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A preliminary evaluation on some plants of Chittagong Hill tracts having significant cytotoxicity

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ABSTRACT

The present study aims in the evaluation of *in vitro* cytotoxic activity of *Sida cordifolia*; *Senna siamea; Acorus calamus; Oroxylum indicum.* The cytotoxic activity of these extracts was assessed by Brine shrimp lethality bioassay method. None of the plants in this assay showed extreme levels of toxicity with all of the plants showing shrimp survival of greater than 50% at the highest concentration tested which was 1000 µg/ml. However, the plants showed certain levels of toxicity. *Sida cordifolia* showed 25 and 34% death at 100 and 1000 µg/ml respectively. Relatively high levels of toxicity were also displayed by *Acorus calamus* which had 27% shrimp death at the highest concentration tested. Other plants displaying some or very little toxicity at concentrations 100 and 1000 µg/ml were *Senna siamea* and *Oroxylum indicum.*

KEYWORDS: *Sida cordifolia*, *Senna siamea, Acorus calamus, Oroxylum indicum,* Brine shrimp lethality bioassay.

INTRODUCTION

Cytotoxicity is the quality of being toxic to cells. The brine shrimp lethality assay is considered a useful tool for preliminary assessment of cytotoxicity. This method also gives a primary idea about the anticancer effect of plant extract. The brine shrimp lethality bioassay is considered a useful tool for preliminary assessment of cytotoxicity. In the United States in 1999, over 1500 people are expected to die of cancer each day, representing an estimated total mortality rate of about 560000. More than twice as many persons than this will be diagnosed with invasive cancer, but, overall, a slight decline in cancer incidence rates has been observed in the USA. Among many recent advances in cancer chemotherapy, phytochemicals play an important role in cancer chemotherapeutic drugs. A search for new anti-cancer drugs has taken many different approaches. The brine shrimp lethality bioassay is efficient, rapid and inexpensive tests that require only a relatively small amount samples. The technique is easily mastered, costs little, and utilizes small amount of test material has been successively employed for *in vivo* lethality bioassay-guide fractionation of active cytotoxic and antitumor agents. For thousands of years, people have relied and survived on the bounty of plants and natural grasslands. Till today, people still rely on plants to maintain a healthy diet. Plants contain many important proteins which humans require for survival, which make plants a necessity. There is a growing interest worldwide in the use of traditional plants for production of medicinal agents [1]. It is estimated that 70-80% of the world's population rely on traditional medicine for primary healthcare needs, most of which are plant derived. This means that around 4 billion people rely on natural products as a source of their primary medicinal needs. It is proved that half of the world's best selling drugs and many potential drugs under development are derived from plants [2]. This implies a tremendous demand for indigenous medicinal plants; therefore, these plants should be protected as a source of both food and medicine. Although plants have immense medical benefits, they are also known to be mutagenic and somewhat toxic [3]. They cause damage to DNA cells, thus influencing the frequency of genetic or heritable diseases. It is important, therefore, that the extracts of plants are tested for their potential to produce cancer and genetic damage (mutations). When mutations occur in the cell of an organism, it may produce irreversible changes in the cell that may ultimately be involved in producing a cancerous growth [4]. All chemicals that produce DNA damage leading to mutations or cancer are described as Genotoxic.

MATERIALS AND METHODS

Sample collection and preparation

Four plant extracts were used in this study. The plants selected for present study were collected from Naramuk, Rajsthali of Rangamati and Khagrachori, Chittagong district of Bangladesh. The leaves were washed thoroughly and shade dried.

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Table 1: Details of plant used in this study

Scientific Name	Family	Common Uses
Sida cordifolia	*Malvaceae*	Ayurvedic medicine [5], anti-inflammatory [6], analgesic [7], anti-cancer [8], antibacterial activity [9, 10], CNS depressive effect [11]
Senna siamea	*Leguminosae*	Anti-plasmodial activity [12, 13, 14], antioxidant and antihypertensive activity [15], laxative activity [16], sedative activity [17, 18]
Acorus calamus	*Araceae*	Analgesic activity [19], anticonvulsant [20], antispasmodic [21], anti-inflammatory [22], antibacterial [23], antiulcer and cytoprotective activity [24]
Oroxylum indicum	*Bignoniaceae*	anti-microbial, analgesic, anti-tissutive, anti-inflammatory activity [25], treatment of leprosy and tuberculosis [26]

Mature leaves were washed several times with distilled water until no foreign material remained and oven-dried at 25^0C for 24 h. The dried leaves were then milled to a fine texture using a food blender (Salton). Samples were stored in capped Schott bottles until further use. All analyses were conducted in triplicate.

Preparation of plant extracts

Fifty grams of the leaf powder was stirred in 200 ml of dichloromethane (CH_2Cl_2). This was placed on a rotary shaker for 24 hours before being filtered using a Whatman no. 1 filter paper. The 200 ml solutions were then concentrated using a rotatory vaccum evaporator (Bibby RE200, Sterlin Ltd., England) including a Buchi 461 water-bath set at a temperature of 50^0C. The crude extracts were freeze dried using a Virtis Benchtop Freezedrier. Aliquots were prepared from dried crude extracts and dissolved in either methanol or dimethyl sulfoxide (DMSO) to give the necessary concentrations for further analyses.

Brine Shrimp Lethality assay

The brine shrimp lethality assay was conducted according to Meyer *et al* [27], with minor modifications.

Principle of the brine shrimp assay

A method, utilizing brine shrimp (*Artemia salina*), is a simple bioassay for natural product research. The procedure determines lethal concentrations of active compounds in brine medium. The activities of a broad range of active compounds are manifested as toxicity to the shrimp. The method is rapid, reliable and has been used for over thirty years in toxicological studies. A positive correlation exists between brine shrimp lethality and human carcinoma.

Sample preparation

Samples were prepared by dissolving 50 mg of plant extract in 5 ml of methanol (Solution A). Solution B was prepared by diluting 0.5 ml of A to 10 ml with methanol. Appropriate amounts of solution (100 µl B, 50 µl A, and 500 µl A for 10, 100 and 1000 µg/ml, respectively) were transferred to 1.25 cm disks of filter paper (Whatman no. 1). The disks were air-dried, placed in vials and then dried further *in vacuo* for one hour. Control disks were prepared using only methanol. Five replicates were prepared for each dose level.

Hatching the shrimp

Brine shrimp eggs were hatched in a shallow rectangular dish (22 x 32 cm) and filled with sea water which was obtained from the ocean. A plastic divider with several 2 mm holes was clamped in the dish to make two unequal compartments. The eggs (50 mg) were sprinkled into the larger compartment which was darkened, while the smaller compartment was illuminated. After 48 hours the phototropic nauplii were collected by pipette from the lighted side, having been separated by the divider from their shells.

RESULT AND DISCUSSION

Brine Shrimp Lethality Assay

The brine shrimp lethality test was conducted on each of the extracts at three concentrations, 10 µg/ml, 100 µg/ml and 1000 µg/ml. Table 2 gives the 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%. None of the plants in this assay showed extreme levels of toxicity with all of the plants showing shrimp survival of greater than 50% at the highest concentration tested which was 1000 µg/ml. However, the plants showed certain levels of toxicity. These plants were *Sida cordifolia* showing 25 and 34% death at 100 and 1000

µg/ml respectively. Relatively high levels of toxicity were also displayed by *Acorus calamus* which had 27% shrimp death at the highest concentration tested. Other plants displaying some or very little toxicity at concentrations 100 and 1000 µg/ml were *Senna siamea* and *Oroxylum indicum.*

Table 2: The mean % shrimp death at plant extracts concentrations of 10, 100 and 1000 µg/ml.

Name of Plant	% shrimp death at concentrations (mean %)		
	10 µg/ml	100 µg/ml	1000 µg/ml
Sida cordifolia	0	25	34
Senna siamea	0	0	5
Acorus calamus	0	17	27
Oroxylum indicum	3	10	13

CONCLUSION

One of the objectives of while conducting this study was to consult an available database that represents the nutritional values of indigenous plants. The quantity of consumption of these plants by humans should be minimized and measures should be taken to ensure that these plants, which are dangerous to animals, do not grow excessively in grazing areas so that the risk of poisoning is reduced. A comparison of the results in this study with documented traditional experiences indicates that most of the traditional plants are safe for human consumption. However the correlation between the knowledge of the rural communities and the scientific proof in this study indicate that science and traditional knowledge needs to harmonized.

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