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Cola Rostrata: Phytochemical and Toxicity Studies

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ABSTRACT: Cola species are used in folk medicine as stimulant, to prevent vomiting, dysentery and suppress sleep. Proximate analysis and phytochemical screening were evaluated using standard methods while acute toxicity study of the crude extract of *Cola rostrata* on swiss albino mice was evaluated by administration of the crude extract. Phytochemical screening showed the presence of flavonoid, phenols, saponin, steroid, tannins, triterpenoid and reducing sugar. The proximate analysis gave moisture content (10.23 ± 0.55 %), total ash value (13.39 ± 0.048 %), acid insoluble ash (2.50 ± 0.18%), water insoluble ash (11.26 ± 0.40%), alcohol soluble extractive (0.86 ± 0.11%) and water soluble extractive (1.44 ± 0.05%). Result of acute toxicity study did not show mortality at 8 g/kg p.o after 24 hours of administration and there was no sign of toxicity or mortality after 14 days of observation. In conclusion, the root bark of *C. rostrata* contains phytochemicals and safe on the basis of acute toxicity testing of the crude extract. © JASEM

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Sterculiaceae is the family name for a group of flowering plants which have been widely utilized in folk medicine in the environment which they are found (Jobstl, 2004; Watson, 1994). The largest genus in this family is Cola, with 100-125 species made up of trees and shrubs which are geographically located in evergreen lowland and forest in continental Africa (Cheek, 2002). In Nigeria about twenty three (23) species (Russels, 1955) are used in traditional medicine as stimulant, to prevent dysentery (Morton, 1992), headache (Seitz, 1992) and to suppress sleep (Niemenak, 2008). For instance, *Cola nitida* is eaten before food to encourage digestion and has been reported to have advantageous effect on the digestive organ especially the liver. Also it has been used as a form of antidepressant (Ojo, 2009). Fresh *Cola acuminata* nut has a bitter and astringent taste when chewed (Rusell, 1955; Purseglove, 1968; Rosengarten, 1984), medicinally it contain caffeine, which act on Central Nervous System (CNS) as stimulant, relieving migraine (Kiple and Ornelas 2000b). Theobromine act as cerebral vasodilator and believe to relieve pain and neuralgia (Hirt and M'pia, 2001). *Cola gigantea* is traditionally called oporoporo, used to improve the supply of blood in the body by maceration of the leaves in water and administered three times a day for one week in small cup (Idu, 2010). *Cola rostrata* is a perennial tree found in lowland rain forest of tropical Africa; in southern Cameroun, Gabon and south-eastern Nigeria. It is popularly known as monkey cola, cockroach kola and the locals called it ochicha (Ebonyi), achicha (Imo), oji ochicha (Abia) (Keay 1960). Its fruits are edible with a sweet taste and it is not known to be used in folk medicine.

The earliest documented record of the use of herbs in Africa date back 3500 years by the Egyptian Ebers papyrus (Levetin and McMahon, 2003). In most cases herbal knowledge were mostly transmitted orally (WHO, 2009). The use of herbs is increasing with 80 % of the rural population in Africa, Asia and Latin America relying on herbal medicines as their source of medicine (WHO, 2002) and in many village markets, herbs are sold alongside vegetables. Due to the development of resistance and untoward effects of synthetic drugs, there is an upsurge in interest on plant-derived medicines in Europe and United State (Sultana *et al*., 2011). Though the direct use of medicinal plants in its crude form has its own

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advantages but the need to identify the phytoactive constituents present in the plant can help in determinig the possible effects that the plant may possess.

The increase in demand for herbs and the urgent need to evaluate nature's repository of chemicals in plants for their potential value in health care have lead to indiscriminant and unscientific collection, misidentification and adulteration which could be accidental or deliberate for financial gain (Ahmad *et al*., 2010; Khan *et al*., 2011; Sultana *et al*., 2011), without any standards for quality of the material. It is important to note that most herbs are not standardized and usually contain many ingredients. This study intends to provide some of the standardizing parameter for the root bark of C. *rostrata* and due to paucity of information with regard to the proximate analysis, phytochemical screening and acute toxicity study, thus this present study was designed to evaluate the proximate parameters, phytochemical content and acute toxicity profile of the root bark of C. *rostrata*.

MATERIALS AND METHODS

Collection of plant material: The root bark of *Cola rostrata K. Schum* was collected on the 30th June, 2011, from Ohanozara local government area in Ebonyi State, Nigeria by Mr. Anoh Kenneth. The plant was identified and authenticated by Ugbogu O. A. And Shansanya of the Forest Research Instituted, Ibadan, Nigeria. A voucher specimen (FHI 109430) was deposited.

The fresh root bark were carefully washed with water to remove earthy material and air-dried for a period of two weeks, after which they were placed in the oven for three hours at a temperature of 50^{o}C, before they were reduced to fine powder with the aid of an electric milling machine. The powdered sample was stored in an air tight container until used.

Extraction and preparation of plant material: Powdered sample (300 g) was macerated at room temperature with 1litre of methanol for 72 hrs (Brain and Turner, 1975). The filtrate was concentrated with a rotary evaporator at 40^{o}C under reduced pressure, to give a dried material, kept in a refrigerator at -4^{o}C until use. The total yield obtained was recorded. The crude extract was partitioned with petroleum ether, chloroform, ethylacetate and butanol.

Phytochemical screening: Chemical tests were carried out on the powdered drug for the qualitative determination of phytochemical constituents as described by Harborne (1973), Trease and Evans (1989) and Sofowora (1993). This involves test for carbohydrate, sugar, glycosides, saponins, alkaloids, tannins, phenol and flavonoids.

Proximate analysis of the powdered drug: The following quantitative parameters were carried using standard methods (African Pharmacopoeia 1986, British Pharmacopoeia 1988, and AOAC 2003); moisture content, total ash, acid insoluble ash, water insoluble ash, alcohol soluble extractive and water soluble extractive.

Acute toxicity profile of Crude extract of C. rostrata: Adult Swiss albino mice (19-31g) were obtained from Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin and were fed standard rodent pellet (Ewu flour mill) approved by Faculty of Pharmacy ethnical committee and the extract was administered orally at doses of 1, 2, 4 and 8 g/kg to group I to IV respectively while the central group received distilled water by the unit. Genral symptoms of toxicity and mortality in each group was observed within 24 hours. Animals that survived after 24 hours were observed for another 14 days for any signs of toxicity (Lorke, 1983).

RESULT AND DISCUSSION

The result of the percentage yield of the extract and fractions (Table 1) gave 6.55, 4.95, 1.24, 0.64 and 0.21 for crude extract, petroleum ether, chloroform, ethylacetate and butanol fraction respectively. The petroleum ether fraction gave the highest yield of 4.95 %, which could be attributed to the high level of lipids such as stigmasterol and sitosterols.

The phytochemical screening (Table 2) of the extract showed the presence of flavonoid, phenols, saponin, steroid, tannins, triterpenoid and reducing sugar while alkaloids was absent. The presence of secondary metabolites in medicinal plants aids in the biological actions for which they are identified in folk medicine. Their presence may impart characteristic odour, taste, colour, medicinal or poisonous properties on the plant (Evans, 1982). Some cola species are rich sources of alkaloids (Blades, 2000; Benjamin *et al*., 1991), but since the geographical distribution of a plants, affect both the morphology and expression of phytochemicals (Folkers *et al*., 2008; Shen *et al*., 2008), this may be the case with *C. rostrata* which lacked alkaloid and is mostly found in South-eastern Nigeria, South Cameroun and Garbon (Keay, 1960). Saponins are known hemolytic (Onning, 1995), and studies have

shown that oral administration of saponin caused diarrhoea, restlessness and histopathological changes in liver and kidney, ultimately leading to death (Lalitha, *et al.,* 1990). Apart from these effects, saponins are known to produce cholesterol lowering effect (Price *et al.,* 1987) which could be beneficial in condition of high blood pressure. Flavonoids are known to have different pharmacological activities but most importantly, its antioxidant nature has been exploited, because of its ability to donate protons (Alan and Miller, 1996). Tannins have been reported to have astringent property (Jobstl, 2004), this have been taken advantage of, in the treatment of wounds (Hupkens *et al*.,1995; Halkes *et al*., 2001). Triterpenoid are the complex oils that have been reported as potent drugs used in treatment of wide range of ailments which include malaria, (Evans, 2002). The presence of triterpenoid will encourage further research for possible new drugs leads in *Cola rostrata.*

Table 3, showed the result of the proximate analysis, moisture content (10.23 ± 0.55%), total ash (13.39 ± 0.048%), acid insoluble ash (2.50 ± 0.18%), water insoluble ash (11.26 ± 0.40), alcohol soluble extractive (0.86 ± 0.11%) and water soluble extractive (1.44 ± 0.05%). The moisture content indicates the stability and susceptibility to microbial degradation (Uraih and Izuagbe, 1990) and account for reactions that take place in the plant (Guisseppe and Baratta, 2000). The level of the moisture content is high when compared with the value set in Africa Pharmacopeia. High moisture content assists in maintaining the protoplasmic contents of cells but make herbs perishable and susceptible to microbial degradation during storage (George, 2008). These may suggest that *C. rostrata* will be prone to microbial attack and could have a short shelve life. The total ash value indicates the level of mineral elements content preserved in the root bark (Antia *et al.*, 2006) and it was recommended that plants with ash content above 8.8 % are useful health wise (Ifon and Bassir, 1980). The total ash value of the powdered drug is high when compared to standard and suggests a high deposit of mineral element in the root bark, implying that it may contain minerals that are very nourishing and suitable for consumption. The level of contamination or adulteration by sand (silicate) can be detected by the level of acid insoluble ash (Rao and Xiang, 2009). Acid insoluble ash of 2.50 % was obtained for the root bark of *C. rostrata* giving an indication of the level of insoluble mineral like silicate in the crude root bark. The alcohol extractive value was observed to be higher than the water extractive value; this is an indication that water could be the best solvent for its extraction. Water and alcohol soluble extractive values aid in the detection of exhausted and already used drugs which could be used as adulterants (Elujoba, 1999).

The crude extract of C. *rostrata* showed no signs of toxicity in the mice after 24 hours. No death was recorded up to the highest dose of 8 g/kg within the 14 days of observation. This indicates the extract is relatively safe by this route of administration. Further toxicity evaluations using mammalian tissues and organs will be necessary in the future.

Conclusion: The root bark of *C. rostrata* contains tannins, triterpenoids, saponins and flavonoids responsible for its ethnomedicinal use activity. The relatively high moisture content could make the plant susceptible to microbial degradation. The study also showed that the oral administration extract of *C. rostrata* is relatively safe at the tested dose in mice.

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Table 1: Percentage (%) yield of the root bark extract and fractions

EXTRACT/Fraction	Percentage(%)yield
Crude extract (methanol)	6.55%
Petroleum ether fraction	4.95%
Chloroform fraction	1.24%
Ethylacetate fraction	0.64%
Butanol fraction	0.21%

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Table 2: Phytochemical screening of the powder root bark of *Cola rostrata*

Carbohydrate	+
Tannin	+
Reducing Sugar	+
Monosaccharide	+
Starch	+
Triterpenoid	+
Saponin	+
Alkaloid	+
Flavonoids	+
Steroid	+

+indicates presence of components -indicates absence of components

Table 3: Percentage (%) values of proximate analysis of the root bark of *C. rostrata*

Parameter	Values ± SEM (%)
Moisture content	10.23 ± 0.55
Total ash value	13.39 ± 0.05
Acid insoluble ash	2.50 ± 0.18
Water insoluble ash	11.26 ± 0.40
Alcohol soluble extractive	0.86 ± 0.11
Water soluble extractive	1.44 ± 0.05

Table 4: Acute Toxicity studies of *Cola rostrata*

Dose (mg/kg)	Mortality ratio	% Mortality
1000	0/5	0
2000	0/5	0
4000	0/5	0
8000	0/5	0

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