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Acute toxicity and phytochemical screening of stem bark extracts of *Entandrophragma angolense* (Welw.) C.DC. (Meliaceae)

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Abstract

The plants are a rich source of drugs because they produce a variety of bioactive molecules, most of which probably act as chemical defence against predators or pathogens. The present study was conducted to determine the phytochemical constituents and the acute toxicity of aqueous extracts, ethanolic and methanolic of stem bark from *Entandrophragma angolense* in rats. The acute toxicity studies were carried out on the basis of the guidelines of the OECD 423. The aqueous extracts, ethanolic and methanolic were administered orally at a single dose of 300, 2000 and 5000 mg / kg body weight to the rats. The extracts showed lesser toxicity at dose of 2000 mg / kg body weight; however at 5000 mg/kg body weight dose the mortality rate was 2/3. Thus, the Lethal Dose 50(DL50) is in the range of 2000 to 5000 mg / kg body weight. These results suggest that the aqueous extracts, ethanolic and methanolic of stem bark of *E. angolense* could be used with some degree of safety by oral route. Phytochemical analysis of aqueous extracts, ethanolic and methanolic of stem bark of *E. angolense* revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, quinones, cardiac glycosides, leucoanthocyanins, phenols and steroids. These extracts contain important bioactive compounds, which justifies the use of this plant in traditional medicine for the treatment of various diseases.

Keywords: *Entandrophragma angolense,* phytochemical analysis, acute toxicity.

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INTRODUCTION

In all developing countries such as Ivory Coast, medicinal plants are the most widely used especially in rural areas to address the public health problems. According to the national program for the promotion of traditional medicine from Ivory Coast, 1421 species of medicinal plants involved in traditional medicine and for the treatment of patients have been identified till today by the Ivorian researchers [1]. *Entandrophragma angolense,* a species of medicinal plants is widespread, occurring from Guinea east to southern Sudan, Uganda and Western Kenya, and south to Democratic Republic of Congo and Angola [2]. The wood, usually traded as 'gedunohor' or 'tiama', is highly valued for exterior and interior joinery. The bark is used in traditional medicine. A decoction is drunk to treat fever and the bark is also used, usually in internal applications, as an anodyne against stomach-ache and peptic ulcers, earache, and kidney, rheumatic or arthritic pains. It is also applied externally to treat ophthalmia, swellings and ulcers [3]. Bark extracts have been reported for moderate antiplasmodial activity; and the compounds 7α-obacunylacetate and 24-methylenecycloarte exhibited pronounced activity against chloroquine-resistant strains of Plasmodium falciparum[4].Methyl angolensate, the major compound isolated from the methanol extract of the stem bark of *Entandrophragma angolense* produced a dose-related inhibition of gastric ulceration[5].Philippus von Hohenheim, known as Paracelsus the famous Swiss, alchemist and physician, said that the only difference between a drug and a poison was the dose [6]. All substances that have medicinal properties may also be toxic, so security issues and medicinal use are closely linked. The present study has been undertaken to estimate the acute toxicity and phytochemical screening of aqueous, ethanolic and methanolic extracts of the stem bark of *Entandrophragma angolense* in rats.

MATERIALSAND METHODS

-Collection of plant material:

The plant material is made up of the stem bark of *Entandrophragma angolense.* The bark has been collected in Abidjan area (south of Ivory Coast)in March 2014 and was identified at National Centre Floristic of University Felix Houphouet-Boigny;-deposited a herbarium specimen of the plant. The bark was dried at room temperature before crushing into powder then stored for further use.

-Plant extraction:

Three types of extract were prepared from the powdered bark of *E. angolense*.

-Decoction

One hundred (100) grams of plant powder were boiled for 20 minutes in 2 litres of distilled water. The cooled decoction is filtered twice on cotton wool and once on Whatman No.1 filter paper. The filtrate is then dried in hot air oven at 50°C for two days, to give the decoction of *E. angolense*.

-Maceration in 70% ethanol.

Ethanolic extracts were prepared according to the method described by Zirihi [7].One hundred grams of plant powder are vigorously agitated at in mixer in 1L of 70% ethanol and then filtered over cotton wool. The filtrate was decanted for 24 hours. The aqueous-alcoholic phase is isolated of the residual deposit, filtered over Whatman No.1paper filter and was dried in hot air oven at 50°C for two days.

-Maceration in methanol

The methanolic extract from the bark of *E. angolense* was prepared by stirring 50 g of finely ground portions in 1.5 L methanol using a magnetic stirrer (IKAMAG RCT) for 48 hours at room temperature. The methanolic extract was filtered over cotton and WhatmanNo.3 filter paper three times and was dried in hot air oven at 50°C for two days [8].

Experimental Animals:

Animals were selected as per the Organization of Economic Co-Operation and Development (OECD)guidelines no.423[9]. Healthy young and nulliparous, non-pregnant Wistar strain female rats weighing from 160-180 mg of 8-12 weeks old obtained from the animal house of Superior Normal School Abidjan(Ivory Coast)were selected, because literature surveys of conventional〖 LD〗$_{50}$ tests show that usually there is little difference in sensitivity between the sexes, but generally females were found slightly more sensitive[10]. A total of 36female rats were used in this study.

The animals are randomly selected, marked to permit individual identification, and kept in plastic cages with wood chips renewed every two days for 5 days prior to dosing to allow for acclimatization to the laboratory conditions(room temperature 25°C (± 3°C), humidity 35to 60%, light and dark period 12/12 hours, bedding clean sterilized husk).

All animals had regular supply of clean drinking water and food.

Acute Toxicity Testing:

The acute oral toxicity of aqueous, ethanolic and methanolic extracts of the stem bark of *Entandrophragma angolense* was determined as per OECD-423 guidelines (acute toxic class method).

The animals were divided into 4 groups of 3 rats each. Two groups received the dose of 300 mg / kg and 2000 mg / kg per body weight of each extract. Then the last group received the same dose of 2000 mg / kg to confirm the first result. Otherwise, if amount 2000 mg/kg body weight did not prove to be toxic, higher dose (5000 mg/kg) is used to determine the toxicity of

plant. When there is no information on the acute toxicity of the test substance, for animal welfare reasons it is recommended to use the starting dose of 300 mg/kg body weight (OECD, 2001). Four groups appear for each extract:

-Group 1→ dose≤ 300 mg/kg
-Group 2→dose ≤ 2000 mg/kg
-Group 3→dose ≤2000 mg/kg
-Group 4→dose ≤5000 mg/kg

The aqueous extracts, ethanolic and methanolic were prepared with distilled water and administered orally at a single dose to the rats. A volume of 2 ml/100g of body weight is used. Rats were maintained into fasting over-night before extract administration without water deprivation, and then they were normally fed 3-4 hours later the substance has been administered. Following the fasting period, the rats were weighed and the concentration was calculated in reference to the body weight. The animals were observed 30min after dosing, followed by hourly observation for 8h and once a day for the next 13 days. All observations were systematically recorded with individual records being maintained for each animal. Surviving animals were weighed and visual observations for mortality, behavioural pattern, changes in physical appearance, injury, pain and signs of illness were conducted daily during the period. When there is no information on the acute toxicity of the test substance, for animal welfare reasons it is recommended to use the starting dose of 300 mg/kg body weight (OECD, 2001).

Phytochemical analysis:

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plant under study were carried with extracts prepared using the standard procedures [11-12-13-14-15].

-Sterols and polyterpenes were detected by the reaction of Liebermann-Buchard. 5 ml of each of the three extracts were evaporated on a sand bath. The residue was dissolved in hot 1 ml of acetic anhydride; we added 0.5 ml of concentrated sulfuric acid to triturate. The appearance at the interphase of a purple or purple ring, turning blue to green indicated a positive reaction.

-The reaction to the ferricchloride (Fecl_3) has been used to characterize polyphenols. 2 ml of each extract (ethanol, methanol and aqueous), we added one drop of alcoholic solution of ferric chloride to 2%. The appearance of a blue-green or blackish coloration more or less dark was the sign of the presence of polyphenols.

-Flavonoids have been sought by there action to cyanidin.2 ml of each extract were evaporated and the residue was taken up in 5 ml of dilute hydrochloric alcohol 2 times. In adding 2-3 magnesium chips, there is a heat release and a pink-orange colour purple. The addition of 3 drops of isoamyl alcohol has intensified this coloration which confirmed the presence of flavonoids.

-Leucoanthocyanins: Carry out the reaction of cyanidin without adding magnesium chips and heat up for 15 minutes in a water bath. In the presence of leucoanthocyanes, it develops a cherry-red or violet.

-Search for catechin tannins is made from reagent Stiasny. 5 ml of each extract were evaporated to dryness. After adding 15 ml of reagent Stiasny the residue, the mixture was kept in a water bath at 80°C for 30 min. The observation of a precipitate in large flakes characterized catechin tannins.

-For gallic tannins, we filtered the previous solution. The filtrate was collected and saturated with sodium acetate. The addition of Fecl_3drops causes the appearance of a blue-black coloration intense, indicating the presence of gallic tannins.

-Quinone substances have been searched from the reagent Bornträeger. 2 ml of each of the three extracts were evaporated to dryness. The residue was triturated in 5 ml of hydrochloric acid 1/5. The triturate was poured into a test tube. The triturate was then heated in a water bath for 30 min. After cooling, it is extracted with 20 ml of chloroform. Ammonia diluted 2-fold (0.5 ml) was added to the chloroform solution. A red or violet colour was the sign of the presence of quinones.

-Alkaloids were characterized from Bouchardat reagent (reagent iodo-iodized) and Dragendorff (reagent iodo bismuthate of potassium). 6 ml of each solution were evaporated to dryness. The residue is taken up in 6 ml alcohol at 60 °. The addition of 2 drops of reagent Dragendorff on the alcoholic solution caused a precipitate or orange color. Adding 2 drops of Bouchardat reagent on the alcoholic solution caused a colour precipitate reddish brown and indicated a positive reaction.

-To find saponins, we contributed in a test tube, 10 ml aqueous total extract. The tube was shaken for 15 s and allowed to stand for 15 min. A height of persistent foam greater than 1 cm indicated the presence of saponins.

-Test for Cardiac glycosides (Keller-Killani test): 0.5 of extract will be dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This will then be underlayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface will indicate the presence of a desoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring while, in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

RESULTS

Acute toxicity tests:

-Clinical signs

Administration of the extracts at the dose of 2000 mg / kg, we observed abdominal constrictions, fast breathing and stillness of some animals during the first 4 hours then after the animals returned to their normal behaviour. For the dose of 5000 mg / kg, in addition to signs mentioned above, the animals showed other signs such as loss of appetite, paralysis of the anterior and posterior leg, weight loss for a week (Table 1).

-Mortality depending on dose

No mortality was recorded in female rats administered by aqueous, ethanolic and methanolic extracts of bark *E. angolense* at doses of 300 mg /kg and 2000 mg /kg as shown in Table 1.

Mortality was observed in group 4 after one week.

-Determination of the LD50

According to the test procedure with a starting dose of 300 mg / kg body weight (OECD 423, annex 2c), it was reported that the LD_50 is between 2000 and 5000 mg / kg.

	Extracts											
	Aqueous				Ethanol				Methanol			
Clinical signs	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4
Stillness	-	X	X	X	-	X	X	X	-	X	X	X
Abdominal constrictions	-	X	X	X	-	X	X	X	-	X	X	X
Fast breathing	-	X	X	X	-	X	X	X	-	X	X	X
Loss of appetite	-	-	-	X	-	-	-	X	-	-	-	X
Weight loss	-	-	-	X	-	-	-	X	-	-	-	X
Paralysis of the anterior and posterior leg	-	-	-	X	-	-	-	X	-	-	-	X
Death	-	-	-	X	-	-	-	X	-	-	-	X

Table 1: Potential toxic effects observed in rats after administration of the bark extracts of *Entandrophragma angolense*
= Absence of signs;
X = Presence of signs or death
G = Group;
-Group 1→ dose≤ 300 mg/kg;
-Group2→dose ≤ 2000 mg/kg;
-Group 3→dose ≤2000 mg/kg;
-Group 4→dose ≤5000 mg/kg

Phytochemical screening:

The main chemical groups identified in aqueous, ethanolic and methanolic crude extracts of the bark of *Entandrophragma angolense* are mentioned in the Table 2.

	Powder extract of the stem bark		
Identified chemicals groups	Aqueous	Ethanol	Methanol
Sterols and polyterpenes	+	+	+
Polyphenols.	+	+	+
Flavonoids.	+	+	+
Leucoanthocyanins	+	+	+
Catechin tannins	+	+	+
Gallic tannins	-	-	-
Quinone	-	+	+
Alkaloids	+	+	+
Saponins	+	-	-
Cardiac glycoside	+	+	+

Table 2: Phytochemical analysis in bark powder extracts of E.angolense

DISCUSSION

In the absence of information on the toxicity of our test material, we started the single oral administration of extracts *E. angolense* at a dose of 300 mg / kg body weight, initial dose recommended by the OCED for reasons relating to the welfare of animals. At this stage, the animals did not develop any visible signs of toxicity. At the highest dose (2000 mg / kg), the acute toxicity tests have shown low toxicity, but no mortality in animals. However the dose of 5000 mg / kg body weight led to mortality in animals. From the experiment conducted in accordance with the OECD Guidelines 423, the results revealed that the aqueous extracts, ethanol and methanol of *E. angolense* were considered toxic at 5000 mg / kg body weight of experimental animals that in the 4 first hours of observation, 2/3 morbidity was observed and a week after 2/3 were found dead. Through this analysis, stem bark of *E. angolense* could be classified in Globally Harmonized Classification System for Chemical Substances and Mixtures (GHS), hazard category 5 with a LD50r anging from 2000 mg / kg and 5000 mg / kg

Samson Amos et al carried out acute toxicity of methyl angolensate, a triterpenoid isolated from *E. angolense* in mice by the oral route and reported LD50 in the range of 145 -172.5 g / kg body weight [16].

The tests of detection of chemical groups responsible for therapeutic effects showed different chemical compounds that have been distributed according to the solvent extraction.

Sterols, polyterpenes, polyphenols, flavonoids, leucoanthocyanins, cardiacglycoside, alkaloids, catechin tannins are present in all extracts except gallic

tannins. The methanolic and ethanolic extracts contain the same chemicals compounds except saponins. However the aqueous extract showed the presence of saponins (Table 2).Unlike the work of Olajide[17], themethanolic extract of *E. angolense* (stem bark) showed the absence of alkaloids, phenols, flavonoids, cardiac glycosides.The antimalarial activity was reported by Bickii (2007), which helps explain the traditional therapeutic uses. The stem bark of *E. angolense,* in aqueous decoctions is used for the treatment of fever or malaria in Cameroon and Ivory Coast. Sterols and polyterpenes have bactericidal properties. The antimicrobial activity from stem bark of *E. angolense* is evaluated against five bacteria species and a fungus namely *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* NCTC 10073, Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 4853 and strains of *Candida albicans* and some test bacteria[18].

CONCLUSION

The aqueous extracts, ethanol and methanol of *E. angolense* showed relatively low toxicity when administered orally at a dose of 2000 mg / kg body weight. These extracts at this dose may, under certain conditions, be a hazard to vulnerable populations. The administration of the extracts at a dose of 5000 mg / kg caused the death of animals. In view of this, the oral LD50of these substances are in the range 2000-5000 mg / kg. However, normality and insignificant changes in the parameters of body weight and health revealed safety of extract at a dose of 300 mg / kg body weight.

For the phytochemical screening of extracts from *E. angolense,* we noted the presence of Sterols, polyterpenes, polyphenols, flavonoids, leucoanthocyanins, cardiac glycoside, alkaloids, tannins, saponins and quinones. These secondary metabolites are bioactive molecules responsible for therapeutic effects. This could justify its widespread use in traditional medicine. Given the phytochemical screening and toxicology studies of the stem bark of *E. angolense*, it is desirable to evaluate pharmacologically other activities unresolved of this plant such as anti-inflammatory activity.

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