

## EVALUATION OF THE LETHAL DOSE OF THE METHANOL EXTRACT OF RHIZOPHORA RACEMOSA LEAF USING KARBER'S METHOD

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### ABSTRACT

**Aim:** To evaluate the median lethal dose (LD<sub>50</sub>) of the methanol extract of *Rhizophora racemosa* leaf commonly used for toothache and dysmenorrhoea in the Niger Delta Area of Nigeria.

**Methods:** The modified arithmetic Karber's method by Aliu and Nwude was used. Various doses of the extract were administered to adult albino rats through intraperitoneal route and the LD<sub>50</sub> was calculated.

**Results:** The result revealed that the LD<sub>50</sub> of the methanol extract was 1583.33 mg/kg which is within safe level of consumption.

**Conclusion:** This research therefore provides a scientific evidence that the traditional medicinal use of this plant is safe within the dose limit established.

**Keywords:** LD<sub>50</sub>, *Rhizophora racemosa*, Mangrove plant, Karber's method, Intraperitoneal.

### INTRODUCTION

Mangroves are a diverse group of predominantly tropical trees, shrubs, palms or ground ferns that generally exceed two feet in height, and which grow primarily in the marine intertidal zone. Mangrove forest is found in silt-salt, saline, (brackish water) habitats worldwide, generally along large river deltas, estuaries and coastal areas. Mangroves are evergreen trees and shrubs that are well adapted to their salt and swampy habitats by having breathing roots (pneumatophores) that emerge from the oxygen-deficient mud to absorb oxygen. *Rhizophora*

*racemosa* G.F.W. Meyer is a species of mangrove plant of the family of Rhizophoraceae. It is found commonly in the Niger Delta region of Nigeria and along the West African coastline (Gerard et al., 2004). In most tropical countries of Africa, high cost of Western medicine and poor health delivery as well as resurgence of phytomedicine have necessitated reliance on the use of traditional plant medicine in the treatment of ailment, often without consideration of the toxic effects that these plant products cause to the body. Mangrove plants have been used in folklore medicine, and extracts from mangrove species have been shown to have inhibitory activity against human, animals

and plant pathogens. Several species of mangrove produce bioactive compounds that may control microbial growth (Ishibashi et al., 1993, and Wu et al., 1997). Several compounds like alkaloids, phenols, steroids and terpenoids have been found and characterized from mangrove and have toxicological, pharmacological and ecological importance (Bandaranayake 1998 and Bandaranayake, 2002). Thus in light of the indiscriminate use of plant medicine without prejudice to the toxic effects of plant extracts of which the leaf of mangrove is one, the present investigation aims to determine the mean lethal dose (LD<sub>50</sub>) of leaf extract of *Rhizophora racemosa* collected from Dodo River in Bayelsa State, Nigeria.

## MATERIALS AND METHODS

### Plant Collection

Fresh young leaves of *Rhizophora racemosa* were collected in June 2012 from Dodo River around Letugbene community (4°5'0"N, 5°33'0"E), in Ekeremor Local Government Area of Bayelsa State of Nigeria.

### Preparation of Plant Materials and Plant Extract

Crude extract of the leaf was prepared according to the procedure described by Kudi et al., (1999) and Samy and Ignacimuthu, (2000) with little modification on the process of drying by using rotary evaporator instead of oven. Fresh leaves of *Rhizophora racemosa* were cleaned with tap water and air-dried under shade at room temperature for 3 weeks. The dry leaves were pulverized with an electric blender to give a fine powder. The powder was dissolved in methanol and left for 72 hours, filtered using Wattman filter paper and the filtrate evaporated to dryness using a rotary evaporator. The dry extract was stored in a refrigerator at a temperature below 4°C. The preparation was reconstituted by dissolving 500mg of the dry extract in 100ml distilled water for use on each day of the experiment (Kudi et al., 1999).

### Animals

Mature, healthy, Albino rats of both sexes weighing between 200-250g were obtained from the Animal House of the University of Port Harcourt. The rats were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities for pelleted food. The animals were maintained in 12 hours light and dark cycle at 28°C ± 2°C in a well ventilated animal house under natural conditions and acclimatized to pharmacology laboratory conditions for 2 weeks prior to the commencement of the experiment. The animals were fed with a standard diet of growers mash supplied by Gee Pee Nigeria Limited and had access to clean drinking water. Saw dust was used as bedding material and changed twice a week.

### Toxicity Range Finding Test

Before the actual LD<sub>50</sub> determination, a pilot study was conducted using ten albino rats (200 - 220g) mainly to select the dose ranges for the subsequent toxicity screening. The extract was administered intraperitoneally to five pairs of rats in ascending and widely spaced dose ranges of (10, 100, 500, 1000 and 2000 mg/kg body weight). The treated rats were observed for signs of toxicity and death over a period of 24 hours. The largest dose that did not kill any rat was noted, as well as the smallest dose that killed all the animals.

### Acute Toxicity Screening

This study was done to determine the median lethal dose (LD<sub>50</sub>) of the methanol extract of *Rhizophora racemosa*. A total of thirty-six Albino rats of both sexes were used for this study.

### Experimental Procedure

The Albino rats of both sexes were divided into six groups (A-F) of six animals each. The groups were given different doses of the extract by intraperitoneal route as follows: Group A: 1000mg/kg, Group B: 1200mg/kg, Group C: 1400mg/kg, Group D: 1600mg/kg, Group E: 1800mg/kg, Group F: 2000 mg/kg. The animals were then observed for 24 h for signs and symptoms of toxicity and death. The LD<sub>50</sub> of the extract was calculated using the arithmetic method of Karber as modified by Aliu and Nwude (1982).

The LD<sub>50</sub> was calculated using the formula:

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

Where LDy = Highest dose (LD<sub>100</sub>)

N = Number of animals per group

Dd = Dose difference

Md = Mean dead

LD<sub>50</sub> = Dose that killed 50% of test animals

LD<sub>100</sub> = Dose that killed 100% (all) the test animals

## RESULTS

None of the animals in Group A showed any clinical or behavioural changes throughout the observation period. However, depression, weakness and loss of appetite in the first 5hr were observed in Groups C, D, E and F animals that were treated with the higher doses of the extract. All animals in Group A were active all through the study. In Group B three of the animals suffered depression, weakness and loss of appetite and they eventually died after 48 hours. The LD<sub>50</sub> of the G. plant extract was determined as described by Aliyu and Nwude (1982) (Table 2 and calculated to be 1583.33mg/kg body weight

Table 1: Toxicity Test

Group	No. per group	No. of Death	Weights (g)	Dose (mg/kg)
1	2	0	200	10
2	2	0	210	100
3	2	0	215	500
4	2	0	220	1000
5	2	2	220	2000

Table 2: Determination of LD<sub>50</sub> of methanol extract of *Rhizophora racemosa* in rats

Dose	No of Death	Mean Death	Dose Diff	Mean Death x Dose Diff.
1000mg/kg	0	0	0	0
1200mg/kg	1	0.5	200	100
1400mg/kg	2	1.5	200	300
1600mg/kg	2	2	200	400
1800mg/kg	4	3.5	200	700
2000mg/mg	6	5.0	200	1000
<b>Sum (Σ)</b>				<b>2500</b>

Applying the formula shown above, the LD<sub>50</sub> could be calculated as follows;

$$LD_{50} = 2000 - \frac{2500}{6} = 1583.33 \text{mg/kg.}$$

The arithmetic method of Karber as modified by Aliu and Nwude (1982) showed that the median lethal dose (LD<sub>50</sub>) of methanol extract of *Rhizophora racemosa* in rats was 1583.33 mg/kg.

## DISCUSSION

Acute toxicity study is one of the initial screening experiments carried out with all compounds in the search for new drugs. The LD<sub>50</sub> determination is one of the methods used in acute toxicity studies and it is that dose which kills 50% of the test group animals. LD<sub>50</sub> values obtained also depend on the route of administration of the drug. Usually the values are found to increase with the following sequence of routes: intravenous, intraperitoneal, subcutaneous and oral (Turner, 1965). Information gathered from the acute toxicity study can have several applications such as providing initial information on the mode of toxic action of a substance, helping arrive at a dose of a new compound or dose determination in animal studies (Akhila et al., 2007). The intraperitoneal route was used to determine the LD<sub>50</sub> in this study. The LD<sub>50</sub> value obtained for the methanol extract of *Rhizophora racemosa* in this study was 1583.33 mg/kg. It has been suggested that any substance with an intraperitoneal LD<sub>50</sub> of above 1000 mg/kg should be regarded as safe (WHO, 1986; Clarke and Clarke, 1975). It can therefore be inferred that, the plant under study is of low toxicity and safe. Although, the extract can be deduced to be safe, some dose dependent toxic manifestations were observed following intraperitoneal administration. This may be due to the effect of one or more of the chemical constituents present in the extract. For example, tannins which are found in adequate quantity in *Rhizophora racemosa* (Gordon and Godwill, 2007; Bandaranayake, 1994, 1998) have been implicated in decreased appetite and reduced activity in rats (Hotellier and Delaveau, 1975; Nwafor et al., 1995).

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