African Journal of Biotechnology Vol. 6 (24), pp. 2752-2757, 17 December, 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Preliminary antidiarrhoeal activity of methanolic extracts of *Securinega virosa* (Euphorbiaceae)

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Accepted 8 November, 2007

Securinega virosa **is used as remedy for diarrhoea in tropical Africa, but has not been investigated for its antidiarrhoeal activity. This study was therefore aimed at investigating the methanolic extracts of the leaves, stem bark and root bark for antidirrhoeal activity, using castor oil-induced diarrhoeal model in mice. The effects of these extracts on perfused isolated rabbit jejunum were also evaluated. The methanolic leaves extract (8 x 10^{-5} – 1.6 x 10^{-3} mg·ml-1) produced a dose-dependent relaxation of the rabbit jejunum, while the methanolic stem bark and root bark extracts (2 x 10^{-5} – 3.2 x 10^{-3} mg·ml-1) produced contraction of the tissue. The methanolic root bark extract produced a dose-dependent protection against the castor oil- induced diarrhoea with the highest protection (100%), obtained at 100 mg·kg-1 comparable to that of loperamide (5 mg·kg-1), the standard agent. The leaves extract also protected the mice but was not dose-dependent. The highest protection (60%) was obtained at the lowest dose (50 mg·kg-1). The stem bark extract did not protect the animal against diarrhoea. The preliminary phytochemical analysis revealed that the three extracts contained similar phytochemical constituents which include alkaloids, tannins, saponins, flavonoids and cardiac glycosides. However, only the leaves extract contained anthraquinone glycosides. The acute toxicity test revealed the median lethal dose (LD_{50}) values for the leaves, stem bark and root bark extracts to be 1265, 288.5 and 774.6 mg·kg-1 respectively. This suggests that the stem bark extract is relatively the most toxic. These results obtained revealed that the leaves and root bark extracts possess pharmacological activity against diarrhoea and may possibly explain the use of the plant in traditional medicine.**

Key words: *Securinega virosa*, antidiarrhoeal, castor oil, isolated tissue, methanolic extracts.

INTRODUCTION

Diarrhoeal diseases are one of the leading causes of childhood morbidity and mortality in developing countries. An estimated 1,000 million episodes occur each year in children under 5 years of age. Diarrhoea causes an estimated 5 million deaths in children fewer than 5 years of age per year (Carlos and Saniel, 1990). The incidence of diarrhoeal diseases still remains high despite the intervention of government agencies and international organizations to halt the trend. The use of herbal drugs in the treatment of diarrhoea is a common practice in many African countries (Agunu et al., 2005). Despite immense technological advancement in modern medicine, many people in the developing countries still rely on traditional healing practices and medicinal plants for their daily healthcare needs (Ojewole, 2004). The World Health Organization (WHO) encouraged studies for the treatment and prevention of diarrhoeal diseases depending on traditional medical practices (Atta and Mouneir, 2004). There is therefore an urgent need for the intensification of research into medicinal plants claimed to be effective in the management of diarrhoea.

A number of medicinal plants are used traditionally in Africa for the management of diarrhoea. One of such medicinal plants is *Securinega virosa. S. virosa* is one of

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the greatest African medicinal plants described as a true "cure all", of which all parts are used as remedies, particularly the root (Neuwinger, 1996). It belongs to the family Euphorbiaceae of the order Geraniales. *S. virosa* is a dense, low branching, many branched shrub, sometimes a small spreading tree up to about 6 m high, although, more commonly 2 - 3 m, evergreen or deciduous. It is widely distributed throughout tropical Africa, also in India, Malaya, China and Australia (Dalziel, 1936). The common vernacular names of *S. virosa* include "Tsuwaawun karee, Gussu, Gwiiwar karee" (Hausa), "Iranje" (Yoruba), "Njisi nta" (Ibo), "Shim shim" (Kanuri), "kartfi-kartfi" (Shuwa arabs) and "Camal, cambe, came" (Fulani).

The leaves have laxative properties and are taken in decoction, while the root decoction is taken for veneral disease (Dalziel, 1936), schistosomiasis and dysmenorrhea in Tanzania (Neuwinger, 1996). The powdered root is used for diarrhoea in Nigeria while the stem bark decoction is used for bloody diarrhoea in Ivory Coast. (Neuwinger, 1996)

The present study was undertaken to investigate the anti-diarrhoeal activity of the methanolic leaves, stem bark and root bark extracts of the plant.

MATERIALS AND METHODS

Collection of plant materials

The whole plant, *S. virosa* was collected from Samaru town in Sabon-Gari Local Government Area of Kaduna State, Nigeria in May 2006. The plant was identified and authenticated by Malam Musa and Umar Gallah of the Herbarium Section in the Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher specimen (NO 918) was deposited at the herbarium for future reference.

Preparation of plant materials

The root and stem of the plant were cleaned and the bark removed separately. The leaves, root bark and stem bark were air dried under shade until constant weights were obtained. The plant materials were then milled into coarse powder. About 100 mg of each powdered plant material was macerated in 500 ml methanol (98%) with occasional shaking. The various extracts were concentrated to obtain the dried extracts. The yields obtained were as follows: leaves extract (13.2%), stem bark extract, (8.5%) and root bark extract (12.7%). The extracts were then stored in desiccators. Solutions of the extracts were prepared freshly for each study.

Animals

New Zealand rabbit (1.65 kg) and Swiss albino mice (18 - 22 g) maintained in the Animal House Facility of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria were used in these experiments. The animals were maintained on standard small animal feeds (Excel feed, Ilorin, Nigeria) and water *ad libitum*. This research was carried out in Ahmadu Bello University in accordance with the rules governing the use of laboratory animals as accepted internationally.

Drugs

Acetylcholine (Sigma chemical, USA), castor oil (Bell Sons and Co., England) and loperamide (Janssen, Germany).

Phytochemical procedure

The extracts were subjected to phytochemical analysis using standard protocol (Silva et al., 1998).

Acute toxicity study

The method previously described by Lorke (1983) was adopted. Briefly, 13 mice were used for each extract. In the first phase, three doses of the methanolic leaves extract (10, 100 and 1000 $mg \cdot kg^{-1}$ were administered to three groups each containing three mice). In the second phase, more specific doses were administered to four groups each containing one mouse. The median Lethal dose (LD_{50}) was determined as the geometric mean of the highest non lethal dose and the lowest lethal dose of which there is 1/1 and 0/1 survival. The same procedure was repeated for the methanolic stem bark and root bark extracts.

Effects on isolated rabbit jejunum

The rabbit was sacrificed by a blow on the head. Segments of the jejunums, about 3.0 cm long were removed and dissected free of adhering mesentery. The intestinal contents were removed by flushing with Tyrode solution of the following compositions in millimoles (mM): NaCl, 136.8; KCl, 2.7; CaCl, 1.3; $NaHCO_3$, 12.0; MgCl, 0.5; $NaPO_4$, 0.14; glucose, 5.5. The tissue was mounted in a 25 ml organ bath containing Tyrode solution maintained at 35°C and aerated with air. An initial tension of 0.5 g was applied to the segments and 60 min equilibration period was allowed while the physiological solution was changed every 15 min. At the end of the equilibration period, the effect of acetylcholine ($2.75 \times 10^{-10} - 8.8 \times 10^{-9}$M) and the methanolic extracts of leaves, stem bark and root bark of *S. virosa* were investigated non-cumulatively. The contact time for each concentration was 1 min which was followed by washing three times. The tissue was allowed a resting period of 15 min before the next addition.

Effects of castor oil-induced diarrhoea on mice

The mice were fasted for 12 h prior to the commencement of the study and were randomly divided into five groups each containing five mice. The mice were treated with either normal saline (10 $mlkg^{-1}$), the methanolic leaves extract (25 -100 mg·kg-1) or loperamide (5 mg·kg-1). All treatment was by intraperitoneal route. 30 min post-treatment, castor oil (0.2 ml / mouse) was given intragastrically. The animals were then placed in individual cages on a clean filter paper. Three hours after the castor oil challenge, the cages were inspected for the presence of characteristic diarrhoeal droppings; absence of which was regarded as protection (Diurno et al., 1996). The same procedure was used for the stem bark and root bark extracts (25 - 100 mg·kg-1).

Statistical analysis

The result of the castor oil-induced diarrhoea was analysed using Chi-square test and differences were regarded as significant with $P < 0.05$.

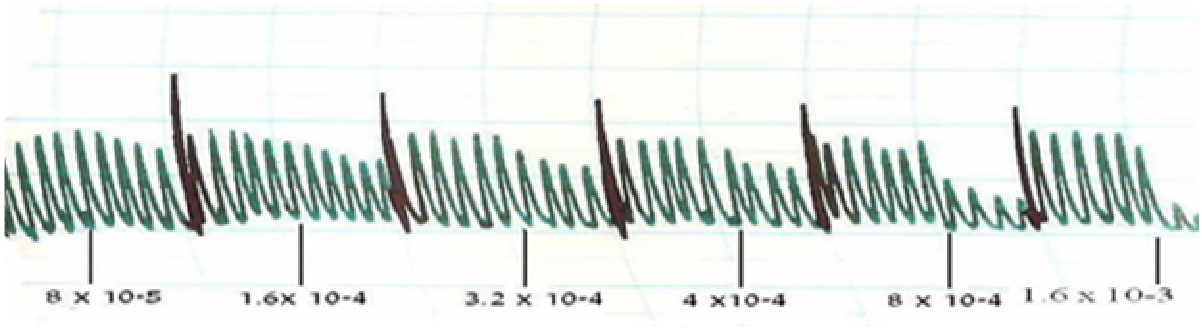


Figure 1. Effects of methanolic leaves extract of *S. virosa* (mg·ml-1) on isolated rabbit jejunum.

Table 1. Phytochemical constituents of the methanolic leaves, stem bark and root bark extracts *of S. virosa*.

Phytochemical constituent	Leaves	Stem bark	Root bark
Carbohydrate	+	+	+
Reducing sugars	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Cyanogenic glycosides	+	+	+
Cardiac glycosides	+	+	+
Anthraquinone	+	-	-
Tannins	+	+	+
Alkaloids	+	+	+
Resins	+	+	+
Steroids/Triterpenes	+	+	+

+ = Present; - = Absent.

RESULTS

Phytochemical analysis

The preliminary phytochemical screening of the extracts revealed the presence of alkaloids, saponins, flavonoids, tannins and resins in all the extracts. However, anthraquinone glycosides were only found in the leaves extract (Table 1)

Acute toxicity study

The median lethal doses of the extracts were as follows: leaves extract, 1265 mg·kg-1; stem bark extract, 288.5 mg·kg-1; and root bark extract, 774.6 mg·kg-1.

Effects of the extract on rabbit jejunum

The effects of the plant extracts on the rabbit jejunum were dose related. The leaves extract relaxed the spontaneous contraction of the rabbit jejunum (Figure 1), while the stem bark and root bark extracts showed contraction. The contractions caused by the root and stem bark extracts were comparable to that of acetylcholine (Figures 2, 3 and 4).

Effects on castor oil-induced diarrhoea

The root bark extract produced a dose-dependent protection against the castor oil induced diarrhoea with the highest protection (100%) obtained at the highest dose tested (100 mg·kg-1) comparable to that of loperamide, the standard anti-diarrhoeal agent. The leaves extract afforded 60% protection at the lowest dose (50 mg·kg-1). There was no protection in the animals treated with the stem bark extract (Table 2).

DISCUSSION

The leaves and root bark extracts exhibited significant anti-diarrhoeal activity against castor oil-induced diarrhoea in mice. The root bark extract had a similar activity to loperamide when tested at 100 mg·kg-1. Loperamide, a drug widely used in the management of diarrhoeal disorders effectively antagonizes diarrhoea induced by castor oil (Niemegeers et al., 1974). Castor oil is made up of 90% ricinoleate (Mekeon et al., 1999) which is metabolized to ricinoleic acid. The active metabolite, ricinoleic acid is responsible for its diarrhoea inducing property (Gaginella and Philips, 1975). It stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin (Galvez et al., 1993).

The experimental studies in rats demonstrated a significant increase in the portal venous PGE_2 concentration following oral administration of castor oil (Luderer et al., 1980). Ricinoleic acid markedly increased the PGE_2 content in the gut lumen and also caused an increase in the net secretion of water and electrolytes into the small intestine (Beubler and Juan, 1979). In previous unpublished studies on the leaves and root bark extracts,

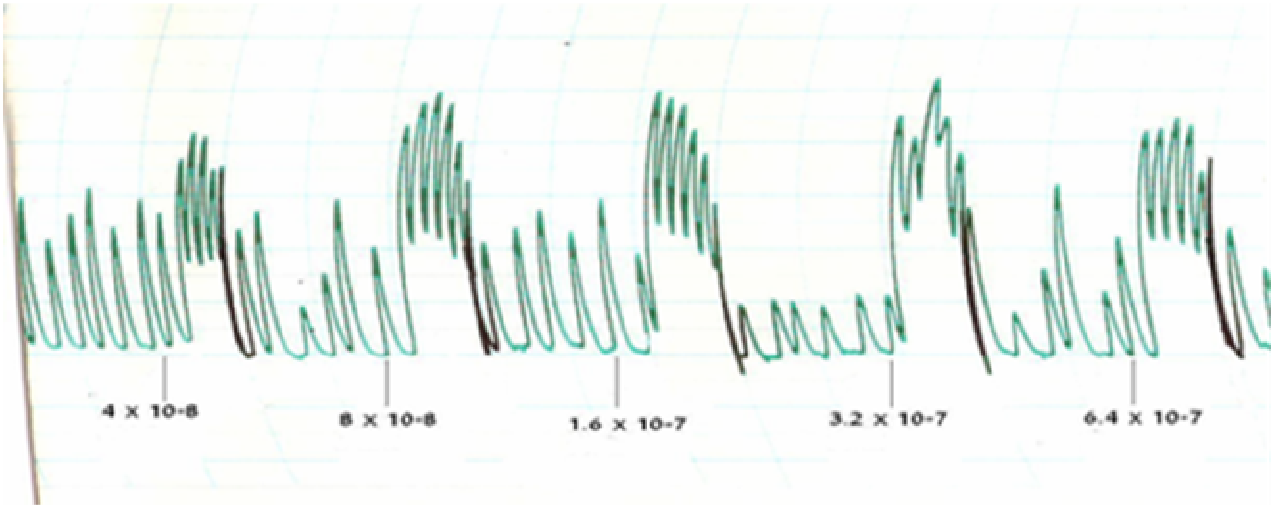


Figure 2. Effects of acetylcholine (mg·ml-1) on isolated rabbit jejunum.

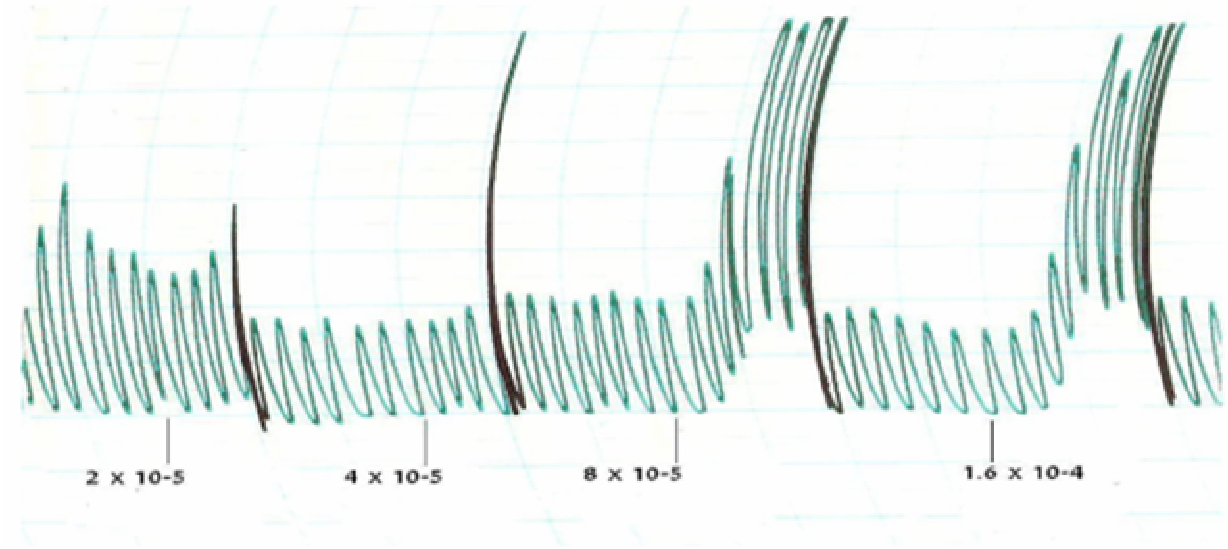


Figure 3. Effects of methanolic root bark extract of *S. virosa* (mg·ml-1) on isolated rabbit jejunum.

both extracts exhibited significant anti-inflammatory activity in the carrageenan-induced rat paw oedema and significantly attenuated acetic acid induced abdominal constriction in mice. Prostaglandins are known to mediate in part in these processes. Based on these observations, it is plausible to suggest that the anti-diarrhoeal effects of methanolic leaves and root bark extracts may be due to the inhibition of prostaglandin biosynthesis. Flavonoids are known to modify the production of cyclo-oxygenase 1 and 2 (COX-1, COX-2) and lipo-oxygenase (LOX) (Christopher et al., 1996; Haruna et al., 1997). Certain flavonoids inhibit inflammatory processes by inhibiting key enzymes involved in the synthesis of prostaglandins processes (Manthey et al., 2001). Early studies have also reported that anti-diarrhoeal activity of medicinal plants may be due to alkaloids, saponins, tannins, sterols and reducing sugar (Galvez et al., 1991; Galvez et al., 1993; Longanga et al., 2000). The anti-diarrhoeal activity of the leaves and root bark extracts is most likely due to the presence of flavonoids singly or in combination with other constituents present. The observed relaxation exhibited by the leaves extract further explains its ability to protect the mice against diarrhoea induced by castor oil. Although, the stem bark extract did not show any anti-diarrhoeal activity in the model, it is possible that its use in traditional medicine may be due to other mechanisms such as antimicrobial activity (Macauder, 1986; Capasso et al., 1988).

The results of this study justify the use of the leaves and root of *S. virosa* as anti-diarrhoeal in traditional medi-

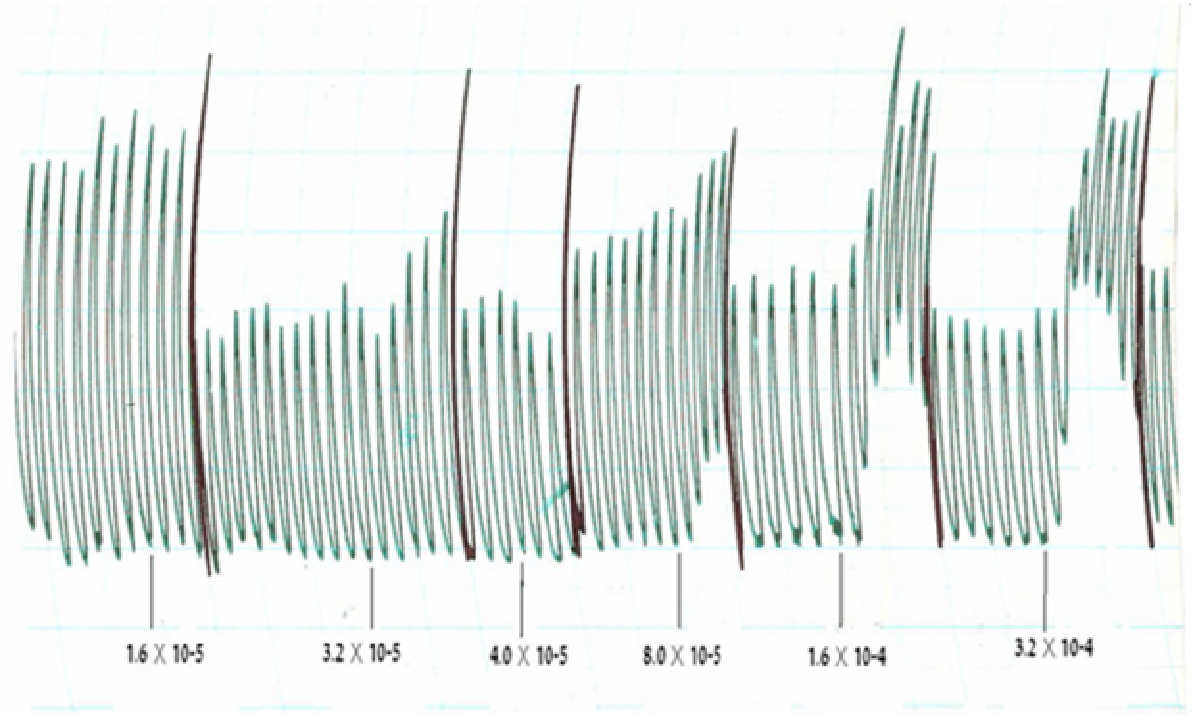


Figure 4. Effects of methanolic stem bark extract of *S. virosa* (mg·ml-1) on isolated rabbit jejunum.

Table 2. Effects of methanolic leaves, stem bark and root bark extracts of *S. virosa* on castor oil-induced diarrhoea in mice.

Treatment	Dose (mg·kg-1)	Number of mice with diarrhoea	Protection (%)
Normal saline	10 mlkg^{-1}	5/5	0.0
Leaves	50	2/5	60
	100	3/5	40
	200	3/5	40
Stem bark	25	5/5	0.0
	50	5/5	0.0
	100	5/5	0.0
Root bark	25	4/5	20
	50	3/5	40
	100	0/5	100
Loperamide	5	0/5	100

Values were considered significant when P <0.05 compared with normal saline group, $n = 5$.

cine. Further work will involve identifying the possible mechanism(s) of action of these extracts and to isolate the compound responsible for the observed effects.

ACKNOWLEDGEMENT

The authors appreciate the technical assistance of Mr. Ibrahim Adamu of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria.

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