PHYTOCHEMICAL AND ANTIMICROBIAL EVALUATIONS OF THE EXTRACTS OF THE ROOTS OF *Commiphora africana* (CAESALPINIACEAE)

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Abstract: *Commiphora africana* is used in traditional medicine for the treatment of various ailments by the natives of northern Nigeria. The air-dried roots were extracted with 95% ethanol using a Soxhlet apparatus to obtain a crude extract. The crude extract obtained was successively partitioned into alkaloid, acid, polar and non-polar fractions. The crude extract and the different fractions were evaluated for phytochemicals and antimicrobial activity. The result of the preliminary phytochemical screening of the crude extract showed that saponins, tannins, reducing sugars, flavonoids, cardiac glycosides, triterpenoids, alkaloids and carbohydrates were detected in the root. The antimicrobial activity of the crude and different fractions was determined against some commercially important microorganisms. The crude extract and fractions exhibited characteristic strong concentration-dependent activity (CDA) against the test organisms, with zones of inhibition of 11-33 mm depending on the concentrations. The result of this study confirmed the documented records of traditional medicinal uses of *Commiphora africana* root.

Keywords: Antimicrobial activity, *Commiphora africana* root, crude extract, fractionation, phytochemicals

Introduction

All plants produce chemical compounds during their normal metabolic activities. These include primary metabolites, such as acetic acid, sugars and residues from fats and secondary metabolites which are complex in structure and in larger quantity. Plant-based drugs have provided outstanding contributions to modern therapeutics. Examples include serpentine isolated from the root of Indian plant *Rauwolfia serpentine* in 1953 which made a remarkable difference in the treatment of hypertension and lowering of blood pressure and vinblastine isolated from the *Catharantus rosesus* (Farnsworth *et al.*, 1967) which is used for the treatment of several illnesses such as leukaemia in children, testicular and neck cancer. Other plant-derived drugs have proven to be effective against mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer (Perumal *et al.*, 1998). The world is now consciously moving towards the herbal medicines or phytomedicines that repair and strengthen body systems and thus help to destroy offending pathogens without toxic side effects.

The domestication, production, biotechnological studies and genetic improvement of medicinal plants, instead of the use of plants harvested in the wild, offers great advantages, since it is now possible to obtain uniform and high quality raw materials which are fundamental to the efficacy and safety of herbal drugs (Calixto, 2000).

The Burseraceae is a plant family that is composed of both trees and shrubs widely distributed in tropical and sub-tropical regions (Watson & Dallwitz, 1992). It consists of approximately 700 species from 18 genera (Weeks *et al.*, 2005). This classification is based exclusively upon their fruit structure (Clarkson *et al.*, 2002). *Commiphora africana* (A. Rich.) Endl., commonly called African myrrh, is a small deciduous tree belonging to the Burseraceae and occurring widely over sub-Saharan Africa. Various parts of the plant are used to treat a wide range of ailments (Hadisa & Jean-Pierre, 2005; Kokwaro, 2009). The leaves and bark have previously been 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 have been reported for the first time from the antimicrobial hexane fraction of the root by some workers (Okwute *et al*., 1990).

In this study we report results of the phytochemical screening of the crude 95% ethanol extract of the root, fractionation of the crude extract into acidic, basic and neutral fractions, followed by the antimicrobial screening of the crude and the fractions (Mitscher *et al.*, 1972) so as to investigate the effect of fractionation on the antimicrobial activity of the crude extract.

Materials and Methods

Fresh roots of *Commiphora africana* were collected from Gaji village, Gwagwalada Area Council, Federal Capital Territory, Abuja, Nigeria, in November, 2014. They were authenticated at the Herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, and a voucher Specimen Number NIPRD/H/6642 was assigned. The roots of *Commiphora africana* were cut into small bits to facilitate drying. The pieces of the roots were air-dried at room temperature for three weeks and then pulverized into fine particle size using a Hammer Mill machine. The powdered plant material was stored in a tightly closed polythene bag from where it was taken for extraction.

The chemicals used were of analytical grade manufactured by BDH Chemicals Poole, London. All the organic solvents used were redistilled before use.

The culture media used for the antimicrobial screening were Mueller Hinton Agar (MHA), Potato Dextrose Agar (PDA), Nutrient Agar (NA) and Mueller Hinton Broth (MHB). All the media were prepared according to the manufacturer's instructions against the following micro-organisms: *Staphylococcus aureus* (*Sa), Bacillus subtilis (Bs), Escherichia coli (Ec), Salmonella typhi (St) and Candida albicans (Ca).* The test organisms used were clinical isolates obtained from the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Methods

Extraction of plant material

The air-dried pulverized root (950 g) of *C. africana* was extracted with 3 L of 95% ethanol using Soxhlet apparatus. The extract obtained was filtered using sterile Whatmann No. 1 filter paper and concentrated with Rotary evaporator to obtain the crude extract.

Fractionation of the crude extract

The fractionation was based on the procedure of Mitscher *et al.* (1972). The crude extract of *C. africana* root was completely dissolved in 0.5 L of dichloromethane and added to 50 mL of 5% HCl in a separating funnel. The solution was then thoroughly shaken and allowed to stand. The organic and aqueous layers were collected separately. The aqueous layer

was repeatedly washed with equal volume of dichloromethane. The bulk of dichloromethane layer (organic layer) collected was combined. The upper aqueous HCl layer was basified with 2M NaOH dropwise to precipitate the base (alkaloids).The basified portion was extracted with 250 mL of dichloromethane three times; the lower dichloromethane layer was evaporated to dryness to give the alkaloids (fraction 1). The original dichloromethane layer containing the neutral and acidic phytochemicals was extracted with an equal volume of 10% Na_2CO_3 twice. The upper layer (acids) containing the sodium salts was collected and acidified with 2M HCl to litmus red and extracted with 250 mL of dichloromethane twice. The lower dichloromethane layer containing the acids was then evaporated to dryness to give the acids (fraction 2). The remaining dichloromethane layer (neutrals) was evaporated to dryness. The residue was dissolved in 90% aqueous methanol and extracted with 250 mL of hexane twice. Both the hexane (upper layer) and aqueous methanol (lower layer) were evaporated to dryness separately to give fractions 3 and 4 (neutral non-polar and polar fractions, respectively). The weight of the total crude extract obtained and different fractions were then recorded.

Preliminary phytochemical screening of crude extract

The crude extract of *C. africana* obtained was subjected to preliminary qualitative phytochemical screening for secondary metabolites such as saponins (Frothing test), tannins, reducing sugars, flavonoids (alkaline reagent test), anthraquinones, cardiac glycosides, triterpenoids (Salkowski's test), alkaloids (Wagner's reagent), steroids (Liebermann-Burchard test), carbohydrates (Molisch's test), proteins and quinones. The screening was based on standard procedures and protocols (Harborne, 1984; Sofowora, 1993; Trease and Evans, 2002).

Antimicrobial activity

Preparation of varying concentrations of the extracts

Various concentrations of the extracts were prepared ranging from 12.5 to 100 mg/mL. This was obtained by taking 1 g of the extract and dissolving it in 10 mL dimethylsulphoxide (DMSO) to give a stock solution of the extract (100 mg/mL). A serial dilution of the 100 mg/mL was carried out in three different bottles containing DMSO to obtain concentrations of 50, 25 and 12.5 mg/mL, respectively.

Sensitivity tests of the crude extract and fractions using agar well diffusion method

The sensivity tests on the samples were based on standard procedures (Magaldi, *et al.,* 2004; Valgas, *et al.,* 2007). The organisms used were standardized using Mc-Farland turbidity standard scale l, to obtain a bacterial cell density of 10^6 colony forming unit per millilitre (cfu/mL). These were standardized by sterilizing the media and autoclaving at 121ºC for 15 min. The standardized inocula were uniformly swabbed into freshly prepared Mueller Hinton agar and potato dextrose agar plates, respectively for the bacterial and fungal growths. Four wells were punched on the inoculated plates with a sterile cork borer (8 mm in diameter). The wells were properly labelled according to different number of the concentrations prepared. The wells were then filled up with the extracts about 0.2 mL per well. The plates were allowed to stay on the bench for 1 hour for the extract to diffuse on the agar. The Mueller Hinton agar plates for bacterial were incubated at 37ºC for 24 h while the potato dextrose agar plates for fungi were incubated at room temperature for three days.

At the end of incubation period, all plates were observed for any evidence of inhibition, which will appear as clear zones that were completely devoid of growth around the wells (zone of inhibition). The diameters of the zones were measured with a transparent ruler calibrated in millimetre (mm)

Sensitivity test of antibiotics against the test organisms

Sensitivity test of antibiotics used as controls was carried out against the test organisms. The antibiotics used were Ciprofloxacin (10 µg), Amoxicillin (30 µg), and Ketaconazole (250 mg). Standardized inocula (organisms) were spread on sterile Mueller Hinton Agar plates with the end of a bent glass rod (spreader). A sterile forcept was used to pick a standard antibiotic disc and placed on the inoculated media. Mueller Hinton Agar was used for bacteria whereas Potato Dextrose Agar medium was used for fungi. The disc was allowed to diffuse on the agar after which the inoculated plates were incubated at 37ºC for 24 h. At the end of incubation period the diameter of the zone of inhibition was measured and results were recorded. Ciprofloxacin and Amoxicillin antibiotics were used against the bacteria (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Salmonella typhi*) while Ketaconazole was used against the fungal isolate (*Candida albicans*) (Jorgensen and Ferraro, 2009; Heatley, 1944).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the extract was determined using tube dilution method (Nijs *et al.,* 2003). Serial dilution of the extract was carried out in test tubes using Mueller Hinton Broth (MHB) and Potato Dextrose Broth (PDB) as diluents. The lowest concentration showing inhibition (clear zone) for each organism when the extract was tested during sensitivity test was serially diluted in test tubes containing Mueller Hinton Broth (MHB) and Potato Dextrose Broth (PDB). Each tube containing the broth and the extract was inoculated with the standardized organisms. A tube containing sterile broth (MHB and PDB) without any organism was used as a control. All tubes were then incubated at 37ºC for 24 h. After the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion. The lowest concentration (dilution) in the series without visible signs of growth was considered to be the minimum inhibitory concentration (MIC).

Determination of minimum bactericidal concentration (MBC)

The results from the Minimum Inhibitory Concentration (MIC) were used to determine the Minimum Bactericidal Concentration (MBC) according to the procedure of Pfaller *et al.* (2004). A sterile wire loop was dipped into the tubes that did not show turbidity in the MIC test and then streaked unto freshly prepared sterile nutrient agar plates. The plates were incubated at 37º C for 24 h. After the incubation period the plates were then examined for the presence or absence of growth. This was done to determine whether the antimicrobial effect of the extract is bactericidal or bacteriostatic.

Results and Discussion

The results of the preliminary phytochemical screening of crude ethanolic extract of the dried powdered roots of *C. africana* are shown in Table 1. The secondary metabolites reported from this investigation were known for their broad spectrum of pharmacological and physiological properties in medicinal applications (Ezekiel *et al.,* 2010).

Table 1: Phytochemical screening of crude ethanolic extract of *C. africana* root

Metabolites	Remarks
Saponins	+
Tannins	+
Reducing sugars	+
Flavonoids	+
Anthraquinones	-
Cardiac glycosides	+
Triterpenoids	+
Alkaloids	+
Steroids	+
Carbohydrates	+
Protein	-
Quinones	-

(+) = Present, (-) = Absent

Flavonoids are very popular because of their anti-cancer, anti-viral, anti-allergic and anti-inflammatory activities (Mahato & Sen, 1997; Valsaraj *et al.*, 1997). Tannins are not only useful industrially; they have antioxidant activity (they prevent the onset of degenerative diseases such as cancer and cardiovascular disease). They also have anti-viral (Lin *et al.*, 2004), antibacterial (Akiyama *et al.*, 2001) and antiparasitic effects and are used for the treatment of small hemorrhage, sore mouth, bronchitis, burns, diarrhea and scars of the skin, wounds and many others. Extracts of cardiac glycosides are useful as diuretics and emetics, as heart tonics for the treatment of congestive heart failure and cardiac arrhythmia (Zhang *et al.*, 2006). Triterpenoids are used for the treatment of cancers (Mahato & Sen, 1997). Alkaloids are use as stimulants, anti-malarial, analgesic, muscle relaxant, anti-tumor, etc. Saponins have proven to be efficient in traditional herbal medicines (Waller & Yamasaki, 1995). Other activities of saponins are reported to include anti-inflammatory (Balandrin, 1996), hypocholesterolemic (Oakenfull, 1999) and immune-stimulating (Klausner, 1988). Carbohydrates provide energy for our body, especially the brain and the nervous system (Rockville, 2010). Reducing sugars are essential for brain function and physical energy.

The results of the antimicrobial screening of crude ethanolic extract and fractions of *C. africana* roots against selected organisms are shown in Tables 2 – 4. From the results the crude extract and fractions of *C. africana* under investigation generally exhibited characteristic strong concentration-dependent activity (CDA) against some of the test organisms, with zones of inhibition ranging from 11 – 33 mm.

Table 2: Results of antimicrobial screening of crude extract and fractions

Organism	Zone of Inhibition (mm)/mg/Ml																			
	Acid				Alkaloid				Polar				Non–polar				Crude			
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5
Sa	25	20	16	12	33	25	20	17	20	18	16	14	18	15	12	0	32	28	22	18
Bs	22	17	12	0	23	19	16	12	28	25	20	17	16	14	11	0	18	16	13	10
Ec	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	11	0	0
St	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	12	0	0
Ca	25	20	18	14	27	25	20	16	21	18	15	0	0	0	0	0	0	0	0	0

Sa = *Staphylococcus aureus;* **Bs =** *Bacillus subtilis;* **Ec =** *Escherichia coli* **St = Ca =** *Salmonella typhi; Candida albicans*

Table 3: Results of inhibitory activity (sensitivity test) of antibiotics used as control on the test organisms

Organism	Zone of Inhibition (mm)		
	Ciprofloxacin (10 µg)	Amoxicillin (30 µg)	Ketaconazole (250 mg)
S. aureus	42	28	-
B. subtilis	43	22	-
E. coli	35	15	-
S. typhi	38	20	-
C. albicans	-	-	38

Table 4: Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against the test organisms

Organism	Concentration (mg/mL)									
	MIC					MBC				
	Acid	Alkaloid	Polar	Non–polar	Crude	Acid	Alkaloid	Polar	Non–polar	Crude
Sa	25	12.5	25	25	12.5	50	25	50	50	25
Bs	25	25	12.5	25	25	50	50	25	50	50
Ec	0	0	0	0	50	0	0	0	0	100
St	0	0	0	0	50	0	0	0	0	100
Ca	25	12.5	25	0	0	50	25	50	0	0

0 = No activity; **Sa =** *Staphylococcus aureus;* **Bs =** *Bacillus subtilis;* **Ec =** *Escherichia coli* **St = Ca =** *Salmonella typhi; Candida albicans*

The crude extract and the fractions were active against the Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* showing that fractionation does not seriously greatly improve on the activity of the crude extract. However, the acid, alkaloid, polar and non-polar neutrals were completely inactive against the Gram-negative bacteria, *Escherichia coli and Salmonella typhi*, whereas, the crude was highly active against those Gram-negative bacteria. Thus, there may be synergism between the constituents in the crude which gets destroyed on separation by the process of fractionation. On the other hand, the crude had no activity against the fungus, *Candida albicans*, while the acid, alkaloid and polar neutral fractions had activity, suggesting synergism between the constituents in the crude extract. The results of this study not only comfirm the documented records of medicinal uses of *C. africana* for the treatment of a number of ailments, but highlights the relevance of activity-guided fractionation in the utilization of the plant for effective management of infections (Hutchings *et al.*, 1996; Lemenih *et al*., 2003).

Conclusion

The presence of the bioactive phytochemicals reported in this study supports the traditional medicinal uses of *Commiphora africana* in the treatment of a large number of illnesses. Activity-guided fractionation can be a powerful tool for the effective utilization of *Commiphora africana* roots in the management of infections as fractions or the crude extract.

References

Akiyama H, Kazuyasu F, Yamasaki O, Oono T & Iwatsuki K 2001. Antibacterial action of several tannins against *Staphylococcus aureus*. *Journal of Antimicrobial Chemother*, 48: 487-491.

Balandrin MF 1996. Commercial Utilization of Plant-derived Saponins: An Overview ofmedicinal, Pharmaceutical and Industrial Applications, In: Waller GR & Yamasaki K (eds) Saponins Used in Food and Agriculture: *Plenum Press*, pp. 1 – 14.

Calixto JB 2000. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (*Phytotherapeutic agents*). *Braz. J. Med. Biol. Res.,* 33: 179-189.

Clarkson JJ, Chase MW & Harley MM 2002. Phylogenetic relationships in Burseraceae based on plastid rps 16 intron sequences. *Kew Bulletin*, 57(1): 183-193.

Ezekiel I, Mabrouk MA & Ayo JO 2010. Study of the effect of hydro-ethanolic extract of *Commiphora africana* (Stem-bark) on sleeping time and convulsion in mice. *Asian J. Medical Sci.*, 2(3): 81-84.

Farnsworth NR, Blowster RN, Darmratoski D, Meer WA & Cammarato LV 1967. Studies on Catharanthus alkaloids IV Evaluation by means of TLC and ceric ammonium sulphate spray reagent. *Lloydia*, 27: 302-314.

Hadissa T & Jean-Pierre D 2005. Use of Medicinal plants for the treatment of oral diseases in Burkina Faso. *Journal of Ethnopharmacology*, 104: 68-78.

Harbone JB 1984. Phytochemical Methods, 2nd edition, Chapman and Hall Publications, London, New York, p. 288.

Heatley NG 1944. A method for the assay of penicillin. *Biochem. J.*, 38: 61-65.

Hutchings A, Scott AH, Lewis G & Cunningham A 1996. Zulu medicinal plants aninventory. University of Natal Press, Pietermaritzeburg, South Africa. In the Leaf Alkaloid Content of Androgenic Diploid Plants of *Datura innoxia*. *Planta Med*., 54: 14-17.

Isyaka M & Okwute S K 2013. Phytochemical, IR spectral and biological studies on the leaf extracts of *Commiphora africana* (Burseraceae). *ChemSearch Journal*, 4(1): 14-17.

Jorgensen JH & Ferraro MJ 2009. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clin. Infect. Dis.*, 49: 1749-1755.

Klausner A 1988. Adjuvants: A real shot in the arm for recombinant vaccines. *Bio/Technology*, 6(7): 773-777.

Kokwaro JO 2009. Medicinal Plants of East Africa: Third edition. Nairobi: University of Nairobi Press, p. 478.

Lemenih M, Abebe T & Olsson M 2003. Gum and resin resources from some *Acacia*, *Boswellia* and *Commiphora* species and their economic contributions in Liban, south east Ethiopia. *Journal of Arid Environments*, 55: 465-482.

Lin, LU, Shu-wen, L, Shi-bo, J and Shu-guang, W 2004. Tannin inhibits HIV-1 entry by targeting gp 41. *Acta Pharmacol. Sin*., 25(2): 213-218.

Magaldi S, Mata-Essayag S & Hartung de Capriles C 2004. Well diffusion for antifungal susceptibility testing. *Int. J. Infect. Dis.*, 8: 39-45.

Mahato SB & Sen S 1997. Advances in triterpenoid research, 1990-1994. *Phytochemistry*, 44: 1185-236.

Mitscher LA, Leu RP, Bathala MS, Wu WN, Beal JL & White R 1972. Antibiotics from higher plants.1. Introduction, rationale, and methodology. *J. Nat. Products* 32: 157.

Nijs A, Cartuyvels R & Mewis A 2003. Comparison and evaluation of Osiris and Sirscan 2000 antimicrobial susceptibility systems in the clinical microbiology laboratory. *J. Clin. Microbiol.*, 41: 3627-3630.

Oakenfull D 1999. Saponins in the treatment of hypercholesterolemia, In: Spiller GA (ed.). Handbook of Lipids in Human Nutrition. CRC Press, pp. 107-112.

Okwute SK, Mitscher LA & Gollapudi SR 1990. Three triterpenes from antimicrobial *Commiphora africana* roots. *J. Chem. Soc. Nig*., 14: 63-66.

Perumal SR, Ignacimuthu S & Sen H 1998. Screening of 34 Indian medicinal plants for antibacterial properties. *J. Ethnopharmacol.*, 62: 173-182

Pfaller MA, Sheehan DJ & Rex JH 2004. Determination of fungicidal activities against yeasts and molds: Lessons learned from bactericidal testing and the need for standardization. *Clin. Microbiol. Rev*., 17: 268-280.

Rockville MD 2010. United States Department of Health and Human Services and United States Department of Agriculture. *Dietary Guidelines for Americans* 7th ed.

Sofowora A 1993. Screening Plants for Bioactive Agents. Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, pp. 134-156.

Trease GE & Evans WC 2002. *Pharmacognosy*. 15th Ed. London: Saunders Publishers, pp. 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.

Valgas C, De Souza SM & Smânia EFA 2007. Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.*, 38: 369-380.

Valsaraj R, Pushpangadan P & Smitt UW 1997. Antimicrobial screening of selected Medicinal plants from India. *J. Ethnopharmacol*, 58: 75-83.

Waller GR & Yamasaki K 1995. Proceedings of an American Chemical Society Symposium on saponins: August 20-24, Chicago, Illinois.

Watson L & Dallwitz, MJ 1992. Onwards. The families of flowering plants: descriptions, illustrations, identification and information retrieval. Version 29th July 2006. http://delta-intkey.com

Weeks A, Dalyb DC & Simpson BB 2005. The phylogenetic history and biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data. *Molecular Phylogenetics and Evolution*, 35: 85-101.

Zhang Y, Li HZ, Zhang YJ, Jacob MR, Khan SI, Li XC & Yang CR 2006. *Steroids*, 71: 712.