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Short Communication

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Cytotoxic Effect Of Five Medicinal Plants Extracts Using Brine Shrimp (*Artemia salina*) TEST

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Abstract

Medicinal plants like *Curcuma longa*, *Curcuma zedoaria*, *Streblus asper*, *Enhydra fluctunas* and *Scoparia dulcis* are commonly available in Bangladesh. This study investigated the effect of ethanol extracts from these five medicinal plants on the viability of Brine Shrimp larvae. The lethal concentrations (LC₅₀ values) of the extracts were determined. LC₅₀ values of *C. longa*, *C. zedoaria* and *S. asper* were less than 250 µg/ml and that of *E. fluctunas* was greater than 250 µg/ml. Among five plants tested, one (*S. dulcis*) appear to be inactive. Results indicate that *C. longa*, *C. zedoaria* and *S. asper* could be potential sources of bio-active compounds.

Key words : Cytotoxicity, Crude plant extracts, *Artemia salina*, *Curcuma longa*, *Curcuma zedoaria*, *Streblus asper*, *Enhydra fluctunas*, *Scoparia dulcis*

Introduction

Modern pharmacologic medicine originally derived its medicines from medicinal plants, fungi, and microorganisms (Yarnell, 2000). On the basis of global survey data from 1997, about 119 plant-derived compounds of known structure are currently used as prescription drugs (Zhu *et al.*, 1996; Zhu *et al.*, 1997). More than half of currently available drugs (Newman and Cragg, 2007) are natural compounds or are related to them, and in the case of cancer this proportion surpasses 60% (Gordaliza, 2007).

Curcuma longa L, is known as 'Holud or Haldi' in Bengal and turmeric in English. Powder of its rhizome is used for its flavoring, as a spice in both vegetarian and non-vegetarian curry preparations and it also has digestive properties. The fresh rhizome is used by the beauticians. Disorders, like anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis (Ammon *et al.*, 1992) sprains and swellings caused by injury (Ammon and Wahl, 1991) are treated by different parts of the plant *C. longa*.

Rhizome of *Curcuma zedoaria* locally known as shoti, is used to make drinks. Hot water extract of the plant have shown moderate anti-mutagenic activity (Lee and Lin, 1988) as well as protective effect on d-galactosamine/ lipopolysaccharide- induced liver injury in mice (Matsuda *et al.*, 1998).

Scoparia dulcis (English name sweet broom-weed) is known as misridana or cinigura in Bangladesh (Yousuf *et al.*, 2009).

The plant is used for hypertension in Taiwan (Chow *et al.*, 1974) and diabetes mellitus in India (Satyanarayana, 1969). Poor antifungal and good antibacterial property have also been attributed to the alcoholic extract of the plant *S. dulcis* (Anwar *et al.*, 1994; Begum *et al.*, 1993).

Streblus asper (known as Harba in Chittagong, Bangladesh) has been used in fever, dysentery, relief of toothache, antingivitis (Gaitonde *et al.*, 1964) and insecticidal activity towards mosquito larvae (Kritsaneepeaboon, 1989). In Bangladesh it (*S. asper*) is used in urinary inflammation and galactagogue (Yousuf *et al.*, 2009).

Enhydra fluctuans (Bengali Name: Helencha) is used as a vegetable with fish curry in Bangladesh. Leaf paste is applied over head as a cooling agent and around the inflamed breast to reduce inflammation. The plant is rich in protein sterols (Krishnaswamy and Prasanna, 1975). β-Carotene (Dewanji *et al.*, 1993) and terpenes (Bohlmann *et al.*, 1982) have been isolated from the plant.

There are several proven methods and protocols to study the biological activity and pharmacological properties of pure compound or a library of compounds. The *in vivo* lethality in a simple zoological organism, such as the brine shrimp lethality test (Meyer *et al.*, 1982), might be used as a simple tool to guide screening and fractionation of physiologically active plant extracts, where one of the simplest biological responses to monitor is either dead or alive. The brine shrimp

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assay has been established as a safe, practical, and economic method for the determination of the bioactivity of synthetic compounds (Almeida *et al.*, 2002) as well as plant products (Meyer *et al.*, 1982; Lhullier *et al.*, 2006; Stefanello *et al.*, 2006) and in toxicological studies. From this point of view, we selected five common medicinal plants of Bangladesh namely, *S. dulcis* (Bondhoni), *E. fluctunas* (Helencha), *C. longa* (Holud), *C. zedoaria* (Shothi) and *S. asper* (Sheora) for investigation of their cytotoxic effects using brine shrimp lethality assay.

Collection of plant materials

Five plants namely *C. longa* (rhizome), *C. zedoaria* (rhizome), *S. dulcis* (shoot), *S. asper* (leaves) and *E. fluctunas* (whole plant) were collected from plantation and residential area of BCSIR laboratories, Chittagong during February to November 2010 depending on availability. The plants were identified by Industrial Botany Research Division of BCSIR laboratories, Chittagong. Voucher specimens (CL-121, CZ-122, SD-123, SA-124 and EF-125) were deposited in the herbarium of BCSIR Laboratory, Chittagong.

Preparation of the crude extracts

Fresh plant parts were washed, cut into pieces and shed dried at room temperature ($28 \pm 3^\circ\text{C}$) and ground into coarse powder and stored in an air tight container. Plant powders were macerated in pure ethanol (95%) individually for 7 days at room temperature ($28 \pm 3^\circ\text{C}$) with occasional shaking. Every seven days later, ethanol extracts were filtered off through a cotton plug and then with a Whatman No. 1 filter paper. The extracts were concentrated under reduced pressure below 50°C through rotary vacuum evaporator (EYELA Rikakikai Co., Ltd., Tokyo). The concentrated extracts were collected in a petri dish and air dried at room temperature. The whole process was repeated three times for each plant. The concentrated extracts were stored at 4°C until use.

Artemia salina hatching

Brine shrimp (*Artemia salina*) eggs obtained locally (Red Jungle Brand, O.S.I., The great Salt Lake, USA.) were hatched in artificial salt water (2.5% commercial NaCl solution) at ($30 \pm 2^\circ\text{C}$), pH between 7 and 8 and at constant illumination (2000 lux). The brine shrimp eggs were incu-

bated in a hatching tank with a water height of 2.54 cm. These hatching conditions correspond to the natural environment (shallow salt water). After an average one day from hatching, the shrimp larvae were used for the experimental bioassay. At this time the larvae still live on their own yolk-sac and received no further food during the experimental time. The living larvae have a high mobility. Nauplii were collected with a Pasteur pipette and kept for an additional 24 h under the same conditions to reach the metanauplii stage.

Biological screening

The samples to be assayed were dissolved in artificial seawater using Twin-80 as emulsifying agent. Five milliliter of test solution with different concentrations (10-80 $\mu\text{g/ml}$ for *C. longa* extract) were taken in five different 25-ml beakers for each sample. About 20 nauplii were added to each set of beakers containing the samples. Controls containing 1.0 % Twin-80 in artificial seawater were included in each experimental set. Twenty-four hours later, the number of survivors was counted, recorded and the lethal concentration 50% (LC_{50} value) and 95% confidence intervals were calculated by Probit analysis (Finney, 1971).

Qualitative phytochemical screening

The ethanolic extract was qualitatively tested for the presence of various phyto-constituents according to the methods described by Parekh and Chanda (2009). Tests for alkaloids performed with Mayer's and Hager's reagent, flavonoids with the use of ammonia and concentrated H_2SO_4 (Sofowara, 1993; Harbrone, 1973), glycoside with NaOH solution and frothing test was done for saponins.

Results and Discussion

Crude extracts and pure substances into toxic were classified into toxic (LC_{50} value $< 1000 \mu\text{g/ml}$) and non-toxic (LC_{50} value $> 1000 \mu\text{g/ml}$) according to reports of Meyer *et al.* (1982). In our test majority of the extracts (four out of five) showed good brine shrimp larvicidal activity. Ethanol extract of *Curcuma longa* was found to be highly effective ($\text{LC}_{50} = 26.63 \mu\text{g/ml}$) whereas *Curcuma zedoaria* and *Streblus asper* showed considerable action and their LC_{50} values were less than $250 \mu\text{g/ml}$ but that of *Enhydra fluctunas* was $332 \mu\text{g/ml}$ (Table I). Natural products play an important role in

Table I: Brine shrimp cytotoxicity of EtOH extracts of the plants

Species	Part used	LC_{50} ($\mu\text{g/ml}$)	95% CI ($\mu\text{g/ml}$)
<i>Curcuma longa</i>	Rhizome	26.63	9-42
<i>Curcuma zedoaria</i>	Rhizome	145.87	128-163
<i>Scoparia dulcis</i>	Shoot	>1000	201-31758
<i>Streblus asper</i>	Leaves	224.74	75-676
<i>Enhydra fluctunas</i>	Whole plant	332.45	272-394

CI: Confidence Interval

chemotherapy (Pezzuto, 1997; Cragg, 1998; Lee, 1999; Schwartzmann, 2000). They offer valuable source of compounds with a wide variety of biological activities and chemical structures provide important prototypes for the development of novel drugs (Cragg, 1998; Verpoorte, 1998; Vuorela *et al.*, 2004). This is also true for anti-cancer agents, most of which have been derived from natural sources; directly as pure native compounds, or as semi-synthetic analogs, or as models for synthetic compounds (Lindholm, 2005). The correlation between the brine shrimp lethality assay and *in vitro* growth inhibition of human solid tumor cell lines shows the value of this bioassay as a pre-screening tool for anti-tumor drug research (Anderson *et al.*, 1991). Qualitative phytochemical analysis results (Table II) shows that all the plant extracts in this investigation possess one or different types of secondary metabolites (alkaloids, flavonoids, glycosides or

explored for prospecting novel antibacterial, antifungal, anti-cancer leads.

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Table II: Results of phytochemical screening of the plants

Species	Name of tests and results				
	General test		General test		Frothing test of Saponin
	Mayer's	Hager's	Flavonoids	Glycosides	
<i>Curcuma longa</i>	-	-	+	+	-
<i>Curcuma zedoaria</i>	+	+	+	+	+
<i>Scoparia dulcis</i>	-	-	-	+	+
<i>Streblus asper</i>	-	-	-	+	+
<i>Enhydra fluctunas</i>	+	+	-	+	+

(+): Present, (-): Not present

saponins). Many new natural compounds of diverse structures, isolated from plant sources (Lee, 1999; Mukherjee *et al.*, 2001), have been considered prototypes, leads or heads of series and their later structural modification has afforded compounds with pharmacological activity and extraordinary therapeutic possibilities (Butler, 2004; Paterson and Anderson, 2005; Koehn and Carter, 2005). The brine shrimp bioassay results of *C. longa*, *C. zedoaria*, and *S. asper* clearly demonstrate the toxic effects of extracts, which could be due to any of the secondary metabolites (alkaloids, flavonoid, glycoside or saponin) of the plants. In toxicity assessment of plant extracts by brine shrimp bioassay, an LC₅₀ value lower than 1,000 µg/ml is considered bioactive (Meyer *et al.*, 1982). In our study all the extracts except *S. dulcis*, have LC₅₀ values less than 1,000 µg/ml; therefore, the rest of the four plant extracts can have bio-active secondary metabolites. Further work is required to isolate and characterize the individual bioactive compound.

Conclusion

The present findings suggested that the utilization of *Curcuma longa* powder in curry and in cosmetic might have some beneficial effect as well as *Curcuma longa*, *Curcuma zedoaria*, *Streblus asper* and *Enhydra fluctunas* could be

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