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Phytochemical and acute toxicity studies on the ethanol roots extract of *Gardenia sokotensis*

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Abstract

Phytochemical study on the crude extract of the roots *Gardenia sokotensis* revealed the presence of alkaloids, glycosides, saponins and steroids and very little or no tannin, and flavonoid, content. The acute toxicity study carried out revealed complete absence of any clinical or behavioural changes that could be suggestive of toxicity in any of the animals in

groups A, B and C following treatment with the crude extract of the roots of the plant. However, depression, weakness and loss of appetite as evidences of toxicity in the first 5hrs were observed in groups D and E animals that were treated with higher doses of the extract. All the animals (Groups D and E) treated with the higher doses of the crude extract of the roots of the plant eventually died 40 – 96 hours post-administration. The LD50 of the *Gardenia sokotensis* root extract was found to be 2400mg/kg body weight when orally administered in rabbits.

Key Words: *Gardenia sokotensis*, phytochemical analyses and acute toxicity

Introduction

In most countries of tropical Africa, prohibitive cost of western medicine and poor health delivery systems have necessitated reliance on use of traditional plant medicine in the treatment of health ailments. Often, folk medicine is used without prejudice to the toxic effects that the use of the plants may cause in the body. *Gardenia sokotensis* is one of the species of plants whose leaves were reported to be widely used in folk medicine (Traore, 1983; Ake-Assi and Guinko, 1991; Dakuyo, 1992). It belongs to the family Rubiaceae. This family comprises a variety of plant species that are widely distributed in the tropics and subtropics (Huchinson *et al.*, 1963; Nielson, 1965). The family is of great economic importance as it includes coffee species, the seeds of which yield coffee; quinine, which is an alkaloid drug, is obtained from Cinchona species and is used as an anti-febrile agent especially in the treatment of malaria (Traore *et al.*, 2006). Many other plant species of this family, such as *Gardenia*, *Ixora*, and *Rondelting*, are used as garden ornaments but more importantly, they have other local uses (Ghazanfar, 1989). *Gardenia sokotensis* is a shrub or small tree that measures up to 8ft in height and has white flowers.

The various parts of the plant, which is found on dry rocky hills of the drier savanna regions of the tropical Africa, were reported to have a promising *in vivo* anti-plasmodial activity (Traore *et al.*, 2006). Traore *et al.* (2006) had established the phytochemical content of the leaves of the *Gardenia sokotensis*. However, in spite of the fact that virtually all parts of *Gardenia sokotensis* have been credited with some medicinal properties, there is dearth of information on the phytochemical composition of its roots and the toxicity effects that may be associated with its use, hence, this study was carried out.

Materials and Methods

Collection Plant Material (*G. sokotensis*)

The fresh roots of the plants strongly suspected to be *Gardenia sokotensis*, which were found around Dutsen Badano in Sokoto State of Nigeria and collected with the assistance of a traditional herbalist. The plant materials were identified and confirmed to be *Gardenia sokotensis* at the Botany Unit of the Department of Biological Sciences in Usmanu Danfodiyo University, Sokoto, Nigeria.

Preparation of Crude Extract

Crude extract of the root was prepared according to the procedure described by Kudi *et al.* (1999) and Samy and Ignacimuthu, (2000). Briefly, the procedure involved drying the roots of the plant in the laboratory for seven days at room temperature. The dried roots were then broken into smaller sizes and then ground into a powder using an electric blender. Five grams of the ground material were mixed with 50ml of 80% ethanol and left over night at room temperature. The preparation was then filtered using Wattman filter paper. The recovered filtrate was concentrated by evaporation in the oven, which resulted in a dark brown, dried crude extract that was used

in this study. The preparation was reconstituted by dissolving 500mg of the concentrated residue in 100ml distilled water for use on each day of the experiment as previously described (Kudi *et al.*, 1999).

Phytochemical Analyses

The extract was evaluated for the presence of alkaloids, tannins, glycosides, saponins, steroids and flavonoids by using the procedures described by Okerulu and Ani, (2001) as follows:

Test for Alkaloids

Exactly one millilitre (1 ml) of 1% Hydrochloric Acid (HCl) was added to 3 ml of the extract in a test tube. The mixture was heated for 20 minutes, allowed to cool, and then filtered. Two drops of Wagner's reagent were added to 1ml of the filtrate. A reddish brown precipitate observed in each extract tested indicated the presence of alkaloids (Okerulu and Ani, 2001).

Test for Tannins

Exactly 1 ml of freshly prepared 10% of Potassium hydroxide (KOH) was added to 1 ml of the extract. A dirty white precipitate was observed, which indicated the presence of tannins (Okerulu and Ani, 2001).

Test for Glycosides

Exactly 10 ml of 50% Tetraoxosulphate (VI) Acid (H₂SO₄) was added to 1ml of the extract in a test tube. The mixture was heated in boiling water for 15 minutes. About 10ml of Fehling's solution was added and the mixture was boiled. A brick – red precipitate was observed in the test mixture, which indicated the presence of glycosides.

Test for Saponins

Presence of saponin was detected using frothing test. In this test, exactly 2 ml of the extracts in a test tube was vigorously shaken for 2 minutes. Frothing observed in each extract tested indicated the presence of saponins.

Test for Steroids

Presence of steroids was detected using Salkowski test in which 5 drops of concentrated H₂SO₄ were added to 1ml of the extracts. Red coloration of the mixture indicated the presence of steroids.

Test for Flavonoids

Exactly 1 ml of 10% Sodium hydroxide (NaOH) was added to 3 ml of the extracts. A yellow coloration observed in each extract tested indicated the presence of flavonoids in all the extracts.

Acute Toxicity Study

Experimental Animals

Eighteen healthy rabbits of both sexes with ages and weights that ranged from 3 to 4 months old and 1.05 to 1.6kg, respectively, were purchased from Sokoto central market, Sokoto, Nigeria. The animals were allowed to acclimatize for 7 days in the animal house of the Department of Biological Sciences Usmanu Danfodiyo University Sokoto. They were fed with fresh vegetable and grower's mash. Water was provided *ad libitum*. Prior to commencement of this study, an approval for the use of these rabbits was sought for from the Ethical Committee on Animal Experimentation of Usmanu Danfodiyo

University and the guidelines on the use of these animals were strictly adhered to.

Animal Grouping and Treatment

The animals were divided into six (6) groups (groups A to F) of 3 rabbits, each. Groups A to E animals were the treatment groups, while group F served as untreated control animals. Animals in groups A, B, C, D and E were orally administered 300, 600, 1200, 2400, 4800 mg/kg doses, respectively, of the crude extract of the *Gardenia sokotensis*, while the control group received an equivalent volume of distilled water. Animals were observed for signs of acute toxicity like behavioral changes and death over 48 hours and LD₅₀ was determined using the Arithmetic method of karber modified by Aliu and Nwude (1982).

Results

Findings from Phytochemical Analyses

Results of the various phytochemical analyses conducted on the extract of *G. sokotensis* were indicative of presence

of appreciable quantities of alkaloids, glycosides, saponins and steroids. The results also indicated presence of minute quantities of tannins and flavonoids in the *Gardenia sokotensis* extract.

Findings from Acute Toxicity Study on the Plant Extract in Rabbits

None of the animals in groups A, B, and C showed any clinical or behavioural changes throughout the observation period. However, depression, weakness and loss of appetite in the first 5hrs were observed in groups D and E animals that were treated with the higher doses of the extract. Attempt at recovery from the toxicity was observed on the 6th hour in two of the animals in group D as they were becoming active, but all the animals in the groups D and E eventually died 40 – 96hours after administration. The LD₅₀ of the *G. sokotensis* extract was determined as described by Aliyu and Nwude (1982) (Table 1) and calculated to be 2400mg/kg body weight.

Table 1: Acute Toxicity (LD₅₀) of *G. sokotensis* in rabbits.

Dose (mg/kg)	Dose difference (mg)	No of dead (n)	Mean dead	Dose difference x mean dead
300		0		
	300	0	0	0
600		0		
	600	0	0	0
1200		0		
	1200	1	1	1200
2400		2	2	4800
	2400	2.5	2.5	6000
4800		3		
Total				7200

The LD₅₀ was calculated using the formula:

$$LD_{50} = LD_y - \frac{\sum (Dd \times md)}{N}$$

Where LD_y = Highest dose

$$\begin{aligned} N &= \text{number of animals per group} \\ Dd &= \text{dose difference} \\ Md &= \text{mean dead} \\ LD_{50} &= 4800 - \frac{(7200)}{3} \\ &= 4800 - 2400 = 2400\text{mg/kg} \end{aligned}$$

Discussion

The presence of alkaloids, glycosides, saponins, and steroids indicates that the plant has some medicinal properties which can be exploited for therapeutic purposes (Harborne, 1973). The substance alkaloid increases the activity against microorganisms (Ahamed *et al.*, 2005) and may also be responsible for the toxic effect observed (Sule *et al.*, 2005). Earlier phytochemical study (Traore *et al.*, 2006) on the leaves of *Gardenia sokotensis* had revealed presence of carotenoids, flavonoids and triterpenes. On the contrary, findings in the present study suggest that the roots of the plant contain very little or no flavonoids.

Leaves of *Gardenia sokotensis* have been reported to be used in the treatment of malaria (Dakuyo, 1992), fever,

asthenia, gastro-enteritis, (Ake Assi and Guinko, 1991) in spite of the fact that only few pharmacological studies had been conducted on the plant (Traore *et al.*, 2006). The established LD₅₀ (2400 mg/kg) of the extract, in this study, when administered orally shows that the ethanol plant extract is of low toxicity and safe for use in the various clinical ailments reported (Clarke and Clarke, 1975; WHO, 1986; Ake Assi and Guinko, 1991; Dakuyo, 1992). This is because it was reported that any substance whose LD₅₀ is above 1000 mg/kg is regarded as safe (WHO, 1986; Clarke and Clarke, 1975). In conclusion, *Gardenia sokotensis* contains the phytochemicals alkaloids, glycosides, saponins and steroids and the plant extract is considered safe for use in the variously reported indications.

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