

## Phytochemical Analysis and Acute Toxicological Study of *Erythrina senegalensis* Ethanolic Leaf Extract in Albino Wistar Rats

Nnama TN\*, Asomugha AL, Asomugha RN, Umeasalugo KE and Mgbemena IO

Nnamdi Azikiwe University of Nnewi, Anambra, Nigeria

\*Corresponding author: Nnama TN, Nnamdi Azikiwe University of Nnewi, Anambra, Nigeria, Tel: +234 808 730 9304; E-mail: nnamatc@yahoo.com

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### Abstract

**Objective:** *Erythrina senegalensis* is used to cure wide range of illnesses such as malaria, gastrointestinal disorders, fever, dizziness, asthma. Given its widespread usage, the study sought to investigate the phytochemical analysis and acute oral toxicity (LD50) of *E. senegalensis*.

**Materials and methods:** Fresh mature leaf samples of *E. senegalensis* were collected and phytochemical analysis was carried out appropriately. The acute toxicity study of the leaf extract was determined using modified Lorke's method. Thirteen adult Wistar rats of both sexes were acclimatized, nine of which were used for the first phase of treatment, and the other four were used for the second phase of acute toxicity testing, while being closely monitored for mortality.

**Results:** Alkaloids, saponins and flavonoids, were found in moderate quantities; tannins and terpenoids were found in trace amounts, while cardiac glycosides and steroids were not found. The acute oral toxicity of *E. senegalensis* was greater than 5000 mg/kg of the experimental rats (LD50>5000 mg/kg).

**Conclusion:** *E. senegalensis* possesses many useful phytoconstituents that contribute to the potency of leaf extract and which made it useful in treatment of many diseases, and that *E. senegalensis* ethanolic leaf extract has low toxicity in rats, especially when administered orally.

Alkaloids; Flavonoids; *Erythrina senegalensis*; Phytoconstituents

### Introduction

Plants usually synthesize aromatic substances, which in many cases serve as the molecules of plant defense against predation by microorganisms, insects, and herbivores. Several of the plants possess medicinal properties. *Erythrina senegalensis* is one of such plants and belongs to the family Fabaceae. It is a thorny shrub used as vegetable, with common names that include 'Minjirya' (Hausa, Nigeria) [1]. Some phytochemicals such as tannins, glycosides, alkaloids, and others have been identified in the *E. senegalensis* stem bark [2]. The leaf has also been shown to contain some phytochemicals [3].

The stem bark and root extracts of *Erythrina senegalensis* have been reported in Nigerian studies to have antimalarial, analgesic, anti-inflammatory, and anti-bacterial actions [4], while in other African studies it has been used to treat female secondary sterility, asthma, liver disorders, gastrointestinal disorders, fever, jaundice, nose bleeding, urinary bilharziasis, eye infections, and body pain [5].

Given the continual consumption of *E. senegalensis* for the maintenance of health and in management of several diseases, and secondly the paucity of information on the leaf extract, the present study is aimed at evaluating the phytochemical constituents and acute oral toxicity (LD50) of the leaf extract of *E. senegalensis*.

### Materials and Methods

#### Ethical approval

Necessary approval was sought and obtained from the Ethical Committee, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus.

#### Collection and authentication of the plant material

Fresh mature leaf samples of *E. senegalensis* were collected in Nnewi, Anambra State, and botanical identification of the plant was done by Mr. Egboka Tochukwu of the Department of Botany, Nnamdi Azikiwe University, Awka.

#### Plant preparation

All the samples of *E. senegalensis* were thoroughly rinsed with running tap water and distilled water before being air-dried at room temperature for 30 days. Then, the plant sample was pulverized to dry powder using an electric grinder into minute pieces and the extract was soaked in absolute ethanol for 4 days with frequent agitation at room temperature. The extract was filtered with Whatman paper No.1 and the residue of fine powder was then re-soaked with a fresh portion of ethanol twice for four days each time at room temperature. The filtrate was concentrated under reduced pressure in vacuum at 45°C

and evaporated to dryness on a rotary evaporator (Model 342/7, Corning Ltd.). The yield of the extract was 17.1% based on dry weight.

### Phytochemical screening

Using a standard method according to Brain and Turner (1975), the crude extracts were subjected to various phytochemical screening for the presence or absence of the following plant secondary metabolites: alkaloids, saponins, flavonoids, tannins, cardiac glycosides, steroids and terpenoids.

**Test for alkaloids:** 2 ml of the extract will be added to 2 drops of Wanger's reagents (solution of iodine and potassium iodide). Precipitation and reddish brown colour will indicate the presence of alkaloids.

**Test for saponins:** 5 ml of extract will be diluted with 20 ml of water and vigorously shaken thoroughly. The formation of emulsion will indicate the presence of saponins.

**Test for flavonoids:** 4 ml of the extract will be shaken with 1 ml of dilute ammonia solution. The yellow colour in the ammoniacal layer will indicate the presence of flavonoids.

**Test for tannins:** Few drops of ferric chloride will be added to 3 ml of the extract. A greenish black precipitate will indicate the presence of tannins.

**Test for cardiac-active glycosides:** 0.2 g of the extract will be dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution followed by the addition of 1 ml of concentrated sulphuric acid. A brown ring at the interface will confirm the presence of cardiac glycoside.

**Test for steroids:** 5 ml of the extract will be extracted with 2.5 ml of chloroform using a separating funnel. Sulphuric acid will be added to form a lower layer. A reddish brown interface will show presence of steroids.

**Test for terpenoids:** 5 ml of the extract will be extracted with 2.5 ml of chloroform using a separating funnel. A 0.5 ml of the chloroform extract will be evaporated to dryness on a water bath. The residue will be reconstituted with 3 ml of concentrated sulphuric acid and heated for 10 min on a water bath. A grey colour will indicate the presence of terpenoids.

### Acute toxicity (LD50)

The acute toxicity study of the leaf extract was determined using modified Lorke's method (1983).

13 Albino wistar rats weighing 98-150 g, were acclimatized for one week, kept in plastic cages at room temperature and fed pelleted diet and water throughout the experimental period.

After acclimatization, the rats were treated in two phases. In the first phase, a total number of 9 adult male and female wistar rats (3 rats per group) received orally in a single administration, *E. senegalensis* extract at the doses of 10, 100, and 1000 mg/kg respectively. The animals were observed for mortality for a period of 14 days post treatment.

After phase 1, there was no mortality in the animals, so the second phase was carried out with the use of a total number of 4 animals. They were grouped into four groups of one animal per group and were dosed orally with *E. senegalensis* ethanolic leaf extract at dose levels of 2000, 3000, 4000 and 5000 mg/kg body weight respectively. The animals were constantly monitored for the next 2 h and over a period of 24 h for mortality.

## Results

### Phytochemical analysis

Sample	Alkaloid	Saponin	Flavonoid	Tannin	Cardiac Glycoside	Steroid	Terpenoid
<i>Erythrina senegalensis</i>	++	++	++	+	--	--	+
-- Absent, + Trace/Mildly present, ++ Moderately present							

**Table 1:** Quantitative and qualitative phytochemical analysis of *E. senegalensis* leaf extract.

### Acute toxicity testing (LD50)

No mortality was observed in any of the animal groups administered the leaf extract at dose levels of up to 5000 mg/kg body weight. The result showed that the acute toxicity of the *E. senegalensis* was greater than 5000 mg/kg body weight (Table 1).

## Discussion

The study revealed that following oral administration of 5000 mg/kg of the *E. senegalensis* ethanolic leaf extract, there was no acute toxicity or instant death in any of the rats treated with acute dose during the observation period. The median lethal dose of the present study was greater than that of Obidah et al. and Tepongning et al. [1,6]. Who reported the LD50 to be greater than 4000 mg/kg and 2000 mg/kg, respectively? Thus, the present study suggested that oral administration of the extract could be considered safe.

In the present study, phytochemical analysis of the ethanolic leaf extract of *E. senegalensis* showed the presence of alkaloids, saponins and flavonoids in moderate quantities. These substances, especially flavonoids were reported to have antimicrobial [7], antibacterial [8], and HIV-inhibitory activities [9]. The alkaloids are known to decrease blood pressure, and are thus responsible for the hypotensive action of *E. senegalensis* [7]. Plants containing saponins are believed to have antioxidant, anti-cancer, anti-inflammatory and anti-viral properties [10]. Other studies reported the presence of alkaloids, flavonoids, polyphenols and reducing sugars. The presence of such biological molecules attempt to justify some of the ethno-medicinal applications of *E. senegalensis* [11-16].

Tannins and terpenoids were found in trace amounts in the present study [2], who also demonstrated the presence of terpenes in plants from the *Erythrina* genus. Nico also reported that tannins tested positive in the *E. senegalensis* leaf extract, amongst other compounds.

Cardiac glycosides and steroids were not present in the phytochemical analysis of the present study [2]. Who reported the presence of cardiac glycosides and steroids in their individual analyses of *E. senegalensis*.

From the reviewed studies, it is understood that the distribution of these biological molecules differs in the part of the plant and it may also be as a result of variation in geographical location.

## Conclusion

The plant *Erythrina senegalensis*, possesses phytochemical constituents such as saponins, terpenoids, flavonoids, tannins and alkaloids in different quantities in leaf extracts, responsible for the bio-activities of the plant. These useful phytoconstituents contribute to the potency of the leaf extract and has made it useful in treatment of many diseases. The acute toxicity tests result of >5000 mg/kg suggested that oral consumption of the leaf extract could be considered safe and would not cause mortality.

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