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ANTI-ULCER POTENTIAL OF *ERYTHRINA SENEGALENSIS* DC STEM BARK

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Abstract
The anti-ulcer property of *Erythrina senegalensis* De Candolle stem bark has been studied. The anti-ulcer property was investigated using the indomethacin-induced ulcer model. Phytochemical tests were carried out to determine the secondary metabolites present in the extract. The toxicity test was done to ascertain the toxicity profile, and the effect of the extract on acetylcholine-induced contraction was investigated. The chemical tests revealed the presence of varying amounts of alkaloids, reducing sugars, glycosides, saponins, flavonoids, proteins, fats and oils. The result indicated that the extract exhibited significant ($p<0.05$) dose – dependent anti-ulcer activity at the two dose levels tested.

Keywords: Anti-ulcer, *Erythrina senegalensis*, stem bark, phytochemical test

INTRODUCTION

In recent years, there has been increased interest in potential sources of biologically active molecules. This is mainly due to widespread resistance to existing ones. Such studies involve ethnobotanical survey as well as bio-assay guided fractionation of extracts. *Erythrina senegalensis* is a tropical, perennial herb that grows up to 10 – 15 meters high, with trifoliate leaves possessing thick spiky petioles. It is an evergreen tree, leaves always available but flowers and seeds have short time of availability. It is locally known as "Nte", and is used in traditional medicine to cure several diseases. In 2005, a survey was performed in three different regions in Mali (West Africa) to collect information on the uses of seven plant species used in traditional medicine. *E. senegalensis* was included in the study (Togola *et al.*, 2005). Several diseases and symptoms were reported by the healers against which *E. senegalensis* was used. The main reported ones were malaria, jaundice, infections, gastrointestinal disorder (gastric ulcer, diarrhea, constipation), amenorrhoea, dysmenorrhoea, sterility, onchocerciasis, body pains (chest pain, back pain, abdominal pain, headache and body weakness).It was also reported to have wound healing and contraceptive properties. Plant parts used include roots, stem barks, leaves, flowers and seeds. It has been used in treating cough, typhoid, and fever (Mann *et* al., 2003). It's significant ($p<0.05$) analgesic and anti-inflammatory properties have been reported (Saidu *et* al., 2000). It is also claimed to have anti-snake venom property (Ozioko, 2009). It's antimicrobial, antiviral, antiparasitic (Taylor *et al.*, 1986; Dastidar *et al.*, 2004; Salvatore *et al.*, 1998), as well as anti-tumor and anti-leukaemic properties (Fomum *et al.*, 1985; Fomum *et al.*, 1987; Matsuda *et al.*, 2007) haven been studied.

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Materials and Methods

Collection and identification of plant material

The stem bark of *E. senegalensis* was collected from Umudiale, Ohom Orba in Udenu Local Government Area of Enugu State, Nigeria. The material was authenticated by Mr. Alfred O. Ozioko, a taxonomist with the Bioresources Development and Conservation Programme (BDCP) Centre, Nsukka.

Animals

These include the in-bred albino mice of either sex (20 – 30 g), adult albino rats of both sexes (140 -210 g) and Guinea pigs (320 -430 g), obtained from the animal house, Department of Zoology, University of Nigeria, Nsukka. The mice and rats were fed on standard pellets marketed by Pfizer Livestock Feeds (Nigeria) Limited. The Guinea pigs were fed on local grass, *Panicum maximum.*

Drugs and Chemicals

The following reagents were used as procured from their manufacturers: Indomethacin (Shanghai Med and Health, China), cimetidine (Synmedic Lab. India), acetylcholine hydrochloride (Sigma, St. Louis, MO, USA), Tween 85 (Janssen, Belgium), Glucose (M & B, England), methanol (Merck, Germany), tetraoxosulphate IV acid (Merck, Germany), ethanol (BDH Chemical Ltd Poole, England), ammonia (Merck, Germany), hydrochloric acid (Merck, Germany), potassium chloride (Hopkins and William, England), sodium carbonate (Reidel-De Haen Ag. Germany).

Preparation of methanol extract

The stem barks were dried under the sun and reduced to coarse powder using Hammer mill. A 30 g quantity of the powdered bark was extracted with 250 ml of methanol using cold maceration technique for 24 h with occasional shaking. The filtrate was concentrated to dryness in a rotary evaporator under reduced pressure.

Phytochemical tests

These tests for the detection of secondary metabolites were performed on the extract following standard procedures (Evans, 1996; Harborne, 1973).

Whole animal experiment

Acute toxicity test

The acute toxicity profile of the extract was assessed using the method described by Lorke (1983).

Test for anti-ulcer property

In this study, gastric ulceration was induced in the experimental animals with indomethacin as described by Urisheani *et al.,* (1979).

Two levels of the extract were employed in the study. Twenty rats, divided into four groups of five animals each were used in this model. Group one was given 5 ml/kg body weight of distilled water and served as negative control; group two received 100 mg/kg body weight of cimetidine and served as positive control, while groups three and four were administered 200 and 500 mg/kg body weight of the extracts respectively. Thirty minutes later, ulcer was induced by administering 30 mg/kg body weight of indomethacin to the rats. All administrations were by oral route. After 8 h, the animals were sacrificed. The stomachs were removed and cut open through the curvature. The stomach was washed under a stream of running water and pinned flat on a cork board for examination using a hand lens. Erosions formed on the glandular portions of the stomach were counted, and each given a severity rating on a 1 – 3 scale based on the diameter of the ulcer and the ulcer index was calculated. The severity ratings were as follows: **1** $\leq$ 1 mm; **2** ›1 mm $\leq$ 2 mm, **3** $\geq$ 2 mm (Suzuki *et al.*, 1976) .The percentage ulcer protection was also calculated for the animals. The overall total divided by a factor of 19 was

designated as the ulcer index (UI) for that stomach.

RESULTS

Table 1: Result of phytochemical tests

Chemical constituent tested	Observation	Inference
Alkaloids	Creamy precipitate (pot), orange ppt, reddish brown ppt. and yellowish ppt.	+
Reducing sugar	Brick red ppt	++
Saponins	Brick red ppt, haemolysis of red blood cells, formation of stable froth	++
Glycosides	Brick red ppt.	++
Flavonoids	Yellowish colour in the ammoniacal layer	+
Proteins	White ppt in Millon's test, yellow colour which changes to orange in Xanthoproteic reaction	+++
Fats and oil	Translucent mark on the filter paper	+++
Tannins	Milky to orange ppt; reddish brown to orange ppt	+

+ = Present, ++ = moderately present, +++ = abundantly present.

The phytochemical analysis of the extract revealed the presence of alkaloids, saponins, tannins, glycosides, flavonoids, reducing sugars, proteins, fats and oil. Proteins and fats/oil are abundant (Table 1.)

Table 2: Result of indomethacin induced ulcer

Drug/substance	Dose (ml/kg or mg/kg)	Mean ulcer index ± SD (mm)	Percentage (%) ulcer protection
Distilled water	5	8.40 ± 0.10	-
Cimetidinne	100	2.53 ± 0.15	69.88
Extract	200	5.53 ± 0.15 *	34.17
Extract	500	2.93 ± 0.11 *	65.12

*significant P< 0.0.5; n = 5.

All the animals that received distilled water developed severe ulceration with mean ulcer index of 8.40 ± 0.10 mm. Those that received cimetidine had ulcer index of 2.53 ± 0.15 mm, while those that received the two doses of the extract, had ulcer indices of 5.53 ± 0.15 mm and 2.93 ± 0.11 mm respectively.

DISCUSSION

The acute toxicity test revealed that even at 5000 mg/kg, the extract did not show any sign of toxicity in mice. This is an indication of high safety profile as substances with LD_{50} above 5000 mg/kg have been classified as being generally safe (Lorke, 1983; Loomis, 1978).

The anti-ulcer study demonstrated that the extract at the two dose levels employed exhibited significant ($p<0.05$) anti-ulcer property in indomethacin-induced ulcer (Table 2). The activity was dose dependent. Indomethacin and related non-steroidal anti-inflammatory drugs induce gastric damage by inhibition of prostaglandin

synthesis, and chronic administration of these drugs is associated with blood loss from the upper gastrointestinal tract, ulcerogenesis, and aggravation of pre-existing peptic ulcer (McCarthy, 1990; Oren and Lugumky, 1994; Dharmani and Palit, 2006). The ulcer inhibitory potency of the extract is an indication of strong cytoprotection and ability to counteract inhibition of prostaglandin synthesis induced by indomethacin.

The abundant proteins in the extract may be partly responsible for the anti-ulcer property. This substance enhances the viscosity of the extract, thereby increasing the viscosity of gastric and intestinal mucus, hence, increased mucosal barrier to gastric acid secretion. It is this impervious protective pellicle on the lining of gastrointestinal walls that will help in resisting the attack of proteolytic enzyme (John and Onabanjo, 1990).

Most of the compounds studied have been isolated from organic extracts of the plant material while traditional healers use water extracts to cure their patients. One may ask how these non-water soluble compounds could be the active principles in phytomedicines normally given as water extract. One probable explanation might be the low minimal concentration (µg range) of these compounds to give effect. Another could be that co-extraction can take place as plant material often contain compounds like saponins that will enhance the solubility of an otherwise non-soluble compound if present in the same mixture. Literature data are far from giving information of all the activities claimed for this plant. Several traditional uses are still to be explored both *in vitro* and *in vivo* bioassay.

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