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ASSESSMENT OF CYTOTOXIC EFFECTS OF METHANOL EXTRACT OF *CALLIANDRA PORTORICENSIS* USING BRINE SHRIMP (*ARTEMIA SALINA*) LETHALITY BIOASSAY

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ABSTRACT
The aim of the present study is to focus on the cytotoxicity of the aqueous and methanol extracts of the root bark of *Calliandra portoricensis* belonging to the family mimosaeae on brine shrimp (*Artemia salina*) using brine shrimp lethality bioassay. The cytotoxicity was reported in terms of lethality concentration (LC_{50}). The shrimps were hatched in sea water exposed to light after 48hours and active shrimps were collected and used for the assay. The extracts were prepared in concentrations of 1000, 100, and 10ppm. 10 active shrimps were added to the 0.5ml diluted test solution and the surviving (larvae) shrimps were counted after 24 hours and lethality concentration LC_{50} was assessed. Aqueous and methanol extracts of *Calliandra portorecensis* exhibited potent brine shrimp lethality LC_{50} of 0.18 and 0.88% respectively. This suggests that brine shrimp bioassay is simple, reliable and convenient method for assessment of bioactivity of medicinal plants and that the methanol and aqueous extracts of *Calliandra portoricensis* contains useful potent bioactive compounds that can be harnessed and purified into useful therapeutic drugs.

KEY WORDS: *Calliandra portoricensis*, brine shrimp lethality, cytotoxicity, lethality concentration LC**50**, *Artemia* nauplii, part per million (ppm)

INTRODUCTION

Medicinal plants are valuable natural resources and are regarded as potentially safe drugs. They have been playing an important role in alleviating human sufferings by contributing herbal medicines in the primary health care systems of rural and remote areas where more than 70% of population depends on folklore and traditional systems of medicines (Rice-Evans, 2004). *Calliandra portoricensis* (Jacq.) Benth is a straggling perennial shrub and belongs to the family mimosaeae (Hutchinson and Dalziel, 1937). *Calliandra portoricensis* belongs to a category of medicinal plants or herbs which has potency of curing or managing diseases. It is used in Nigeria folklore medicine as a laxative/worm expeller (Adesida, 1976) and an abortifacient in human beings (Ayensu, 1978). *It* has also been reported to possess antimalarial, anticonvulsant, antidiarrheal, antisplasmodic, antipyretic, antirheumatic and analgesic activities in humans (Akah and Nwaiwu, 1988; Aguwa et al., 1988; Adesina, 1982). In addition, It has been reported to exhibit anticholigenic, antacid, antiulcer, molluscidal and ovucidal activities in laboratory animals (Aguwa *et al*., 1988) as well as in the traditional management of sickle cell anaemia and prostate cancer in Africa(Orishadipe *et al*., 2010). The plant extracts equally have antimicrobacterial activities against the following organisms: *Escherichia coli, Staphyloccocus aureus, Streptococcus faecium* and *Candida albicans* (Adesina, 1982) and contains phytochemical constituents such as tannin saponins, flavonoids, cardiac glycosides (Aguwa and Lawal, 1998; Orishadipe *et al.,* 2004). *Artemia salina* the brine shrimp is an invertebrate component of the fauna of saline aquatic and marine ecosystem (Lewan *et al*., 1992). The brine shrimp bioassay is a simplest, less expensive and easily achievable method replacing cell lines bioassay in order to determine the toxicity of plants extracts by the estimation of their medium lethality concentration LC_{50} (Meyer *et al*., 1982; Piccardi, *et al*., 2000). This method is normally conducted to draw inferences on the safety of the plant extracts and to further depict trends of their biological activities and considered as a useful tool for the preliminary assessment toxicity (Solis *et al.,* 1993). It has also been used for the detection of the LC_{50} for a series of other toxins including cyanobacterial toxins (like microcystins, anatoxins) and other plant extracts (Jaki *et al.,* 1999; Lagadic and Caquet, 1998). The Artemia nauplii have in the past 3 decades been used to test general toxicity (Persoone and Wells, 1987) in teratology screens (Sleet and Brendel, 1983, 1985; Acey and Tomlison, 1988; Kerster and Schaffer, 1983) and in ecotoxicology (Sorgeloos *et al*., 1978; Persoone and Wells, 1987). Furthermore, from the pharmacological perspective, a good correlation has been found with brine shrimp lethality test to detect anticancer compounds in plant extracts (Solis *et al*., 1993; Meyer *et al*., 1982; Mackeen *et al*., 2000). However, the present study aimed at determining the cytotoxicity effect of plant *Calliandra portoricensis* using brine shrimp (*Artemia nauplii*).

MATERIALS AND METHODS

Plant collection and extraction

About 500g root of *C. portoricensis* was collected and authenticated from the medicinal plant garden of the Botany Department University of Ibadan, Nigeria. The root was freshly harvested, washed and the peeled barks were air-dried and pulverized using a hammer mill (Trapp TRF 80, Trapp Metallurgical, Brazil), and thereafter powdered at room temperature. The powdered samples (500g each) were suspended and extracted in 2.5L of methanol (w/v) and kept at 25ºC for 3 days. The extracts were filtered through Advantech -4B filter paper (Tokyo Roshi Kaisha Ltd., Japan). The extraction of the residue was repeated twice under the same conditions. The methanol extract was first dried using a vacuum rotary evaporator (N-1000; EYLA, Tokyo, Japan) in a water bath at 40°C. Dried samples were weighed and kept at 4°C until use.

Chemical reagents

All solvents used were analytical grade from Sigma-Aldrich Chemicals, U.K. The crude methanol extract *Calliandra portoricensis* root bark was extracted using sequentially n-hexane, chloroform, ethylacetate and methanol as solvents. For the aqueous, the powdered root bark were macerated with distilled water using chloroform as a preservative. The extract was concentrated, collected and stored in refrigerator at 4ºC. The aqueous and methanol extracts obtained from the above methods were used for the cytotoxicity study.

Hatching of *Artemia salina* shrimps

70 mg of shrimp eggs was sprinkled into container containing 250ml distilled water of sea water. Container was placed beside a light ray precisely the window blind for rays of light and proper ventilation, After 48 hours, brime shrimp larvae were collected by dropping pipette. About 4.5ml of brine solution (sea water) into each test tube.20 mg each of the extracts was dissolved in 2 ml of sea water. The corresponding concentrations were 1000ppm, 100ppm and 10ppm respectively. The 0.5ml diluted test solution of the extracts was added to the test tubes. Ten (10) active brine shrimp (nauplii) were transferred into each of these vials using Pasteur pipette. Replicates of each of the dose levels were prepared, using seawater as control Number of survivors, deaths, and nauplii with sluggish movement were recorded after 24 hours.

RESULTS AND DISCUSSION

The brine shrimp lethality assay was carried out to determine the lethal concentration of the aqueous and methanol extract of *Calliandra portoricensis* root bark. The concentrations of the extracts was prepared in part per million (PPM). It was observed that the aqueous extract of *C. portoricensis* significantly, killed the brine shrimp with percentage lethality/mortality of 96.67, 86.67, and 80% at a concentration of 1000, 100, and 10ppm respectively (Table 1). While for the methanol extract percentage mortality/lethality was 100, 83.30, and 90% at concentrations of 1000, 100, and 10ppm respectively (Table2).compared to the control group. The LC_{50} of the aqueous and methanol extracts was found to be 0.18% and 0.88% which is within the range of 0-100 considered to be very toxic.

TABLE 1: Shows result for brine shrimp lethality of aqueous extract of *Calliandra portoricensis* root bark

Dose level ppm	Initial Nauphili	Number survive After 24hrs.	Number died after 24hrs	Average Number died after 24hrs	% mortality/lethality
1000	30	1	29	29	96.67
100	30	4	26	26	86.67
10	30	6	24	24	80
Control	30	21	9	9	30

TABLE 2: Shows result for brine shrimp lethality of methanol extract of *Calliandra portoricensis* root bark

Dose level ppm	Initial Nauphili	Number survive after 24hrs.	Number died after 24hrs	Average Number died after 24hrs	% mortality/lethality
1000	30	0	30	30	100
100	30	5	25	25	83.33
10	30	3	27	27	90
Control	30	21	9	9	30

The brine shrimp lethality bioassay represents a rapid, low-cost simple bioassay for testing plant extract's bioactivity which in most cases correlates reasonably well with cytotoxic and antitumor properties of plant extracts (McLaughlin *et al*., 1993). The brine shrimp bioassay was proposed by Michael *et al*.,(1956) and later developed by Vanhaeck *et al*. (1981) as well as Sleet and Bbrendel (1983). The cytotoxicity bioassay of plant extracts using brine shrimps is regarded as an important tool useful for the preliminary assessment of toxicity and it has been used for the detection of fungal toxins, cyanobacterial toxins like microcystins, anatoxins etc, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of dental materials (Jaki *et al.,* 1999; Harwig and Scott,1971;McLaughlin *et al*., 1991;Martinez *et al*., 1999;Barahona and Sanchez-Fortun,1999; Pelka *et al*., 2000).

The findings of this research have shown that the aqueous and methanol extracts of *Calliandria portoricensis* is toxic to brine shrimps on exposure for 24 hours in a dose dependent manner in which the tested animals were killed at the highest dose (1000ppm) in most of the samples. This

conforms to the work of Sed mak (1997) and Chou *et al* (2004) who conducted a brine shrimp lethality assay with the extract of *M. aeruginosa* isolated from the Solvene pond in Central Europe. The result of their work showed that the tested animals (brine shrimp) were killed at various doses of the extract. The LC_{50} values obtained for the aqueous and methanol extracts from this work are 0.18% and 0.88% which falls within the lethality range (0-100) of biological compounds which is considered very toxic.

The significant lethality of the brine shrimp due to the aqueous and methanol extracts *Calliandra portoricensis* root bark indicates the presence of potent cytotoxic components which warrants further investigation.

CONCLUSIONS

The brine shrimp lethality bioassay is considered as a useful tool for the preliminary assessment of toxicity and for the isolation of bioactive compounds from plant extracts. It can be deduced that the methanol and aqueous extracts of *Calliandra portoricensis* contains useful potent bioactive compounds toxic that can be harnessed and purified into useful therapeutic drugs. Although, the brine shrimp lethality cytotoxicity bioassay is rather inadequate as to the elucidation of the mechanism of action but it offers a front line screen for the establishment of the LC_{50} of any plant extracts which can be backed up by a more specific and expensive bioassays, once the active compound has been isolated.

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Conflict of interest

The authors declare that there are no conflicts of interest and that the authors of this manuscript have no financial or personal relationship with any organisation which could influence the work.