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Phytochemical analysis and cytotoxic activity of the root extract of *Commiphora africana* (Caesalpiniaceae)

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Abstract
Commiphora africana is traditionally used for the treatment of several ailments because of its pharmacological activities. The dried roots were extracted with 95% ethanol to obtain a crude extract which was investigated for its phytochemicals and acute oral toxicity. The qualitative phytochemical investigation of the crude extract demonstrated the presence of several secondary metabolites which included saponins, tannins, reducing sugars, flavonoids, cardiac glycosides, triterpenoids, alkaloids and carbohydrates. The crude extract was also screened *in-vivo* for cytotoxicity using mice and it exhibited low toxicity in the assay. There were no records of mortality even at the highest concentration of 5000 mg/kg. The presence of the phytochemicals in the crude ethanolic extract provides the scientific evidence for the efficacy of *Commiphora africana* root in traditional medicinal applications.

Keywords: *Commiphora africana* root, phytochemicals, acute oral toxicity, mortality

1. Introduction
Natural products, particularly from plants, have played a vital role in the discovery of drugs. Some of these drugs are completely derived from natural products, while some natural products serve as leads for novel drugs development (Cowan, 1999) [6]. One of the most important roles herbal medicines play in modern drug development is the identification of plants with useful therapeutic compounds (Mendonca-Filho, 2006) [19]. This field of science provides us with the opportunity to explore the activities phytochemicals have, identify the bioactive components, estimate the appropriate dosages, as well as describe the best methods of extraction and conservation of the compounds. Up to now, only a few methods have been developed to identify the potential plants for drug development.
Commiphora a The 95% ethanolic crude extract of *C. africana* showed low toxicity against mice of different body weights at various concentrations in the assay. This agrees with the general experimental observation that *Commiphora* species are commonly toxicologically harmless plants with the exception of five species, namely *C. erlangeriana, C. staphyleifolia, C. unilobata, C. guidotti and C. boiviniana* (Neuwinger, 1996) [20] which are not covered in this assay. From the experimental results, they were no records of mortality even at the highest concentration of 5000 mg/kg. However, a prominent clinical sign (drowsiness) was observed in the second phase of the assay which increased with increase in concentration from 1200 to 5000 mg/kg. From the result of the clinical signs observed, there was the possibility of mortality at concentrations far higher than 5000 mg/kg. Since the LD_{50} concentration values would therefore be greater than 5000 mg/kg, they will be of no practical interest. It is important to note that the failure of the plant extract to demonstrate acute *in vivo* cytotoxicity during the general screening process does not necessarily imply a total absence of inherent medicinal value. The possible presence of antagonistic interactions between the different plant constituents in crude preparations may be responsible for the poor toxicity of the crude. There may be high cytotoxic activities exhibited by the different components of the crude extract such as the triterpenoids which have been reported previously by some workers as constituents of the root (Okwute *et al*., 1989) [22].
Commiphora africana, popularly known as African myrrh, is a small deciduous tree belonging to the family Burseraceae (=Caesalpiniaceae). It is widely used in many parts of northern Nigeria as an incensing, insecticidal and antiseptic fumigant and commonly found in Angola, Botswana, Burkina Faso, Chad, Eritrea, Ethiopia, Kenya, Mali, Mauritania, Mozambique, Namibia and Niger, among many other countries (Dalziel, 1937) [7]. The different parts of this plant are used to treat a wide range of ailments. Its edible fruits are chewed for the treatment of typhoid fever, stomach problems, tooth-ache and as an astringent drug for bleeding gum. The bark is used to manage malaria and when chewed together with the plant species

Nicotiana tobacum (tobacco) the resulting macerated substance is applied onto snake bite wounds for treatment. An infusion made from its bark is given to livestock to treat the foot and mouth disease (Hadisa & Jean-Pierre, 2005; Kokwaro, 2009) [9, 13]. The resin is used to suppress convulsions and for covering and disinfecting wounds. The resin when burnt acts as an insecticide and as an aphrodisiac, and is traditionally believed to drive-off evil spirit (Dalziel, 1937) [7].

Previous studies on the plant included the preliminary phytochemical screening of the hydro-ethanolic extract of the stem bark which revealed the presence of flavonoids, tannins, anthraquinones, cardiac glycosides, triterpenoids, saponins, alkaloids and reducing sugars. The extract was also found to possess anti-inflammatory and analgesic effects on rodents (Ezekiel, *et al.,* 2010) [8]. The essential oil from the hydro-distillation of the leaf from the West African country, Benin Republic displayed anti-oxidant activity (Ayeodoun *et al.*, 1998; Ma *et al.,* 2004; Alvessi *et al.,* 2005) [3, 17]. From the chemical examination of the antimicrobial n-hexane extract of the root of *Commiphora africana* three triterpenes were isolated. They included α-amyrin, β-sitostenone and an unidentified hydroxy-carboxylic acid (Okwute *et al.,* 1989) [22]. A number of lupane-type triterpenoids have recently been found to possess cytotoxic and anti-tumor properties (Tran *et al.,* 2011) [28]. In this study we report the results of the phytochemical and acute toxicity screenings of the crude root extract of *C. africana* which was previously found to be strongly active against *Staphylococcus aureus* and *Mycobacterium smegmatis* as a potential source of drugs for the management of infections and inflammations (Okwute *et al.,* 1989) [22].

Materials and methods

Plant materials and preparation

The fresh root of *Commiphora Africana* was collected from Gaji village, Gwagwalada Area Council, Federal Capital Territory, Abuja, in November, 2014. It was authenticated at the Herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja where a voucher Specimen, Number NIPRD/H/6642, was then deposited. 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 Hammer Mill machine. The powdered plant material was stored in a tightly closed polythene bag and later subjected to extraction.

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

Extraction of plant material

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

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. This screening was done using standard procedures (Harborne, 1984; Sofowora, 1993; Trease and Evans, 2002) [10, 26, 29].

Acute oral toxicity screening

Experimental animals

The experimental animals were mice obtained from the Pharmacology Department of the School of Pharmaceutical Sciences, Ahmadu Bello University, Zaria and were acclimatized in cages under standard environmental conditions of light/dark cycles (12 hours/12 hours) and temperature (23±1 ^{o}C). The animals had free access to tap water and a standard pellet diet, except for a short fasting period of four hours before and after the oral administration of single dose of the *C. africana* crude ethanolic root extract. The study was performed in accordance with the wide-reaching established pattern of laboratory animal use and care (OECD, 2001) [23].

Preparation of stock solution

The stock solution was prepared according to Lorke's method (Lorke, 1983) [16]. Various concentrations of the extracts were prepared ranging from 100 mg/mL to 1 mg/mL. The crude plant extract (0.2g) was dissolved in 2 mL of distilled water to give a stock concentration of 100 mg/mL. The stock solution (0.2mL) was then dissolved in 1.8 mL of distilled water to give 10 mg/mL and this was then diluted to 1mg/mL by dissolving 0.2 mL in 1.8 mL of distilled water. Normal saline was used as a control.

Toxicity Screening

The acute oral toxicity study was carried out *in vivo* in two phases. The animals were divided into the control and three treated groups consisting of three animals each for the first phase while the second phase was made up of the control and four treated groups consisting of one animal each. All the animals were subjected to four hours of fasting prior to treatment and their respective body weights taken. The control group received only normal saline and the treated groups received 10, 100, and 1000 mg/kg of *C. africana* ethanolic root extract for the first phase while 1200, 1600, 2900 and 5000 mg/kg of *C. africana* ethanolic root extract was administered for the second phase. The mice in the first phase were observed for one hour after the treatment, and then intermittently for 24 hours following treatment. The process was the same for the treated mice in the second phase. The mice were then carefully monitored for clinical signs such as weakness or drowsiness, aggressiveness, loss of weight, diarrhea, discharge from eyes and ears, noisy breathing and the number of deaths in each treated group and the control. The observation was carefully recorded and result documented.

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 results of the preliminary phytochemical screening of the crude extract showed that the root was very rich in saponins, tannins, reducing sugar, flavonoids, cardiac glycosides, triterpenoids, alkaloids and carbohydrates. These secondary metabolites reported from this investigation are known for their broad spectrum of pharmacological and physiological properties in medicinal applications (Ezekiel, *et al.,* 2010) [8].

Flavonoids are known for their health related properties which are based on their antioxidant activities. These properties have been found to include anti-cancer, anti-viral, anti-allergic and anti-inflammatory activities (Mahato & Sen, 1997; Valsaraj *et al.,* 1997) [18, 30]. Apart from industrial applications, herbal preparations containing tannins are used for the treatment of small hemorrhage, sore mouth, bronchitis, burns, and scars of the skin, wounds and many other ailments. They are also used for the treatment of diarrhea. Tannins are considered antioxidants and they prevent the onset of degenerative diseases such as cancer and cardiovascular disease. They have also been reported to have anti-viral (Lin *et al.,* 2004) [15], antibacterial (Akiyama *et al.,* 2001) [1] and antiparasitic effects. Extracts of cardiac glycosides have been reported for their use as diuretics and emetics, as heart tonics for the treatment of congestive heart failure and cardiac arrhythmia (Zhang *et al.,* 2006) [32]. Triterpenoids are used for the treatment of cancers (Mahato & Sen, 1997) [18]. Alkaloids, on the other hand, are popular for their use as stimulants, anti-malarial, analgesic, muscle relaxant and anti-tumor. This work has reported the third occurrence of alkaloids in *Commiphora africana* showing that alkaloids occur in the stem bark (Banso and Mann, 2006) [5], the leaves (Isyaka and Okwute, 2013) [11] and now in the root. Saponins exhibit a variety of biological activities and have been investigated toward the development of new natural medicines and support the efficacy of traditional herbal medicines (Waller & Yamasaki, 1995) [31]. Other interesting biological applications for various specific saponins include their uses as anti-inflammatory (Balandrin, 1996) [4], hypocholesterolemic (Oakenfull, 1996) and immune-stimulating (Klausner, 1988) [12] whose properties are widely recognized and commercially utilized. The primary function of carbohydrates is to provide energy for the body, especially the brain and the nervous system (Rockville, 2010) [24]. Reducing sugars are essential for brain function and physical energy. The level of reducing sugars in wine, juice, and sugarcane are indicative of the quality of these food products, and monitoring the levels of reducing sugars during food production has improved market quality (Leotério *et al.,* 2015) [14].

Table 2 shows the result of the *in vivo* acute toxicity assay of the crude ethanolic root extract of *C. africana*. The 95% ethanolic crude extract of *C. africana* showed low toxicity against mice of different body weights at various concentrations in the assay. This agrees with the general experimental observation that *Commiphora* species are commonly toxicologically harmless plants with the exception of five species, namely *C. erlangeriana, C. staphyleifolia, C. unilobata, C. guidotti and C. boiviniana* (Neuwinger, 1996) [20] which are not covered in this assay. From the experimental results, they were no records of mortality even at the highest concentration of 5000 mg/kg. However, a prominent clinical sign (drowsiness) was observed in the second phase of the assay which increased with increase in concentration from 1200 to 5000 mg/kg. From the result of the clinical signs observed, there was the possibility of mortality at concentrations far higher than 5000 mg/kg. Since the LD_{50} concentration values would therefore be greater than 5000 mg/kg, they will be of no practical interest. It is important to note that the failure of the plant extract to demonstrate acute *in vivo* cytotoxicity during the general screening process does not necessarily imply a total absence of inherent medicinal value. The possible presence of antagonistic interactions between the different plant constituents in crude preparations may be responsible for the poor toxicity of the crude. There may be high cytotoxic activities exhibited by the different components of the crude extract such as the triterpenoids which have been reported previously by some workers as constituents of the root (Okwute *et al*., 1989) [22].

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

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

Key: (+) = Present, (-) = Absent

Table 2: Results of i*n vivo* acute oral toxicity screening

Parameters	*C. africana* crude ethanolic root extract								
	Doses in mg/kg body weight				Doses in mg/kg body weight				
	Result of the phase 1 investigation				Result of the phase 2 investigation				
Conc.	0 (control)	10	100	1000	0(control)	1200	1600	2900	5000
Body weight	20	23	19	20	20	21	20	19	22
(g)		22	21	18					
		20	21	21					
Number of deaths	0/1*	0/3*	0/3*	0/3*	0/1*	0/1*	0/1*	0/1*	0/1*
Clinical signs	x	x	x	x	x	+	++	+++	++++
		x	x	x					
		x	x	x					

Key: *Number of mice which died/number of mice used, x: No clinical sign, +: Drowsiness.

Conclusion

The present study has investigated the chemical and biological activities of *Commiphora africana* ethanolic roots extract and revealed that it is rich in phytochemicals. The anti-cancer/tumor, analgesic/anti-inflammatory, insecticidal, anthelmintic, antidiabetic, anti-oxidant, antibacterial and antifungal properties of the plant may largely be due to the presence of secondary metabolites which may include flavonoids, tannins, anthraquinones, cardiac glycosides, triterpenoids, saponins, alkaloids and reducing sugars found in the plant extracts. The toxicity study revealed that the plant was non-toxic against the test animal. It is important to note that even though there may be no evidence of cytotoxicity *in vivo*, the possibility of *in vitro* cytotoxicity may not be excluded. Yet, it is safe and can be recommended for human consumption either as herbs or as food. All the constituent

compounds have previously been known to possess pharmacological activities and are therefore potential sources of new drugs and other therapeutic substances. Thus, the findings in this study support the traditional medicinal uses of the plant.

References

1. Akiyama H, Kazuyasu F, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action ofseveral tannins against *staphylococcus aureus*. Journal of antimicrobial chemother. 2001; 48:487-491.
2. Alvessi F, Alitonou GA, Sohounhloue DK, Bassiere Jean-Marie, Menut Chanta. Aromatic plants of West Tropical Africa. XV. Chemical and Biological Evaluation of Leaf Essential Oil of *Commiphora africana* from Benin. Journal of Essential Oil Research. 2005; 17(5):569.
3. Ayeodoun MA, Sohounhloue DK, Menut st A, Lamty G, Molangui T, Casanova J *et al*. The aromatic plants of Tropical West Africa.VI. α-oxobisbolene as main constituent of the leaf essential oil of *Commiphora africana*(A. Rich) Engl, from Benin. Journal of Essential Oil Research. 1998; 10(1):105-107.
4. Balandrin MF. Commercial Utilization of Plant-derived Saponins: An Overview of semedicinal, Pharmaceutical and Industrial Applications, In: Waller, G.R. and Yamasaki, K. (eds) Saponins Used in Food and Agriculture: Plenum Press. 1996, 1-14.
5. Banso A, Mann A. Antimicrobial alkaloid fraction from *Commiphora africana* (A. Rich). J. Pharm. and Biores. 2006; 3(2):98-102
6. Cowan MM. Plant Products as Anti-microbial Agents. Clinical Microbiology Reviews. 1999; 12(4):564-582.
7. Dalziel JM. The useful plants of West Tropical Africa. Africa Crown Agents for Colonies, London, 1937, 179.
8. Ezekiel I, Mabrouk MA, Ayo JO. Study of the Effect of Hydro ethanolic Extract of *Commiphora africana* (Stem bark) on Sleeping Time and Convulsion in Mice. Asian Journal of Medical Sciences, 2010; 2(3):81-84.
9. Hadissa T, Jean-Pierre D. Use of medicinal plants for the treatment of oral diseases in Burkina Faso. Journal of Ethno-pharmacology. 2005; 104:68-78.
10. Harbone JB. Phytochemical Methods, 2nd edition, Chapman and Hall Publications, London, New York. 1984, 288
11. Isyaka MS, Okwute SK. Phytochemical, IR Spectral and Biological Studies on the Leaf Extracts of *Commiphora africana* (Burseraceae). Chem Search Journal. 2013; 4(1):14-17.
12. Klausner A. Adjuvants: a real shot in the arm for recombinant vaccines. Bio/Technology; 1988; 6(7):773-777.
13. Kokwaro JO. Medicinal plants of East Africa: Third edition. Nairobi: University of Nairobi press. 2009, 478.
14. Leotério Dilmo MS, Silva Paulo, Souza Gustavo, Alves Aline de A, Belian Mônica, Galembeck André *et al*. Copper–4, 4′-dipyridyl coordination compound as solid reagent for spectrophotometric determination of reducing sugar employing a multicommutation approach. *Food Control* (European Federation of Food Science and Technology; International Union of Food Science and Technology) 2015; 57:225-231.
15. Lin LU, Shu-wen L, Shi-bo J, Shu-guang W. Tannin inhibits HIV-1 entry by targeting gp 41. *Acta Pharmacol Sin.* 2004; 25(2):213-218.
16. Lorke D. A New Approach to Practical Acute Toxicity Testing. *Arch. Toxicol*. 1983; 54:275-287.
17. Ma J, Jones SH, Hecht SM. A dihydroflavonol glucoside from *Commiphora africana* that mediates DNA strand scission. *J. Nat. Products.* 2004; 68(1):115-117. PMID:1567933[PubMed-indexed for MEDLINE]
18. Mahato SB, Sen S. Advances in triterpenoid research, 1990-1994. *Phytochemistry*, 1997; 44:1185-236.
19. Mendonca-Filho FF. Bioactive phytocompounds: new approaches in the phytosciences, in Modern Phytomedicine, I. Ahmad, *et al*., Eds., edWeinheim: Wiley-VCH, 2006, 1-24.
20. Neuwinger HD. African Ethnobotany: Poisons and Drugs. Chapman & Hall, London, 1996.
21. Oakenfull D. Saponins in the treatment of hypercholesterolemia, In: Spiller GA (ed.) Handbook of Lipids in Human Nutrition. CRC Press; 1999, 107-112.
22. Okwute SK, Mitscher LA, Rao SG. Triterpenes from Antimicrobial *Commiphora africana* (*Burseraceae*) Root: A Preliminary Report. J. Chem. Soc. Nigeria, 1989; 14:63-66.
23. Organization for Economic Co-operation and Development (OECD) Guideline for testing of chemicals: Acute oral Toxicity-Fixed Dose Procedure, 420. Dec 17th, 2001.
24. Rockville MD. United States Department of Health and Human Services, and United States Department of Agriculture. Dietary Guidelines for Americans, 7th ed, 2010.
25. Sajjad A, Farhat U, Anwar Z, Muhammad A, Farman U, Abdul S. Evaluation of *Rumex hastatus* D. Don for cytotoxic potential against HeLa and NIH/3T3 cell lines: Chemical characterization of chloroform fraction and identification of bioactive compounds. BMC Complementary and Alternative Medicine BMC Series, 2016. http://doi.org/10.1186/s12906-016-1302-y
26. Sofowora A. Screening Plants for Bioactive agents. Medicinal Plants and Traditional Medicine in Africa, 2nd ed., Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria. 1993, 134-156.
27. Suquin K, Guangying C, Changri H, Hong. Chemical constituents from the roots of Xanthium sibiricum. Natural Products Research, 2011; 25(13):1243-9. DOI:10.1080/14786419.2010.539182
28. Tran DT, Ping-Chung K, Chun-Shu Y, Chiang S, Le TMH, Tran VT *et al*. Chemical constituents of the leaves of *Glochidion obliquum* and their bioactivity. Archives of Pharmacal Research. 2011; 34(3):383-389.
29. Trease GE, Evans WC. *Pharmacognosy*. 15th ed., London: Saunders Publishers. 2002, 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.
30. Valsaraj R, Pushpangadan P, Smitt UW. Antimicrobial screening of selected medicinal plants from India, J. Ethnopharmacol. 1997; 58:75-83.
31. Waller GR, Yamasaki K. Proceedings of an American Chemical Society Symposium on Saponins. Chicago, Illinois, 1995, 20-24.
32. Zhang Y, Li HZ, Zhang YJ, Jacob MR, Khan SI, Li XC *et al*. Steroids, 2006; 71:712.