoid, 2α, 3β, 21β, 23,28-pentahdroxy 12-oleanene had previously been reported to exhibit reasonable repellent activity against *Tribolium castaneum* (Alam *et al.*, 2014). This is the first study on the insect repellent activity of *Commiphora africana*, though the burnt parts have been known traditionally to keep off mosquitoes (Dalziel, 1937).

The benzene and chloroform extracts on chromatographic purification gave semi- pure fractions CAB and CAC, respectively. They were subjected to IR and GC-MS analysis for the purpose of their identification (Table 4).

Table 4: IR and GC- MS data for compounds CAB and CAC

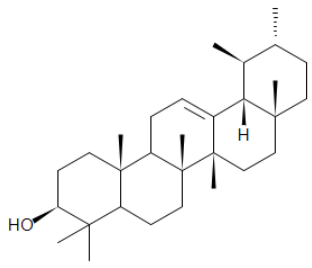
Compound	IR(CM^{-1})	GC/ RT(mins)	MS (m/z)
CAB	3401(OH, weak), 2931(C-H) 1722(C=O), 1452(C=C), 1377, 1266, 1176, 1031(C-O)	17.824	454(M^+), 394, 351, 255, 228, 213, 173, 159, 145, 133, 119, 105, 93, 81, 69, 43(base peak)
CAC	3361(OH, strong), 2941(C-H) 1696(C=C), 1445(CH2), 1028(C-O)	29.567	426(M^+), 411, 218(base peak), 203, 189, 175, 161, 135, 122, 109 95,81, 69, 44, 41

The IR spectrum of compound CAB showed a weak absorption band at 3401 for an alcoholic hydroxyl group, 2931 for carbon-hydrogen bond stretching of a multi-methylene system,a fairly strong carbonyl band at 1722 for an α,β-unsaturated or a cyclo-alkane ester, an absorption band at 1422 for C=C skeleton and other bands at 1377, 1266 and 1031 for C-O absorptions. The IR spectrum therefore suggests that compound CAB is either a hydroxy-ester or a mixture of two compounds, an ester and an alcohol of the triterpenoid type (Achika *et al*, 2016, Anjoo and Ajay, 2011). The GC-MS showed a very weak molecular ion (M^+) peak at 454 corresponding to a molecular formula, $C_{31}H_{50}O_2$). Based on NIST Computer GCMS Library Data it is suggestive that fraction CAB may be stigmasterol acetate, 1. In agreement with this structure the other important fragment ions included the major peak at m/z 394, suggesting the loss of an acetate unit (59+1) from the molecular ion and the fragment ion m/z 255 corresponding to cleavage of the side chain at C-17 from the fragment ion m/z 394(-139). The loss of the isopropyl group (m/z 43) from the fragment ion m/z 394 to give m/z 351 was observed. The above IR and MS spectral analyses coupled with some similarities with those reported for stigmasterol and β-sitosterol by other workers Anjoo and Ajay, 2011, Kenkata and Indra, 2012) support the suggestion that fraction CAB is stigmasta-5,22-diene-3β-ol,acetate,1. The hydroxyl absorption band at 3401 in the IR spectrum of CAB may therefore be due to some other triterpenoids such as sitosterols or stigmasterols as impurities.



1

The IR spectrum of fraction CAC showed a strong absorption band at 3361 for alcoholic hydroxyl group, a strong C-H absorption band at 2941 and weak absorptions at 1696 for C=C and 1028 for C-O vibration as reported for some triterpenoids and derivatives (Achika *et al.*, 2016, Okoye *et al.*, 2014, Krishnam *et al.*, 2014). The GC-MS spectral analysis gave MS data with significant fragment ions. A molecular ion peak occurred at m/z 426(M^+), corresponding to a molecular formula, $C_{30}H_{50}O$. The peak at m/z 411 is as a result of loss of methyl (15 units) from the molecular ion, m/z 426(M^+). An important fragmentation is the splitting of the molecular ion (m/z 426) to give m/z 204/203 and m/z 232 fragment ions. The loss of a methyl group(-15 units) from fragment ion m/z 232 leads to the generation of the base peak, m/z 218, which is typical of triterpenes lacking oxygen function in rings C and D or E (Thomas and Willhalm, 1964). The above spectral characteristics are in agreement with NIST standard computer MS data and literature spectral properties for α-amyrin,2 (NIST Mass Spectral Library,2014, Okwute *et al.*, 1989, Narender *et al.*,2008, Okoye *et al.*, 2014).



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Thus, while α-amyrin had previously been reported from the root of *Commiphora africana*, this is the first report of stigmasterol acetate from the species and probably from the genus. The amyrins and stigmasterols, and their derivatives have been found to have a number of pharmacological activities including antimicrobial (Achika *et al.*, 2014) and anti-inflammatory (Okoye *et al.*, 2014, Krishnam *et al.*, 2014).

CONCLUSION

The root of *Commiphora Africana*, a plant used traditionally as a pesticide and in ethno-medicine for the management of a number of infections has been suggested in this study to possess insect repellent activity and therefore can be used to protect stored crops in agriculture and in the home against termites. The extracts were also shown to possess antimicrobial activity against some pathogens of industrial significance. In addition, two triterpenes α-amyrin and stigmasterol acetate, were identified in the extracts. Of the two triterpenes, stigmasterol acetate is being reported for the first time from the species and probably from the genus. These compounds and other phytochemicals in the extracts may account for the pesticidal and medicinal uses of the plant.

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