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ACUTE TOXICITY, PHYTOCHEMICAL CONSTITUENTS AND *IN-VITRO* ANTI-OXIDANT ACTIVITY OF CRUDE METHANOLIC STEM BARK EXTRACT OF *CASSIA SIEBERIANA* DC.

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ABSTRACT

This study investigated the acute toxicity, phytochemical constituents and in-vitro anti-oxidant activity of the crude methanolic stem bark extract of Cassia sieberiana DC, a plant whose parts (root, stem bark and fruit pulp) are used traditionally for the treatment and management of some diseases. Fresh stem bark of C. sieberiana were collected, dried, powdered, extracted using 80% methanol by cold maceration method and the yield was calculated. The acute toxicity in albino rats, phytochemical analysis and in-vitro anti-oxidant activity of the extract were evaluated following standard procedures. Results showed that the median lethal dose (LD_{50}) of the extract was 3,379.33 mg/kg body weight (bw) in albino rats, and the extract contains high levels of tannins, moderate levels of flavonoids, saponins and carbohydrates, and low levels of alkaloids. At 400 µg/ml concentration, the extract exhibited anti-oxidant activity comparable to that of ascorbic acid. It was concluded that the crude methanolic stem bark extract of C. sieberiana is relatively non-toxic, however, caution must be exercised in its use since 500 mg/kg dose and above resulted in the death of some rats. The anti-oxidant property of the extract was attributed to the presence of bio-active constituents in the extract either acting alone or in combination with each other.

Keywords: *Cassia sieberiana*, stem bark extract, acute toxicity, phytochemicals, anti-oxidant activity

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INTRODUCTION

Herbal medicines are widely accepted and used for the treatment and prevention of various diseases in Africa and other parts of the world [1, 2]. Plant-derived drugs constitute the bulk of therapeutic agents dispensed by native doctors, and recipes used in traditional medicine are mainly derived from herbal medicinal products [2]. Reports have shown that a large percentage of compounds and substances currently used as drugs were derived from plants, and about 80% of these compounds are used in the same or related manner as their traditional ethnomedical use [3]. Although herbal medicines are widely used for the treatment and prevention of various diseases, some of the time, information on their toxicity, phytochemical constituents and anti-oxidant activity are not known.

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Acute toxicity is defined as the adverse change(s) occurring immediately or shortly following a single or short period of exposure to a substance, or as adverse effects occurring within a short time following administration of a single dose of a substance or multiple doses given within 24 hours [4]. Acute toxicity is usually measured by median lethal dose (LD_{50}), which is defined as the statistically derived dose, that when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period [5, 6, 7]. Acute toxicity testing provides information on the biological activity, mechanism of action and hazard identification of a substance/herbal formulation being tested. It also serves as a dose-finding exercise for long-term studies [7, 8, 9].

Phytochemistry as used in the field of ethno-medicine and phytopharmacology involves the study of biologically active chemical compounds of plants that are of medicinal and pharmacological value [10]. Phytochemical analyses are used to screen and assess the biologically active components of herbal formulations for both quality control and for elucidation of their therapeutic mechanisms of action [9, 10].

Anti-oxidants are molecules that inhibit the oxidation of other molecules. Oxidation reactions in living organisms produce free radicals which can initiate chain reactions that may cause damage or death to cells. Anti-oxidants terminate these chain reactions by removing free radical intermediates and inhibiting other oxidation reactions [11, 12]. Insufficient levels of anti-oxidants or inhibition of the anti-oxidant enzyme in living organisms cause oxidative stress that may lead to injury and death of cells [12, 13]. Oxidative stress is known to be involved in the development of many diseases or may exacerbate their symptoms [12, 14]. Anti-oxidants and foods and herbal formulations containing anti-oxidants are used pharmacologically to prevent, manage and/or treat such diseases in which oxidative stress play critical roles [11, 12, 14]. The assessment of anti-oxidant activity of herbal formulations used traditionally for treatment of certain diseases gives an insight into their possible mechanism of action and further scientifically validates their use for the treatment of those ailments [9, 14].

Cassia sieberiana DC is a savanna plant of the family Caesalpiniaceae [15]. It is commonly known as African laburnum or drumstick tree in English and casse de sieber or casse-flute in French [15]. In Nigeria, the plant is variously known as “gama fada” or “malga” in Hausa; “ifo” or “aridanworo” in Yoruba; “malgahi” in Fufulde; “marga” or “shuwa” in Kanuri and “apagan’’ in Edo languages [16]. Different parts of *C. sieberiana* are traditionally used for the treatment of many illnesses in the tropics [16]. Extracts of the roots, stem bark and fruit pulp have been used traditionally in Nigeria for the treatment of inflammatory conditions, fever, joint pains, malaria, diarrhea, leprosy, bilharzias, stomach pains, and diabetes mellitus [17]. In Senegal, Uganda and Coted’Ivoire, decoctions of the root or infusion of the whole plant are used as purgative and diuretic, and recommended for the treatment of haemorrhoids, bilharzias, leprosy, dropsy, intestinal worm infestations, diabetes mellitus and numerous childhood illnesses [15, 16]. There is little or no information in available literature on the acute toxicity, phytochemical constituents and anti-oxidant activity of extracts of *Cassia sieberiana*. Hence the present study was designed to evaluate the acute toxicity, possible phytochemical constituents and in-vitro anti-oxidant activity of crude methanolic stem bark extract of Cassia sieberiana DC.

MATERIALS AND METHODS

Plant Material

Fresh samples of the stem bark of Cassia sieberiana were collected from Adoka in Benue State, Nigeria in April 2010. The plant was identified and authenticated by a plant taxonomist at the Department of Botany, University of Nigeria, Nsukka where voucher specimens were deposited for reference purposes.

Plant Extraction

One thousand grams of dried and powdered *Cassia sieberiana* stem bark were extracted with 80% methanol using the cold maceration method for 48 hours.. The extract was filtered with Whatman no. 1

filter paper, and concentrated *in vacuo* with a rotary evaporator. The percentage yield of the extract was 4.88% weight/weight. The residual extract was dissolved in distilled water and used for the study.

Experimental Animals

A total of 36 male albino rats (*Rattus norvegicus*) of 12 weeks of age obtained from the Laboratory Animal House of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, were used for the study. They were housed in steel cages at room temperature and fed standard rat chow (Grand Cereals Ltd., Jos, Nigeria). The animals had free access to food and water. Guidelines for the humane handling of animals were followed all through the study.

Experimental Procedures

Acute Toxicity Test

This was carried out using a total of 36 male albino rats. The rats were randomly assigned to six groups A-F of six rats each. The extract was administered to five groups of rats (A-E) at the doses of 250, 500, 1000, 2000 and 4000 mg/kg body weight (bw) *per os* respectively. Group F (control) was given distilled water at the dose of 10 ml/kg bw. Signs of toxicity such as excitement, dullness, sedation, anesthesia, wretches, pupillary dilation, and death were checked within 48 hours. The trend-line equation relating the dose of the extract with the percentage mortality was $Y = 68.514X - 46.367$, where Y = dose of extract and X = percentage mortality (figure 1). By substituting 50 in place of X in the equation, the LD_{50} was calculated [5].

Phytochemical analysis

Test for the presence of tannins, flavonoids, alkaloids, saponins, carbohydrates, starch, and reducing sugars were carried out on the crude extract following standard procedures [18, 19]. One gramme of the crude extract was dissolved in 100 ml of distilled water in a beaker, and was used to test for the presence of the phytochemicals.

Estimation of total anti-oxidant activity of the crude extract

The free radical scavenging activity of the crude extract was analyzed by 1, 1-diphenyl 2-picryl hydrazyl (DPPH) assay [20], and confirmed by the Fehling's reducing anti-oxidant power (FRAP) assay [21].

Data Analysis

Data obtained from the study were analyzed using a one way analysis of variance (ANOVA), and variant means were separated by post-hoc analysis using the least significant difference (LSD) method. Significance was accepted at $p < 0.05$. Results were presented in form of tables of means with standard deviations, bar charts and graphs.

RESULTS

The results of the acute toxicity test showed that at the doses of 500, 1000, 2000, and 4000 mg/kg bw, some of the rats were dull and weak. After 48 hours of observation, there was no mortality (0% mortality) in the group treated with distilled water (control) and the group treated with 250 mg/kg bw dose of extract. Mortality was recorded at the doses of 500 mg/kg bw (16.6% mortality), 1000 mg/kg bw (33.3% mortality), 2000 mg/kg bw (33.3% mortality) and 4000 mg/kg bw (33.3% mortality) (Table 1). The LD_{50} was calculated to be 3,379.33 mg/kg bw, using the trend-line equation (Figure 1).

The phytochemical analysis of the crude extract revealed the presence of high levels of tannins (+++), moderate levels of flavonoids, saponins, carbohydrates and reducing sugars (++), and low levels of alkaloids (+) (Table 2).

The DPPH anti-oxidant values of the extract were 39.77 ± 1.37, 59.15 ± 3.20, 59.50 ± 3.36, 61.69 ± 0.07 and 59.72 ± 1.72 % at concentrations of 10, 50, 100, 200, and 400 µg/ml respectively, while the DPPH anti-oxidant values of the ascorbic acid standard were 73.64 ± 1.82, 74.10 ± 0.10, 74.62 ± 3.46, 77.16 ± 2.12, and 79.21 ± 2.78 % at concentrations of 10, 50, 100, 200, and 400 µg/ml respectively (Figure 2).

Table 1. The percentage mortality of rats treated with different doses of the methanolic stem bark extract of Cassia sieberiana.

Dose of extract (mg/kg b.w.)	No. of rats given the dose	Number (%) mortality
200	6	0
250	6	1 (16.7)
500	6	2 (33.3)
1000	6	2 (33.3)
2000	6	2 (33.3)
4000	6	2 (33.3)
Control (Distilled water at 10 ml/kg b.w.)	6	0

Table 2: The phytochemical constituents of the crude methanolic extract of the stem bark of Cassia sieberiana

S/No.	Test	Observation	Levels present*
1	Test for tannins		
	Lead sub-acetate	Brownish precipitate	+++
	Dilute sulphuric acid	Yellowish coloration	+++
	Ferric chloride	Greenish brown coloration	+++
2	Test for alkaloids		
	Meyer's reagent	Slight yellowish precipitate	+
	Wagner's reagent	Intense yellowish precipitate	+
	Dragendorff's reagent	Dirty yellowish precipitate	+
3	Test for flavonoids		
	Sodium hydroxide	Reddish brown coloration	++
	Ammonia + H2SO4	Intense yellowish coloration	++
4	Test for saponins		
	Frothing test	Stable froth/ foaming	++
	Emulsifying test	Emulsions	++
5	Test for carbohydrates		
	Molisch reagent	Brownish deposit	++
6	Test for starch		
	Iodine solution	No color change	-
7	Test for reducing sugar		
	Fehling's solution I and II	Blue-black coloration with brick red precipitate on heating.	++

*+++ = high; ++ = moderate; + = low; - = absent

The DPPH anti-oxidant activity values of the ascorbic acid standard were significantly ($p<0.05$) higher than those of the extract at all the concentrations tested (Figure 2). When the different concentrations of the extract were compared, the DPPH anti-oxidant activity of the extract at 50, 100, 200 and 400 µg/ml were significantly ($p < 0.05$) higher than the values obtained at 10 µg/ml (Figure 2). In contrast, there were no significant ($p > 0.05$) differences between the DPPH anti-oxidant values of the ascorbic acid standard at varied concentrations (Figure 2).

The FRAP of varied concentrations of the extract were 0.13 ± 0.07, 0.43 ± 0.16, 0.68 ± 0.33, 1.33 ± 0.20 and 1.89 ± 0.30 µM at concentrations of 10, 50, 100, 200, and 400 µg/ml respectively, while those of the ascorbic acid standard was 2.00 µM (Figure 3). The FRAP of the ascorbic acid standard was significantly ($p<0.05$) higher than that of the extract at the concentrations of 10, 50, 100 and 200 µg/ml, but there was no significant ($p > 0.05$) difference between the FRAP of the ascorbic acid standard and that of the extract at 400 µg/ml (Figure 3).

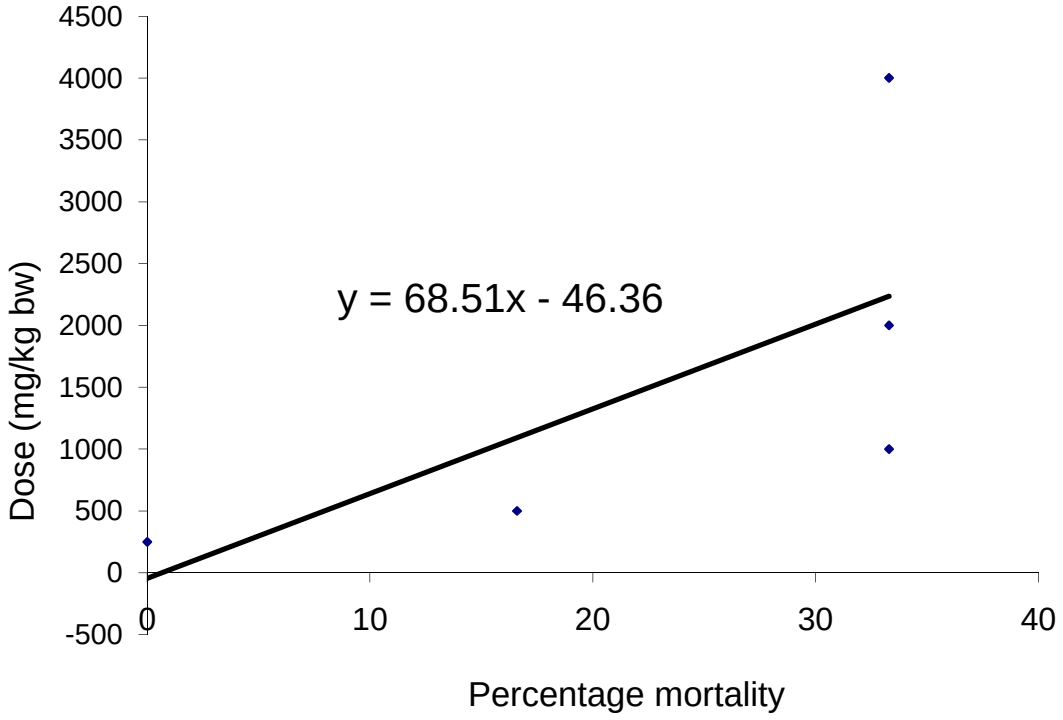

Figure 1. The scatter plot, trend line and trend line equation relating the dose of the crude extract and percentage mortality in the acute toxicity test of the methanolic extract of the stem bark of Cassia sieberiana

DISCUSSION

The LD_{50} of 3,379.33 mg/kg bw obtained for the methanolic stem bark extract of Cassia sieberiana in this study falls within the World Health Organization's (WHO) category of substances "unlikely to present acute hazard in normal use", thus the extract is considered to be safe by WHO acute hazard ranking [22]. This may imply that the methanolic stem bark extract of *Cassia sieberiana* is safe for use as treatment of ailments and diseases for which it is used. Compared to an earlier report [23] that recorded an oral LD_{50}

of 1,950 mg/kg b.w. for the aqueous pods pulp (fruit) extract of *C. sieberiana* in rats, it could be inferred that the methanolic extract of the stem bark of *C. sieberiana* as used in this present study may be safer for acute use than the fruit extract. The methanolic stem bark extract used in this present study may be considered to be extremely safe when compared to the aqueous leaf extract of this plant with an oral LD_{50} of 24.4 mg/kg b.w. [24]. These significant variations in the LD_{50} reported for different parts of the same plant are worthy of note and should be considered before any part of the plant is recommended for use.

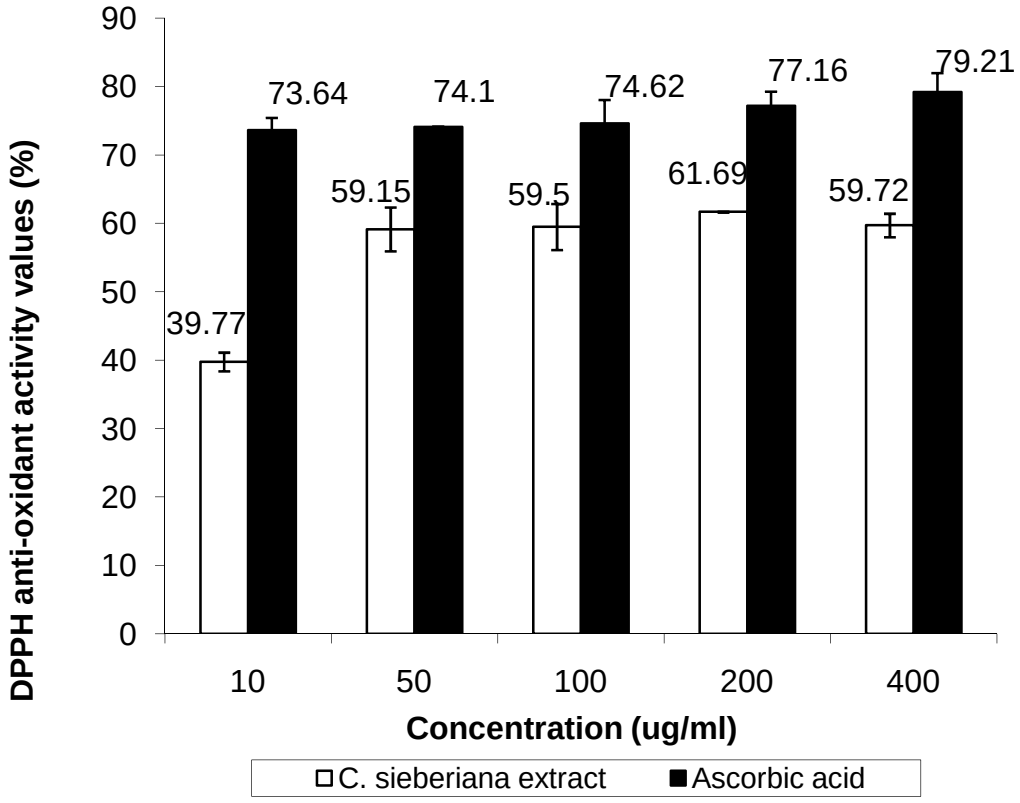

Figure 2. The 1,1-diphenyl 2 picryl hydrazyl (DPPH) anti-oxidant activity values of varying concentrations of the crude methanolic extract of the stem bark of Cassia sieberiana and ascorbic acid control.

The phytochemical analysis which revealed the presence of tannins, alkaloids, flavonoids, saponins, carbohydrates and reducing sugars in the crude methanolic stem bark extract is in agreement with an earlier report [17] on the same plant extract. The results of this present study is also in agreement with the reports of Toma et al [23] who also obtained tannins (+++), alkaloids (+), saponins (+++), cardiac glycosides (++), steroids (+), flavonoids (+), phlobatannins (++), reducing sugars (++) and cyanogenic glycosides (+), in extracts of the pods pulp of *C. sieberiana*. When compared to the earlier report on the pods pulp extract of *C. sieberiana* collected from Maiduguri in Borno state of Nigeria [23], this present study on the stem bark extract showed similar concentrations of tannins, alkaloids and reducing sugars, however, a higher concentration of flavonoids and lower concentration of saponins was recorded for the stem bark in this present study compared to that of the pods pulp (fruit) extract [23]. These differences imply that variations may occur in the bioactive compounds contents of different parts of same plant. These variations may also be accounted for by differences in the environment in which the plants are grown [25].

The presence of these phytochemicals recorded in this present study on *C. sieberiana* stem bark extract may partly account for its traditional medicinal uses and efficacy in handling ailments for which it is commonly recommended. Heterogeneous phytoconstituents of crude extracts have been reported to have synergistic effects [26]; this may explain why the extract of the stem bark of *C. sieberiana* is used in the tropics for the treatment and management of many illnesses [15, 16, 17]. These heterogeneous phytoconstituents, most especially flavonoids and tannins, possess the ability to reduce free radical formation and scavenge free radicals in vivo [27, 28]. These phytochemicals present in this extract are known to exhibit a wide range of biological activities, which may include anti-microbial, anti-allergic, anti-oxidant and anti-inflammatory properties [29]. It is also known that certain molecules which have structures like flavonoid, alkaloid or tannin have anti-inflammatory effects [30. 31]; this may justify the use of the stem bark extract of *C. sieberiana* for the treatment of inflammatory conditions in Nigeria traditional medicine.

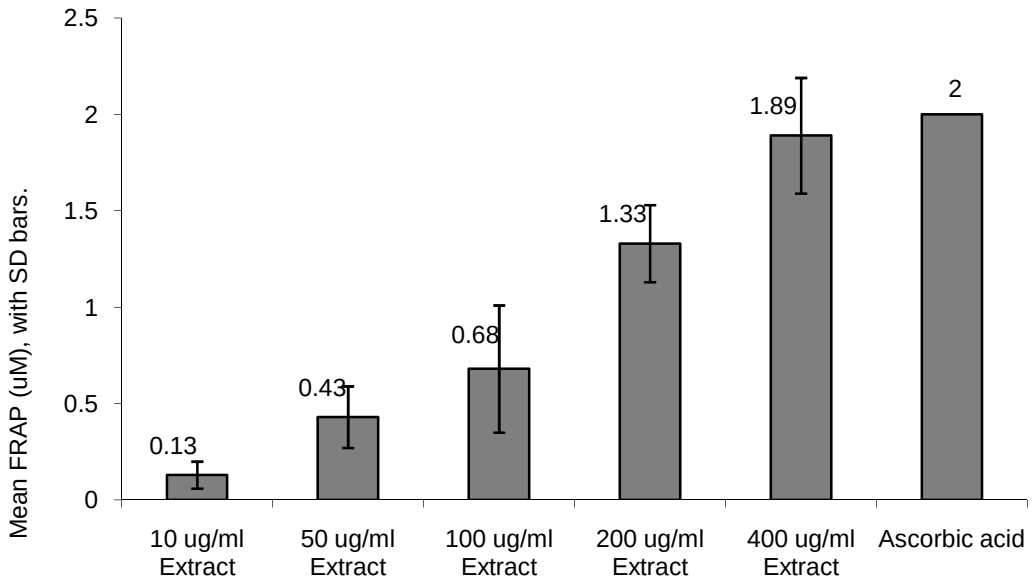

Figure 3. The Fehling's reducing anti-oxidant power (FRAP) of varying concentrations of the crude methanolic extract of the stem bark of Cassia sieberiana and ascorbic acid control

The free radical scavenging activity of the methanolic stem bark extract of *C. sieberiana* as determined by the DPPH and FRAP assay confirm that the extract exhibits anti-oxidant activity. The anti-oxidant properties of the extract may be attributed to its flavonoid and tannin contents [28, 29]. The traditional use of *Cassia sieberiana* extract in the management of diseases associated with oxidative stress such as diabetes mellitus may be attributed to the ability of its flavonoid and tannin contents to scavenge hydroxyl radicals, superoxide anion radicals and lipid peroxyl radicals [32]. Anti-oxidants scavenge free radicals in the body and this ward off diseases and promote general well-being. The possible specific health benefits of the anti-oxidant activity of the *C. sieberiana* extract may include slowing down of ageing, prevention of cancers, glaucoma and muscular degeneration, reduction of risk of cholesterol oxidation and heart diseases, enhancement of immunity, management of diabetes mellitus, relief from allergies, asthma, stroke, gum diseases, arthritis, and high blood pressure [27, 28, 29].

In conclusion, the results obtained from the study showed that the methanolic stem bark extract of *C. sieberiana* is relatively non-toxic, especially when used below 500 mg/kg bw. The extract also possesses anti-oxidant activity which could be attributed to the presence of flavonoids and/or tannins acting alone or in conjunction with other constituents in the extract. These findings may justify its use in traditional medicine.

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