

ASSESSMENT OF BIOACTIVITY OF *Desmostachya bipinnata* (L.) Stapf USING BRINE SHRIMP (*ARTEMIA SALINA*) LETHALITY ASSAY

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Summary

Medicinal plants constitute an important component of flora and are widely distributed in India. The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds which can lead to the development of novel and safe medicinal agents. *Desmostachya bipinnata* Stapf is an official drug of ayurvedic pharmacopoeia, belongs to the family of Poaceae/Gramineae. The various parts of this plant were used extensively in Indian traditional and folklore medicine to cure various human ailments like wounds, asthma, thirst, jaundice, vaginal discharges, vesicle calculi, diseases of bladder and skin eruptions, also used as analgesic, antipyretic, anti-inflammatory, diuretic etc. Based on the ethno-pharmacological literature, this medicinal plant which was used in traditional medicine in India was collected. In the present study, four different concentrations of hydro-alcoholic extract of *Desmostachya bipinnata* (10, 100, 500 and 1000 ppm) was screened for cytotoxicity In Vivo using brine shrimp lethality test in comparison to vehicle control. The results were expressed as mean percentage death of shrimp at 24 hours. In this study, hydro-alcoholic extract of *Desmostachya bipinnata* failed to show extreme levels of toxicity with all doses of the plant showing shrimp survival of greater than 50% at the highest concentration tested which was 1000 µg/ml. However, the plant showed certain level of toxicity of 17.4 and 42 % death at 500 and 1000 ppm respectively with an LD₅₀ value of 1215.929 ppm. So, the results of present study revealed that *Desmostachya bipinnata* show variable levels of toxicity at high concentrations and the quantity of consumption of this herb by humans should be minimized and measures should be taken to avoid poisoning to animals and safe for human consumption.

Key words: *Artemia salina*, brine shrimp lethality test, *Desmostachya bipinnata*, cytotoxicity.

Introduction

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures (1).

Complementary system of medicine includes namely Ayurveda, Siddha, Unani, Kaempo, and Chinese medicine has gained its popularity in recent years (2). Now Ayurvedic system of medicine became an integral part of alternative medicinal systems of India (3). The demand of herbal medicine is increasing day by day due to their efficacy, rare chances of side effects in the treatment and good faith of society on herbal medicine and also their products (4). Some of these herbs have been subjected to the isolation of the active ingredients (chemical compounds) and their subsequent modification. A large proportion of such medicinal compounds have been discovered with the aid of ethno-botanical knowledge of their traditional uses. The rich knowledge base of countries like India and China in medicinal plants and health care has led to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development programs in the pursuit of discovering novel drugs. India is a varietal emporium of medicinal plants and it is one of the richest countries in the world as regards genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. More over the agro climatical conditions are conducive for introducing and domesticating new exotic plant varieties. At present majority of the people are relying for their primary health care on traditional medicine (5).

In continuation of our efforts to verify the efficacy of traditional medicine we have collected *Desmostachya bipinnata*, based on the ethno-pharmacological information. *Desmostachya bipinnata* Stapf is an official drug of ayurvedic pharmacopoeia, belongs to the family of Poaceae/Gramineae. The various parts of this plant were used extensively in Indian traditional and folklore medicine to cure various human ailments and used traditionally as analgesic, antipyretic, wounds, anti-inflammatory, asthma, thirst, jaundice, vaginal discharges, vesicle calculi, skin eruptions, vomiting, aphrodisiac, diuretic, diseases of the blood and sedative to pregnant uterus, cooling, sweet, astringent, and galactogogue and also useful in dysentery, diarrhoea, urinary calculi, dysuria, menorrhagia and other diseases of bladder and skin diseases (6, 7). In order to study the toxicity of this medicinal plant we performed brine shrimp lethality bioassay which based on the ability to kill laboratory cultured brine shrimp (*Artemia nauplii*). The brine shrimp assay was proposed by Michael et al. (8), latter developed by Vanhaecke et al. (9) and Sleet and Brendel (10). The aim of this method is to provide a front-line screen that can be backed up by more specific and more expensive bioassays once the

active compounds have been isolated. Since its introduction in 1982 (11), this *in vivo* lethality assay has been successively employed for preliminary assessment of toxicity, bioassay-guide fractionation of active cytotoxic and antitumor agents such as trilobacin from the bark of *Asimina triloba* (12), *cis*-annonacin from *Annona muricata* (13), ent-kaur-16-en-19-oic acid from *Elaeoselinum foetidum* (14) and is considered a useful tool for and it has been used for the detection of fungal toxins (15), plant extract toxicity (16), heavy metals (17), pesticides (18), cytotoxicity testing of dental materials (19) and used for the detection of cyanobacterial toxins like microcystins, anatoxins etc (20).

The brine shrimp assay is very useful tool for the isolation of bioactive compounds from plant extracts (21). The technique is attractive because it is easily mastered, simple, rapid, efficient and inexpensive test that utilizes small amount of test material (toxin) to perform the test in the microwell scale. In the present study, four different concentrations of hydro-alcoholic extract of *Desmostachya bipinnata* (10, 100, 500 and 1000 ppm) was screened for assessment of its cytotoxicity In Vivo using brine shrimp lethality test in comparison to vehicle control, based on their ethno-pharmacological information and the results obtained were described.

Materials and Methods

Plant Material

The plant *Desmostachya bipinnata* L. Stapf was collected in the month of November-2010 in and around the Charlapally Village, Nalgonda District, Andhra Pradesh, India. After collection the plant material was shade dried and stored suitably for further future use. The plant material was taxonomically identified by the field botanist, authenticated by Dr.T.Shankara Chary, Head of Botany Department, Government Degree College for Women, Nalgonda, Andhra Pradesh, India. The voucher specimen (No: DBP/GDCWN/54/2010) was deposited in the college herbarium for future reference.

Preparation of Extract

The whole plant of *Desmostachya bipinnata* was shade dried, cut into small pieces and powdered coarsely. The powder was passed through sieve no. 40 and stored in air tight container for further use. About 500 gm of air dried powdered material was extracted with 70% of Methanol as solvent in a Soxhlet extractor (50-60°C) and this extraction was carried out until the extractive becomes colorless and drop of solvent from the siphon tube does not leave residue when evaporated. Then extract was filtered through vacuum filter into dish and the filtrate was concentrated to dryness in vacuum evaporator under controlled temperature (40-50°C). Dried extracts were stored in glass desiccators, subjected for cytotoxicity studies.

Brine Shrimp Eggs

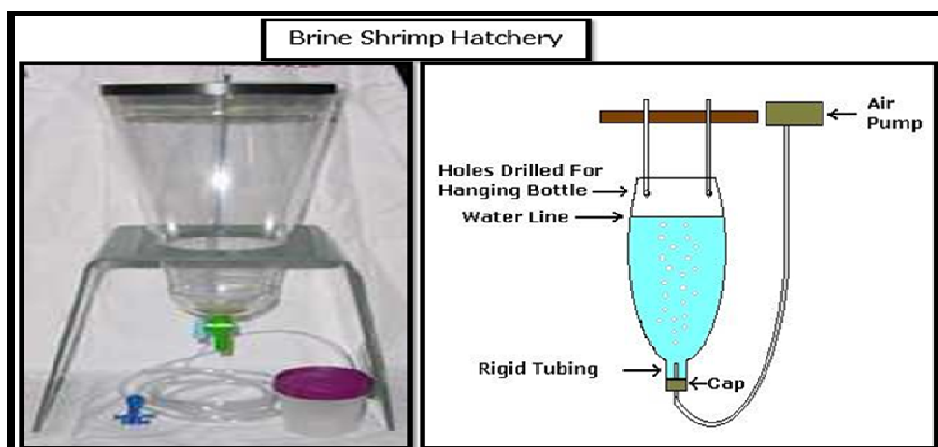
Brine shrimp eggs (*Artemia salina*, Sanders™ Great Salt Lake, Brine Shrimp Company L.C., U.S.A.) were purchased from tropical fish dealers (Ramnath Breeding Farms, Hyderabad-35, Andhra Pradesh, India). Each egg is as small as a grain of fine

sand. A teaspoonful contains many thousands of eggs. The eggs will remain viable for several years if kept cool and dry.

Hatching of Brine Shrimp Eggs

Brine shrimp eggs were hatched in sterile artificial sea water prepared from commercial sea salt 40 g/L (4%) and adjusted to pH 8.5 using 1N NaOH under constant aeration for 48 h in a suitable hatchery as shown in figure 1. The plastic chamber was divided into two unequal compartments using a divider with several holes was used for hatching. The eggs were sprinkled into the larger compartment which was darkened, while the smaller compartment was illuminated. After 48 hours incubation at room temperature (30-37°C), newly hatched free-swimming pink-coloured nauplii (larvae) were pipetted from the brighter side or harvested from the bottom outlet. As the cyst capsules floated on the surface of another side, this collection method ensured pure harvest of nauplii. The freshly hatched free-swimming nauplii were used for the bioassay.

Figure 1: Brine Shrimp Egg Hatchery and its Components.



Cytotoxicity bioassay

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of Hydro-alcoholic extract of *Desmostachya bipinnata*, a medicinal plant of India. Brine shrimp cytotoxicity assay was performed by using the methodology of Ahmad et al. (22). Freshly hatched brine shrimp (*Artemia salina*) larvae (nauplii) were used as test organisms. Different concentrations that is 1000, 500, 100, 10 and 0 ppm (control) of Hydro- Alcoholic extract of *Desmostachya bipinnata* were prepared in Distilled Water and used against brine shrimp larvae. The survival rate of these larvae was observed against all concentrations of extract. For this purpose, ten nauplii were drawn through a glass capillary and placed in each vial containing 4.5 ml of brine solution. In each experiment, 0.5 ml of the plant extract was added to 4.5 ml of brine solution to adjust the final volume to 5 ml and incubated at room temperature (30-37°C) for 24 hours under the light. Test was performed in triplicate. After this, the numbers of dead (non-

motile) nauplii in each well were counted under the microscope or against a lighted background as shown in figure 2. Analysis of the data was performed by probit analysis on a Finney computer program to determine the lethal concentration to half of the test organisms (LC_{50}).

Figure 2: Observing dead (non-motile) nauplii in each well under the microscope.



Lethality concentration determination

The percentage lethality/ mortality was calculated (by Abbott's formula) and determined by comparing the mean surviving larvae of the test (Extract) and control (Vehicle) tubes. EPA probit analysis program (Version 1.5) used for calculating LC_{50}/EC_{50} value.

Statistical analysis

Lethality assay was evaluated by using probit analysis on a Finney computer statistical program to determine the lethal concentration to half of the test organisms (LC_{50}) values and 95% confidence intervals (23). The percentage lethality was calculated from the mean of triplicate measurements of survival larvae of extracts treated tubes and control.

Results and Discussion

When screening for biologically active plant constituents, the selection of the plant species to be studied is obviously a crucial factor for the ultimate success of the investigation. Plants used in traditional medicine are more likely to yield pharmacologically active compounds (24). The *in vivo* brine shrimp lethality test (BST), detects a broad range of biological activities and a diversity of chemical structures. One basic premise here is that toxicology is simply pharmacology at a higher dose, thus if we find toxic compounds, a lower, non-toxic, dose might elicit a useful, pharmacological, perturbation on a physiologic system.

The cytotoxic activity of *Desmostachya bipinnata* hydro-alcoholic extract was investigated *in vivo*, tested against the brine shrimp (*Artemia salina*) at four

concentrations of 10µg/ml, 100µg/ml, 500µg/ml and 1000µg/ml. The results were given in **Table 1** and expressed as mean percentage death of shrimp at 24 hours. According to literature in order for a test compound to be considered highly toxic it needs to show shrimp death of 50% or less. For a compound to be considered slightly toxic it needs to show cell death of between 50 – 70%. *Desmostachya bipinnata*, in this assay failed to show extreme levels of toxicity with all doses of the plant showing shrimp survival of greater than 50% at the highest concentration tested which was 1000 µg/ml. However, the plant showed certain level of toxicity. This plant was showing 17.4 and 42 % death at 500 and 1000 ppm (µg/ml) respectively.

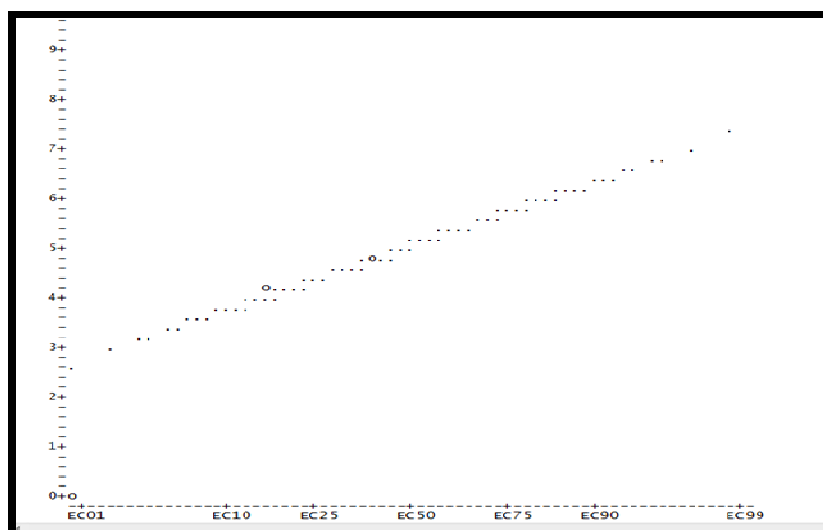
Table 1: The mean % shrimp death at 10, 100,500 and 1000 ppm (µg/ml) concentrations of *Desmostachya bipinnata* hydro-alcoholic extract.

Plant	% shrimp death at concentrations (mean %)*					
	0 (control)	ppm	10 ppm	100 ppm	500 ppm	1000 ppm
<i>Desmostachya bipinnata</i>	0	0	0	17.4	42	

*Values are expressed as Mean % shrimp death of triplicate measurements.

Brine shrimp cytotoxicity assay has been considered as Pre-screening assay for antimicrobial, antitumor, anti-malarial, antifungal and insecticidal activities. Brine shrimp assay is suggested to be a convenient probe for the pharmacological activities in plant extracts (25). In the present study, *Desmostachya bipinnata* shown an LD₅₀ value of 1215.929 ppm (µg/ml) and the corresponding Plot of adjusted probit and predicted regression line was shown in **figure 3**.

Figure 3: Plot of adjusted probit and predicted regression line.



Based on my investigation and the results from literature, it was found that *Desmostachya bipinnata* is regularly consumed by the African population and this herb contain adequate amounts of protein and do not exhibit any toxic properties, however the results of this study showed that *Desmostachya bipinnata* show variable levels of toxicity at high concentrations and that was may be due to the presence of active constituents useful for the utilization of cytotoxic principles of this extract. The quantity of consumption of this herb by humans should be minimized and measures should be taken to avoid poisoning to animals. A comparison of the results in this study with documented traditional experiences indicates that this traditional herb safe for human consumption.

Conclusion

Although the brine shrimp lethality assay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the bioactivity of the plant extracts. In the course of our studies, the brine shrimp lethality assay actually has proven to be a convenient system for monitoring biological activities of several plant species that are used in the Indian traditional medicine. Out of the four concentrations of hydro-alcoholic extract screened for toxicity against the brine shrimp, none of shown extreme levels of toxicity with all doses of the plant showing shrimp survival of greater than 50% at the highest concentration tested. However, the results of this study showed that *Desmostachya bipinnata* show variable levels of toxicity at high concentrations and the quantity of consumption of this herb by humans should be minimized and measures should be taken to avoid poisoning to animals. A comparison of the results in this study with documented traditional experiences indicates that this traditional herb safe for human consumption. Even though, the present study on the crude extract of *Desmostachya bipinnata* is an addition to the scientific literature, detailed investigations for the pharmacological activities and active ingredients could provide leads to interesting pharmaceuticals of plant origin.

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