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Chrozophora senegalensis A. Juss brine shrimp activity

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Abstract

Chrozophora senegalensis is a medicinal plant with widespread use in Northern Nigeria by the local populace for treatment of ailments and diseases such as diarrhea, malaria and dysentery. The study evaluated the Cytotoxicity of *C. senegalensis* crude methanol extract using brine shrimp lethality test. The plant sample was air dried at room temperature, pulverized to powder and subjected to maceration with methanol. The marc was removed by double filtration using cotton wool and Whatmann No.1 filter paper. The filtrate obtained was concentrated on a rotary evaporator at 45 °C. The crude extract was partitioned between hexane and ethyl acetate and subjected to Cytotoxicity assay using brine shrimp lethality test. The study revealed that the *C. senegalensis* extracts are highly cytotoxic to the brine shrimp larvae with LC50 of 4.68 µg/mL and 0.76 µg/mL for the hexane and ethyl acetate fractions respectively. The results suggest that the ethylacetate fraction is more cytotoxic than the hexane fraction. However, the study had shown that *C. senegalensis* contains bioactive principles which could be useful as antitumor agent and partly justify its use in traditional medicine practice.

Keywords: *Chrozophora senegalensis*, brine shrimp, cytotoxicity, lethal concentration

Introduction

Chrozophora senegalensis of the family Euphorbiaceae is a herb or an under shrub often found prostrate on river banks in the Savanna regions of West Africa from Senegal to Nigeria. The genus *Chrozophora* consists of 8-7 species distributed across Pakistan, India, West Africa and the Mediterranean region and are indicated for several conditions such as skin disorders, diarrhea, jaundice, fever, migraine, menstrual problems and as worm expeller in traditional medicine [1]. Several biological activities such as antioxidant activity, antimicrobial, antitumor activity, antiviral and antihelminthic had been attributed to the genus [2]. Examples include *C. tinctora*, *C. rottleri* and *C. plicata*. The phytochemical and pharmacological studies on the genus also revealed the presence of phytoconstituents such as alkaloids, flavonoids, saponins, tannins and steroids [1]; strong antioxidant activity and broad spectrum of antimicrobial and antiviral activities were also reported [3]. From the foregoing, the family Euphorbiaceae may serve as a potential source of many useful drugs that can contribute to improvements in healthcare delivery.

The plant *C. senegalensis* of the genus *Chrozophora* is traditionally used for the cure of ailments and diseases such as diarrhea, malaria and dysentery in Northern Nigeria. It is also used in the treatment of boils and as body wash for expectant mothers as well as a remedy for intestinal pains [4]. Jigam *et al.* [5] reported the *in vivo* antimalarial activity of *C. senegalensis* in mice in which the parasitaemia level was suppressed by 51.80%. The crude methanol leaf extract was found to be active against *B. subtilis*, *Staphylococcus aeruginosa* and *S. typhi*. Similarly, the *in-vitro* antimicrobial activities of *C. senegalensis* extract and fractions against *B. subtilis* (NCTC 8326 B76), *E. coli* (ATCC 11775), *P. aeruginosa* (ATCC 10145) and *S. aureus* (ATCC 021001) and laboratory isolates of *Aspergillus flavus*, *Aspergillus niger*, *C. albicans* and *S. typhi* were assessed using agar plate diffusion and broth dilution methods [6]. The ethanol extract, ethylacetate extract and n-butanol extract inhibited the growth of all bacterial isolates and *Aspergillus flavus*. The ethanolic and ethylacetate extracts recorded minimum inhibitory concentrations (MIC) of 0.39 and 3.125 mg/mL against *S. typhi* and *E. coli* while the n-butanol and aqueous extracts had MICs of 3.125 and 1.563 mg/mL respectively against *S. typhi*. The aqueous extract was found to be active against *S. typhi* and *B. subtilis* [6]. These results indicate the usefulness of *C. senegalensis* in the management of some diseases and ailments associated with these bacterial and fungal pathogens. Studies had also shown that the genus *Chrozophora* contains a variety of phytotoxins such as diterpene esters, alkaloids and the ricin-type toxins. These reports confirm the existence of active principles in *C. senegalensis* which serves as the basis for any observed pharmacological

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activity [7]. This therefore underscores the need to evaluate the brine shrimp lethality test of the crude extract fractions of *C. senegalensis*. Additionally, to the best of our knowledge, there are no reports yet on the brine shrimp lethality test of *C. senegalensis* crude extracts. Consequently, we report for the first time the brine shrimp lethality test (cytotoxicity) of *C. senegalensis* crude extracts.

Materials and Method

Collection and Identification of Plant Material

C. senegalensis was collected in November from Gwani Village Yamaltu-deba Local Government Area of Gombe State, Nigeria. The plant was identified by Dr. Daniel A. Zhigila of Biological Science Department Gombe State University, Nigeria. The plant was air dried at room temperature. The dried plant sample was pulverized to powder. The powdered plant sample was kept in a polythene bag under cool and dry condition until required for use.

Extraction and Preparation of Test Solution

The powdered plant sample of *Chrozophora senegalensis* (1 Kg) was extracted using maceration procedure with intermittent shaking at room temperature for a period of 7 days. The extract was filtered with Whatman No.1 filter paper. The filtrates were combined and concentrated on a rotary evaporator at 45°C to obtain the crude extract. A stock solution of concentration 10,000 µg/mL was prepared by dissolving 20 mg of extract in 0.2 mL dimethylsulfoxide and 1.8 mL normal saline to yield a solution of 10,000 µg/mL. The stock solution was diluted with brine solution (artificial sea water) to produce working solution. [8].

Brine shrimp Test method

The brine shrimp test was carried out according to the method described by Kwaji *et al.* [8]. Briefly, ten shrimp larvae in 4.5 mL brine solution was added to 0.5 mL crude extract at concentrations of 5000, 500, 50 and 5 µg/mL respectively. Final test concentrations are 1000, 100, 10 and 1 µg/mL. After 24 hr incubation, the percentage mortality at each concentration was evaluated. This was performed in triplicate. Results were evaluated using probit analysis.

Result and Discussion

The result revealed the LC₅₀ value for hexane and ethyl acetate extract are 4.68 µg/mL and 0.76 µg/mL respectively (Fig. 1 and Fig. 2). According to Meyer *et al.* [9], LC₅₀ <1000 µg/mL indicates the presence of cytotoxic constituents which may serve as antitumor agent(s). This shows that the hexane and ethyl acetate crude extract fractions of *C. senegalensis* are highly cytotoxic. Jamil *et al.* [10] reported the brine shrimp lethality test for the leaf extract of *Chrozophora hierosolymitana* Spreng. The study revealed LC₅₀ values ranging from 171.55-523.80 µg/mL while the phytochemical screening of its extracts showed the presence of alkaloids, cardiac glycosides, terpenoids, phlobatannins, anthraquinones, flavonoids and saponins. In a separate study, the cytotoxicity of *Lantana camara*, *Chromolaena odorata* and *Euphorbia hirta* were assessed using brine shrimp lethality test. Results showed that the ethanol extracts were potent against the brine shrimp larvae with LC₅₀ of 55 µg/mL, 10 µg/mL and 100 µg/mL respectively [11]. Relative to the preceding studies cited above, the extracts of *C. senegalensis* for both the hexane and ethyl acetate displayed better cytotoxic activity. This observed activity could be due to presence of a compound or the combined effect of the extract phytoconstituents. The

study therefore validates the use of *C. senegalensis* in traditional medicine practice.

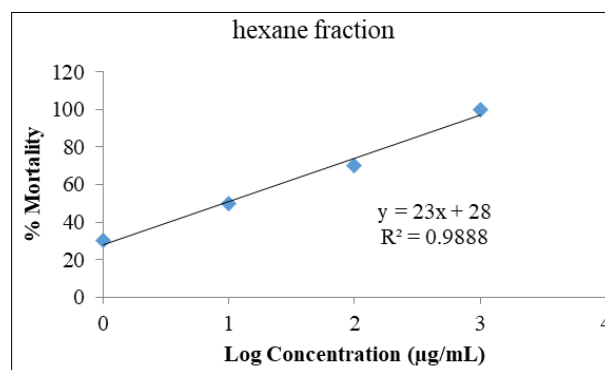


Fig 1: Percentage Mortality vs Log concentration of hexane extract

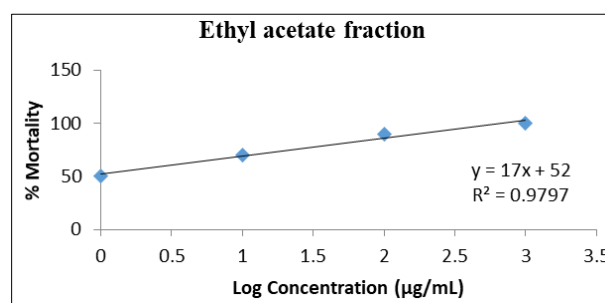


Fig 2: Percentage Mortality vs Log concentration of

Conclusion

The study had shown that *C. senegalensis* is strongly cytotoxic with LC₅₀ of 4.68 and 0.76 µg/mL for hexane and ethyl acetate fractions respectively. This shows that the ethylacetate fraction is much more cytotoxic than the hexane fraction. This high toxicity might partly explain the widespread use of *C. senegalensis* for several conditions such as skin disorders, jaundice, diarrhea, fever, amenorrhea, intestinal pains and as a worm expeller in traditional medicine practice. Therefore this high toxicity indicates the presence of bioactive phytochemical compounds with useful pharmacological properties.

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Conflict of Interest

None.

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