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

Okwute SK and Ochi IO

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 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. uniolobata*, *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 LD50 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* (=Caesalpiniaeaceae). 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 tabacum (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; Kokwara, 2009) [9, 11]. 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) [17].

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) [10]. 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) [5, 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-sitosterone and an unidentified hydroxy-carboxylic acid (Okwute et al., 1989) [23]. A number of lupane-type triterpenoids have recently been found to possess cytoxic 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) [23].

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 °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) [19]. 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) [3], 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) [10]. 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. erlangerviana, C. staphylefolia, C. unilobata, C. guidotti and C. boiviniana (Neuwing, 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 LD50 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.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Remarks</th>
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<tr>
<td>Saponins</td>
<td>+</td>
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<td>Tannins</td>
<td>+</td>
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<td>Reducing sugars</td>
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<td>Flavonoids</td>
<td>+</td>
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<td>Anthraquinones</td>
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<td>Cardiac glycosides</td>
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<td>Triterpenoids</td>
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<td>Alkaloids</td>
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<td>Steroids</td>
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<td>Carbohydrates</td>
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<tr>
<td>Protein</td>
<td>-</td>
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<td>Quinones</td>
<td>-</td>
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Key: (+) = Present, (-) = Absent

Table 2: Results of in vivo acute oral toxicity screening

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C. africana crude ethanolic root extract</th>
<th>Doses in mg/kg body weight (mg)</th>
<th>Doses in mg/kg body weight (g)</th>
<th>Result of the phase 1 investigation</th>
<th>Result of the phase 2 investigation</th>
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<td>Number of deaths 0/1</td>
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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 anticancer/tumor, analgesic/anti-inflammatory, insecticidal, anthelmintic, anti-diabetic, 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
14. Leotério Dilmo MS, Silva Paulo, Souza Gustavo, Alves Aline de A, Belian Mônica, Galembek 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.