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Acute toxicity studies and phytochemical screening of aqueous and ethyl acetate stem bark extracts of *Securidaca longipedunculata* Fresen (Polygalaceae)

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Abstract

Acute toxicity assessment of chemicals and herbal products is usually conducted to predict toxicity and provide guidelines for selecting safe doses for humans. The present study was aimed to determine the phytochemical constituents and medial lethal dose (LD_{50}) of aqueous and ethyl acetate extracts of *Securidaca longipedunculta* stem bark using the basic phytochemical screening, Up and Down Procedure (UDP) and Lorke's method. The result shows that glycosides, steroids, terpenoids, alkaloids flavonoids, saponins, anthraquinones, phenolic compounds and carbohydrates were all detected in both extracts. The medial lethal dose (LD_{50}) of the ethyl acetate extract was greater than 5, 000 mg/kg body weight, while that of aqueous extract was found to be above 2, 000 mg/kg body weight. It can be concluded that the aqueous and ethyl acetate stem bark extracts of *Securidaca longipedunculta* were slightly toxic and practically non-toxic respectively.

Keywords: Acute toxicity, chemicals, herbal products, humans and safe dose.

1. Introduction

The median lethal dose (LD_{50}) test was first introduced in 1927 by J. W. Trevan in order to estimate the dose of a test substance that produces 50 % death in a given species of animals. It is usually the first test conducted for every chemical before further toxicity tests are evaluated. It is used for estimating the potential hazards of substances on humans. Although, the major endpoint of LD_{50} is death, non-lethal acute effect may occur as signs of toxicity depending on the substance being investigated [1].

Results obtained from acute toxicity test may vary greatly from species to species, and sometimes from laboratories to laboratories. The median lethal dose is not tested on humans and relation to humans is only a guess because the human lethal dose may not be predicted exactly from animal studies [1].

Some of the signs and symptoms that should be observed and recorded during acute toxicity testing include analgesia, tremors, increased motor activity, anesthesia, tonic extension, salivation, clonic convulsions, muscle spasm, loss of righting reflex, writhing, hyperesthesia, ataxia, depression, sedation, stimulation, hypnosis cyanosis etc [2,3]. The endpoints of ocular acute toxicity test include redness, hemorrhage, ulcerations, discharge, blindness and swelling, while those of derma toxicity test include erythema and edema [1].

Plants are the oldest known health care products, because people all over the world have been utilizing traditional medicine for the treatment and/or management of a variety of ailments since ancient times [4].

In African countries, the use of herbal drugs prepared and dispensed by the traditional healers without formal training in the drug formulation and preparation is still very common, therefore, there is the need to screen the safety and efficacy of those herbal products [5].

Securidaca longipedunculata belongs to the family Polygalacea. It is commonly known as violet tree, fibre tree and Rhodesian violet, while its local names are Sanya or Uwar Magunguna (Hausa) and Ipeta (Yoruba) [6]. It is a multipurpose plant with a long history of use in African traditional medicine to treat sexually transmitted infections, hernias, coughs, fever, diarrhea, dysentery, typhoid constipation, headaches, rheumatism, stomach ache, malaria, tuberculosis, pain, epilepsy, pneumonia, skin infections etc. It was reported to have many pharmacological activities which include antimicrobial, antioxidant, antiparasitic, antidiabetic, antiinflammatory, antimalarial, insecticidal, pesticidal and anticonvulsant properties [7].

Most of the toxicological studies on *S. longipedunculata* evaluated the LD_{50} of the root bark and whole root extracts alone. It was reported by Auwal *et al* [8] that the aqueous root bark extract of the plant was slightly toxic to albino rats with an LD_{50} of 771 mg/kg, while Agbaje and Adekoya [9] reported an LD_{50} of 3160 mg/kg when administered orally to laboratory animals. Also, acute toxicity studies of the aqueous whole root extract on mice revealed LD_{50} values of 1740 mg/kg and 20 mg/kg for the oral and intraperitoneal application routes respectively [10], while Dapar *et al* [11] reported an LD_{50} of 37 mg/kg when aqueous root extracts were administered orally to albino rats.

However, to the best of our knowledge, the medial lethal dose (LD_{50}) of *S. longipedunculata* stem bark has not been evaluated; therefore, the main objective of this work was to assess the toxicity index of the aqueous and ethyl acetate stem bark extracts of this important medicinal plant.

2. Experimental Work

2.1 Animals

Adult Swiss albino mice of both sexes, weighing 20 to 35 g were obtained from the animal house of the Department of Pharmacology, Bayero University Kano, Nigeria and maintained under normal laboratory conditions of humidity, temperature and light for 7 days before the experiment and allowed free access to food and water. All experiments performed on the laboratory animals in this study were approved by the Local Ethical Committee for animal experimentation in the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Bayero University, Kano, Nigeria.

2.2 Collection, Identification and Preparation of Plant Material

The plant was collected from Gwaram Local Government Area, Jigawa State, Nigeria. It was identified in the field using taxonomic characters and then taken to the Herbarium of Ethnobotany Unit of Bioresources Development Centre, Kano for authentication. A reference voucher number, BDCKN/EB/1898 was deposited in the Herbarium.

The powdered stem bark (100 g) was successively macerated with ethyl acetate and distilled water (500 ml each) for 48 hours, the mixture was shaken occasionally. The filtrate obtained was evaporated to dryness at 40 ^{0}C using a rotary evaporator and a water bath.

2.3 Preliminary Phytochemical Screening

The Preliminary Phytochemical screening of the aqueous and ethyl acetate extracts was conducted using the standard laboratory procedures [12-16].

2.4 Median Lethal Dose (LD_{50}) Determination

2.4.1 Lorke's Method

This was conducted in two phases using the method described by Lorke [17]. In the initial phase, mice were divided into 3 groups of three mice each. The first group received the extract (*i.p*) at a dose of 10 mg/kg body weight, followed by 100 mg/kg and 1000 mg/kg to the second and third group respectively. The animals were then observed for 24 hours for signs and symptoms of toxicity and death.

In the final phase, mice were divided into 3 groups of one mouse each, the extract was then administered to group 1, 2 and 3 at the dose based on the result of the first phase (Table 2 and 3) as suggested by Lorke [17]. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

2.4.2 Up and Down Procedure (OECD TG 425)

This was conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals 425. Two groups of 4 mice were dosed orally with 2000 mg/kg and 5000 mg/kg body weight of aqueous and ethyl acetate extract respectively using the up and down procedure. The animals were then observed for 48 hours for signs and symptoms of toxicity and death. The LD_{50} was estimated to be above the limit range 2,000 or 5,000 mg/kg. Subsequently, all animals were observed for the next 14 days for any delayed toxic effects [3, 18-20].

3. Results

3.1 Preliminary Phytochemical Screening

The preliminary phytochemical screening of aqueous and ethyl acetate extracts of *S. longipedunculata*

stem bark revealed the presence of glycosides, steroids, terpenoids, alkaloids, flavonoids, anthraquinones, saponins, phenolic compounds and carbohydrates in both extracts (Table 1).

Table 1: Phytochemical Constituents of Aqueous and Ethyl Acetate Extracts

Phytochemicals	Extracts	
	Ethyl acetate	Aqueous
Tannins	-	-
Anthraquinones	+	+
Glycosides	+	+
Saponins	+	+
Phenolic compounds	+	+
Flavonoids	+	+
Alkaloids	+	+
Terpenoids	+	+
Steroids	+	+
Carbohydrates	+	+

Key
+ = Present
- = Absent

3.2 Median Lethal Dose (LD_{50})

3.2.1 Lorke's Method

The results of the acute toxicity study of the ethyl acetate extract of *S. longipedunculata* stem bark resulted in no mortality and other behavioural changes (Table 2). Thus, the LD_{50} was estimated to be above 5000 mg/kg body weight.

Table 2: Acute Toxicity Study of Ethyl Acetate Extract

First Phase		Second Phase	
Dose (mg/kg)	**Mortality**	**Dose (mg/kg)**	**Mortality**
10	0/3	1600	0/1
100	0/3	2900	0/1
1000	0/3	5000	0/1

On the other hand, deaths were recorded in animals administered with the aqueous extract of *S. longipedunculata* stem bark (Table 3). Behavioural changes observed include loss of appetite, restlessness and general weakness, and the median lethal dose (LD_{50}) of the extract was estimated to be 2154.07 mg/kg body weight.

Table 3: Acute Toxicity Study of Aqueous Extract

First Phase		Second Phase	
Dose (mg/kg)	**Mortality**	**Dose (mg/kg)**	**Mortality**
10	0/3	1600	0/1
100	0/3	2900	1/1
1000	0/3	5000	1/1

3.2.2 Up and Down Procedure (UDP)

There was no any sign of toxicity/behavioural changes and mortality when the laboratory animals were administered with 5000 mg/kg and 2000 mg/kg body weight of ethyl acetate and aqueous extracts respectively. Thus, the LD_{50} of the ethyl acetate extract was estimated to be above 5000 mg/kg body weight, while that of aqueous extract was estimated to be above 2000 mg/kg body weight.

4. Discussion

The assessment of acute toxicity of an unknown substance is the first step in toxicological investigation, it predicts the safety margin for any substance and hence the choice of doses for further studies [17, 21]. The intraperitoneal and oral acute toxicity studies of the ethyl acetate extract of *S. longipedunculata* stem was estimated to be above 5000 mg/kg body weight, thus the extract could be considered as practically non-toxic [17, 22].

From the result of an acute toxicity test, a conclusion can be made on the toxicity status of the test substance. Substances with LD_{50} below 5 mg/ kg are considered to be highly toxic while substances with LD_{50} above 15,000 mg/kg are termed relatively harmless [22].

The intraperitoneal and oral acute toxicity studies of the aqueous extract of *S. longipedunculata* stem was estimated to be greater than 2000 mg/kg body weight. According to Loomis and Hayes [22], substances with LD_{50} of 500-5000 mg/kg body weight are considered to be slightly toxic. The acute toxicity studies of the aqueous whole root extract of *S. longipedunculata* revealed LD_{50} values of 1740 mg/kg and 20 mg/kg for the oral and intraperitoneal application routes respectively [10]. The difference in LD_{50} between the aqueous whole root and stem bark extracts may be due to differences in the phytochemical constituents in both parts of the plant.

In toxicity assessment of chemicals, there is no doubt that the best test species for humans are humans since accurate extrapolation of animal data directly to humans may not be guaranteed due to interspecies variation in anatomy, physiology and biochemistry [23]. However, due to ethical reasons, such chemicals are to be tested using animal models before they are subjected to trials in humans [24].

5. Conclusion

The present study has reported for the first time the acute toxicity index of stem bark extracts of *S. longipedunculata*, and it can be concluded that the aqueous and ethyl acetate extracts of *S longipedunculta* stem bark were slightly toxic and practically non-toxic respectively.

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