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Full Length Research Paper

Bioactivity of crude extracts of *Euphorbia kamerunica* Pax using brine shrimp (*Artemia salina*) lethality assay

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Medicinal plants are gaining recognition worldwide in the treatment and control of diseases. In advance nations a good number of plants are screened for their pharmacological activities but less than 25% of plants native to Africa which are been used to treat diseases have been evaluated. In the present study hexane, diethyl ether, ethyl acetate, methanol and aqueous extracts of *Euphorbia kamerunica* Pax were screened for their cytotoxicity and lethality using the brine shrimp (*Artemia salina*) assay. Three concentrations (10, 100, 1000 ppm) of each extract were applied in the bioassay and the LC_{50} determined. The least LC_{50} value of 0.00 was obtained for hexane extract and the highest value of 13.87 was obtained for aqueous extract using the Finneys probit analysis which indicates high lethality of the extracts and presence of potent cytotoxic compounds in the plant extracts.

Key words: *Euphorbia kamerunica*, *Artemia salina*, cytotoxicity, insecticidal compound.

INTRODUCTION

Medicinal plants are gaining increasing attention in the world in the health care delivery system. Ethno- botanical knowledge of traditional uses of plants has help in identification and isolation of active compounds that are of benefit to mankind. China, India and Africa have varieties of medicinal plants which their large population depends upon for their health care (Farnsworth and Soejarto, 1991). Knowledge of medicinal plants used in traditional health care has aroused the interest of pharmaceutical companies into research and developmental programs in the pursuit of novel drugs.

The *Euphorbiaceae* are family of poisonous plants that have both medicinal and hazardous implications. The compounds responsible for the toxic properties of this family had been identified to be lectins and esters of certain diterpenes alcohols, with phorbol derivatives, tiglane, dephnane and ingenene diterpene esters (Dagane et al., 1992; Gundidza et al., 1993; Neuwinger 2004; Evans and Taylor, 1983; Milillo et al., 1993). The toxic diterpenes esters cause inflammation of the skin, with reddening and formation of edematous swellings. On geting to the eyes, these poisonous lattices cause inflammation of the cornea and conjunctiva which sometimes leads to blindness. When the extracts of this plants are taken internally, poisoning with severe gastroenteritis, vomiting and colicky diarrhea were observed (Frohne and Pfander, 1984). Many species of the family are used in folk medicine as drugs or as raw materials for medicinal preparations (Wiriyachitra et al*.,* 1985), in agriculture and horticulture (Hecker et al*.,* 1979) and as alternative Industrial resources (Calvin, 1980).

Euphorbia kamerunica is a cachus-like plant that prefers dry, rocky country. Its distribution extends from Guinea to Cameroon (Keay, 1989). Fishermen commonly use this plant for fishing in fresh water bodies in the region. *E kamerunica* contains skin irritant, co-carcinogenic principles and other constituents responsible for health hazards to both human and grazing livestock (Gundidza et al*.,* 1993).

Fai and Fagade (2005) determined the toxicity of *E. kamerunica* on *Oreochromis niloticus* and the compounds responsible for this toxicity identified (Dagane et al., 1992; Gundidza et al., 1993; Neuwinger 2004; Milillo et al., 1993). The brine shrimps lethality assay of this plant is yet to be determined. Brine shrimp assay is an indicator used in determining the cytotoxicity and insecticidal properties of compound and it is very useful preliminary tool for isolation of bioactive compounds from plant

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Table 1. Bioactivity of *Euphorbia kamerunica* extracts using brine shrimp (*Artemia salina*) lethality assay.

Extract	Conc. 1000 ppm	% mortality	Conc. 100 ppm	% mortality	Conc. 10 ppm	% mortality	LC_{50},µg/ml, 24h
HX	0,0,0	100	0,0,0	100	0,3,0	90	0.00
DEE	0,0,0	100	0,2,0	93	1,2,2	83	0.32
EA	0,0,0	100	3,0,1	86	4,2,2	73	1.61
Me0H	0,0,0	100	1,2,2	83	4,3,2	70	3.01
AQ	0,0,0	100	3,2,2	76	5,5,6	46	13.87

Key: HX = Hexane; DEE = Diethyl ether ; AQ = Aqueous; EA = Ethyl acetate; Me0H = Methanol.

extracts (Vanhecke et al*.,* 1981; Sleet and Brendel, 1983; Sam 1993). Based on the wide application of *E. kamerunica* in traditional medicine, it is necessary to confirm the safety or otherwise of its extracts consumption. The presence of cytotoxic and insecticidal compounds from result of brine shrimp assay is also inferred. The effects of different extracts on *Artemia salina* (Brine shrimp) were studied under 24 h observatories.

MATERIALS AND METHODS

Plant collection and authentication

E. kamerunica Pax succulent branches were collected from the Botanical gardens of the University of Ibadan, Ibadan, Oyo State, Nigeria. The plant sample was identified and authenticated at the herbarium of the Department of Botany and Microbiology, University of Ibadan. A voucher specimen with number UIH 22278 was deposited in the herbarium.

Plant extraction

The fresh stem of *E. kamerunica* was cut and pounded using mortar and pestle. Cold extraction was carried out by pouring distilled water on 750 g of the sample and allowed to stand for 48 h. The water is decanted and fresh water added again and left for another 24 h. The two extracts are combined and dried under rotary. Hot extraction of other batches of the plant was done using Soxhlet with redistilled hexane, diethyl ether, ethyl acetate and methanol (AOAC, 2005). Extracts were concentrated under rotary and stored in samples bottles in the refrigerator prior the assay.

Brine shrimp lethality assay

The modified method of Krishnaraju et al . (2005) was employed in this study. Brine shrimps (*Artemia salina*) eggs were hatched in an improvised hatchery made of plastic dish filled with natural sea water from Bar Beech, Lagos. The hatchery with the eggs was placed in light for 48 h.

The stock solution was prepared by dissolving 0.02 g extract in 2 ml dimethyl sulphoxide (DMSO). 1.8 ml of the brine was added to 0.2 ml of the stock to give 1000 ppm solution. Subsequent concentrations of 100 and 10 ppm were obtained from this. Ten *nauplii* were drawn through a glass capillary and placed in test tube containing 4.0 ml of brine solution and 0.5 ml of plant extract concentration and made up to 5 ml with brine solution. Tests for each concentration were done in triplicate. A control experiment containing 5 ml of brine solution with two drops of DMSO and ten *nauplii* was set along side. The experiments were maintained at room temperature for 24 h under light and the surviving larvae counted.

Statistical analysis

The lethality of the extracts was calculated from the mean survival larvae of extract treated tubes and control using the arithmetic graphic method of Reish and Oshida (1986) and Finney's Probit analysis was used to determine the LC_{50} of each extract. The toxicity is expressed by this LC_{50} which is defined as concentration of extract that kills 50% of the shrimps within 24 h. Percentage mortality was calculated as number of dead *nauplii* divided by initial number of *nauplii* (10) multiply by 100.

$$\% \text{ mortality} = \frac{\text{No. of dead } nauplii}{\text{Initial no. of live } nauplii} \times 100$$

Toxicity of the extract against the brine shrimps was determined by a statistically significant decrease in the survival of brine shrimps exposed to plant extract relative to the survival of shrimps in the control and Statistcal Chi -square (χ^2) test was employed to check if there is any significant difference between the observed and expected mortality with the hypothesis, H_0: Observed mortality is same as the expected mortality of five (5) extractants, H_1: not H_0 at = 0.05.

RESULTS AND DISCUSSION

The bioactivity of different extracts of *E. kamerunica* against *A. salina* is shown in Table 1. At 1000 ppm for all the extracts, the ten brine shrimps used in the triplicate tests died after 12 h, which gave 100% mortality and by implication 1000 ppm of all the extracts are highly toxic to the brine shrimps.

At 100 ppm, 100% mortality was recorded for hexane extract. The remaining extract showed mortality between 76-96% which is still very high. At 10 ppm, hexane extract still showed the highest percentage of mortality, it has 90% mortality while aqueous had the least mortality, 46%. This goes to say that hexane extract has the highest toxicity of all the extracts, even at 10 ppm, the LC_{50} value was 0.00. Diethyl ether extract is next in toxicity with LC_{50} value of 0.32 while ethyl acetate and methanol has 1.61 and 3.01, respectively. Aqueous extract had the least mortality with high LC_{50} value of 13.87. The result of

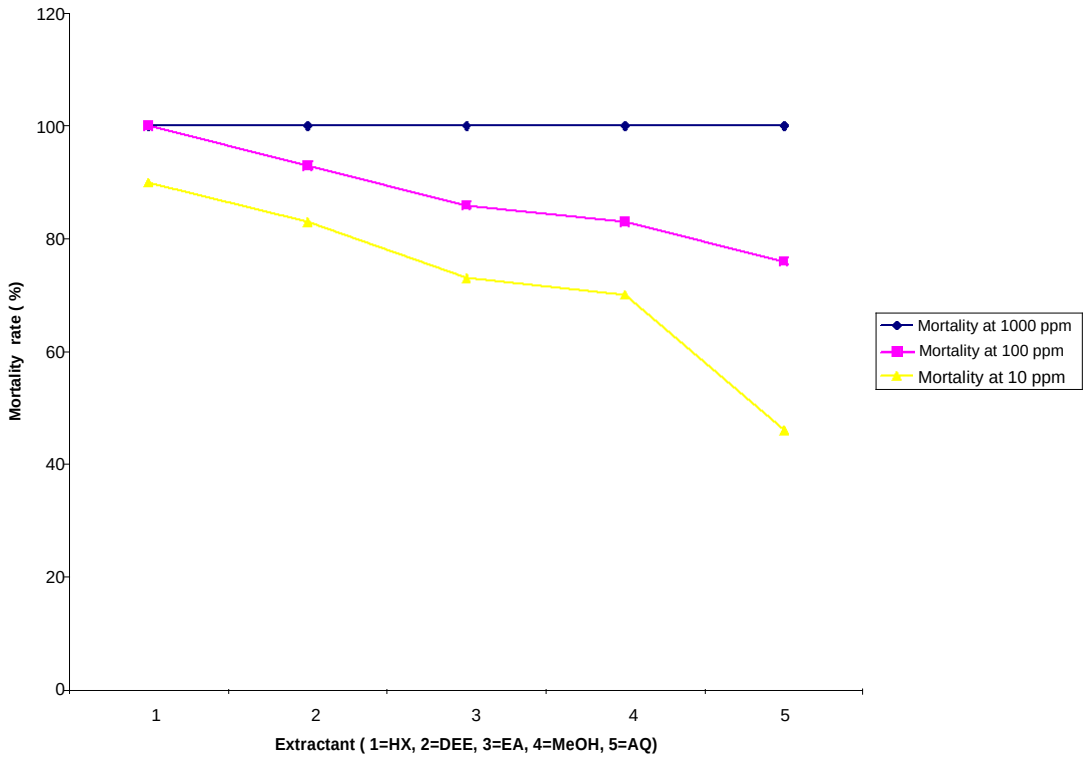

Figure 1. Graph of mortality at different values of concentration.

this study showed that toxicity activity of the plant extracts decreases with increase in polarity of solvent of extraction. This observation can be rationalized in terms of polarity of compounds being extracted by each solvent and in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in different solvent media used in this assay. Brine shrimp lethality assay is a rapid inexpensive and simple bioassay for testing plant extracts bioactivity, the result in most cases correlate with cytotoxic and antitumor properties of the plant.

Table 1 also show that hot extraction is more efficient in extracting the bioactive component than cold when the LC_{50} of the other four extracts is compared to that of aqueous extract done in the cold. Although all the five crude extracts of *E. kamerunica* displayed high toxicity values with hexane extract having the least LC_{50} value (0.00 µg/ml), while the aqueous extract had the highest (13.87 µg/ml) . The difference in the LC_{50} of aqueous extract which was done in cold to that of the organic solvents that were done in hot is much. This simply means that apart from solvent affinity of the active compounds, the method of extraction employ is also important. The traditional healers employ the two me-thods in extraction based on their experience in handling the plants for the desire purpose and are usually done in aqueous and alcohol.

The graph in Figure 1 shows that as the polarity of solvent of extraction increases, brine shrimp toxicity decreases. This implies that most of the potent cytogenic compounds in this plant have affinity for non- polar solvents and are more effectively extracted by these solvents. The degree of lethality was also directly proportional to the concentration of the extracts. Maximum mortalities took place at 1000 ppm and least mortalities were at 10 ppm. The significance of low LC_{50} value recorded for the extracts is indicative of the presence of potent cytotoxic and insecticidal compounds which need to be investigated further. There was no much difference in the observed and expected mortality of the extractants that is, the solvent except at 10 ppm (Table 2).

Conclusion

The low LC_{50} values obtained from the different extracts of *E. kamerunica* plant corroborates its folk medicinal uses in the treatment of leprosy and it high toxicity to both man and animals. This property is attributed to cytogenic and toxic compounds present in the plant. Low LC_{50} value indicates possibility of the presence of antitumor and insecticidal compounds in an extract, therefore low LC_{50} (that is, < 20 µg/ml) found for this plant extract shows the possibility of potent antitumor and insecticidal compounds presence in the plant (Krishnaraju et al. 2005) . The presence of these compounds is of importance in pharmaceutical industry and to the generality of mankind, although caution needs to be sounded to natives who consume the extract of *E. kamerunica* indiscri-

Table 2. Observed and expected frequency of extractant and mortality rate.

Extractant	Mortality at 1000 ppm	Mortality at 100 ppm	Mortality at 10 ppm	Total
Hexane	100(111.54)	100(97.71)	90(80.75)	290
Diethyl ether	100(106.15)	93(92.99)	83(76.860	276
Ethyl acetate	100(99.62)	86(87.26)	73(72.12)	259
Methanol	100(97.31)	83(85.24)	70(70.45)	253
Aqueous	100(85.38)	76(74.80)	46(61.82)	222
Total	500	438	362	1300

Expected mortality in parenthesis.

indiscriminately,.particularly at high concentration. This caution is due to high toxicity displayed by all the extracts either in the cold or hot. Further works on the plant is on in order to isolate and characterise the active compounds.

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