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In-vitro Cytotoxicity and Antioxidant Studies of *Elaeocarpus serratus*

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ABSTRACT

The pet-ether soluble fraction (PEF), Carbon tetrachloride soluble fraction (CTSF) and Chloroform soluble fraction (CFSF) of the methanolic extract of stem bark of Elaeocarpus serratus were investigated for cytotoxic activity using brine shrimp lethality bioassay .The methanolic extract was also evaluated for possible antioxidant activity using nitric oxide scavenging activity and reducing power assays. In Elaeocarpus serratus, the LC_{50} value were found to be 14.94, 0.831 & 3.288 µg/mL in pet-ether soluble fraction (PEF), Carbon tetrachloride soluble fraction (CTSF) and Chloroform soluble fraction (CFSF) of the methanolic extract respectively. The extract showed significant antioxidant activity in nitric oxide scavenging activity and reducing power assay. In nitric oxide scavenging activity, the IC_{50} value of Elaeocarpus serratus extract was 89.325µg/mL while the IC_{50} value of ascorbic acid was 47.684µg/mL. It was found that scavenging of nitric oxide by the extract was concentration dependent.

Key words: *Elaeocarpus serratus***,** Cytotoxicity, Antioxidant*,* Reducing power.

INTRODUCTION

Elaeocarpus serratus Linn. (*Elaeocarpaceae)* locally known as Singhali Jolpai, is a medium sized to big tree tree, planted for its edible fruits and timber in different areas of Bangladesh.Leaves contain myrceicitrin, myrecetin, mearnsetinand ellagic acid.Fruits contain tannin and large amount of plant acids.Ethanolic extract of leaves is diuretic and cardiovascular stimulant and are also used in rheumatism. Fruits are prescribed in diarrhea and dysentery. (Ghani, 1998). Recently, interest has increased considerably in finding naturally occurring antioxidants in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity .In addition, natural antioxidants have the capacity to improve food quality and stability and can also act as nutraceuticals to terminate free radical chain reactions in biological systems and thus may provide additional health benefits to consumers (Brand-Williams et al., 1995). Again brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, antiviral, pesticidal and anti-tumor etc. of the plant extracts (Meyer et al., 1982; McLughilin et al., 1998).In the present study, we evaluated the antioxidant and cytotoxic activity of leaf extracts of *E. serratus.*

MATERIALS AND METHODS

Chemicals and drugs

Ascorbic acid was obtained from SD Fine Chem. Ltd., Biosar, India. Trichloroacetic acid and ferricchloride was obtained from Sigma Chemical Co. TBA (thiobarbituric acid) was obtained from Koch-Light Ltd, Suffolk, UK. Potassium ferricyanide from May and Backer, Dagenham, UK.

Plant material

The plant *Elaeocarpus serratus* was collected from Dhaka in the month of May 2007. A voucher specimen for this collection has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh.

Extraction

The powdered plant sample (500 g) was soaked in 1.5 L of methanol for 16 days and then filtered through a cotton plug followed by Whatman filter paper number 1.The volume of the filtrate was

concentrated with a rotary evaporator at low temperature (40°-50°C) and reduced pressure. The crude methanolic extract was dissolved in 10% aqueous methanol to make the mother solution which was partitioned off successively by three solvents namely pet ether (3x100 ml), carbon tetrachloride (3x100 ml) and chloroform (3x100 ml). All the three fractions and the residual hydromethanol fraction were subjected to dryness under reduced pressure. The dried extracts thus obtained were used for cytotoxic investigation.

BRINE SHRIMP LETHALITY BIOASSAY

Brine shrimp lethality bioassay (McLaughlin *et al.*, 1998; Meyer et al., 1982; Persoone, 1980) technique was applied for the determination of general toxic property of the plant extractives. Brine shrimp eggs collected from pet shops was used as the test organism. Seawater was taken in the small tank. Shrimp eggs were added to one side of the tank, and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was provided throughout the hatching time. The hatched shrimps were attracted to the lamp through the perforated dam and with the help of a pasteur pipette 10 living shrimps were added to each of the vials containing 5ml of seawater.

Preparation of positive control group

Vincristine sulphate was used as the positive control. Measured amount of vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 μg/ml from which serial dilutions were made using DMSO to get 10μg/ml, 5μg/ml, 2.5μg/ml, 1.25μg/ml, 0.625μg/ml, 0.313μg/ml, 0.157μg/ml, 0.079μg/ml and 0.040μg/ml. Then the solutions were added to the premarked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water.

Preparation of negative control group

100 μl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii. If the brine shrimps in these vials show a rapid mortality, then the test is considered as invalid as the nauplii died due to some reasons other than the cytotoxicity of the compounds.

Preparation of test groups

20mg of sample was dissolved in 2ml of DMSO to obtain a solution having concentration of 10μg/μl. From that test solution different volumes were added to premarked glass vials or test tubes containing 5ml of seawater and 10 shrimp nauplii, so as to make the final concentration of samples in the vials or test tubes 200μg/ml, 100μg/ml, 90μg/ml, 80μg/ml, 70μg/ml, 60μg/ml, 50μg/ml, 40μg/ml, 30μg/ml, 20μg/ml and 10μg/ml.

Counting of nauplii

After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

TESTS FOR ANTIOXIDANT ACTIVITY

Assay of Nitric oxide scavenging activity

The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM) in phosphate buffered saline (PBS) was mixed with different concentrations of methanolic extract of *Elaeocarpus serratus* dissolved in methanol and incubated at room temperature for 150 minutes. The same reaction mixture without the methanol extract but the equivalent amount of methanol served as the control. After the incubation period, 0.5ml of Greiss reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride was added. The absorbance of the chromophore formed was read at 546nm (Sreejayan & Rao, 1997).

Reducing power

The reducing power of *Elaeocarpus serratus* was determined according to the method previously described by Oyaizu (Oyaizu, 1986). Different concentrations of *Elaeocarpus serratus* extract (25 – 500 μg) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5ml) and FeCl3 (0.5 ml. 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the reference.

RESULTS AND DISCUSSION

The results of Brine shrimp lethality bioassay are given in Table 1.

Table 1: Brine shrimp lethality bioassay of methanolic extract of *E. serratus*

Sample	LC_{50} (μg/ml)	Regression Equation	R^2
Vincristine Sulfate (Positive control)	0.812	y = 33.219x + 52.781	0.9717
Pet-ether soluble extract	14.94	y = 27.381x + 17.845	0.9404
Carbon tetrachloride soluble extract	3.288	y = 20.334x + 51.635	0.9065
Chloroform soluble extract	0.831	y = 27.381x + 35.845	0.9341

Brine shrimp lethality bioassay was employed to explore a number of novel antitumor, antibacterial and pesticide of natural origin (Mayer *et al*., 1982). In this cytotoxic activity study, mortality of the nauplii was observed in all the experimental groups. Control group nauplii remained unchanged (no lethality/mortality), is indicative of the cytotoxicity of both the extracts. The rate of mortality of the nauplii found to be increased with increased concentration of the sample. A plot of log concentration of the test sample versus percentage of mortality on a graph paper showed an approximately linear correlation between them. From this graphical plotting, the LC_{50} value were found to be 14.94, 0.831 & 3.288 μg/mL in pet-ether soluble fraction (PEF), Carbon tetrachloride soluble fraction (CTSF) and Chloroform soluble fraction (CFSF) of the methanolic extract respectively. The Chloroform soluble extract was found to show significant activity against the Brine shrimp nauplii. The positive response obtained in this assay suggests that the extracts may have bioactive compounds. The result of NO scavenging activity of the plant extract is shown in Fig 1.

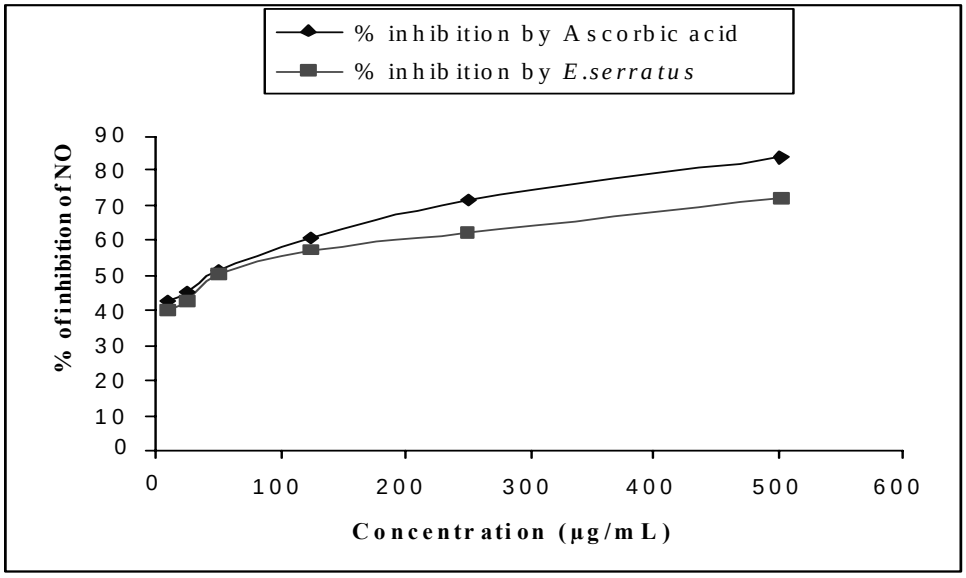

Figure 1: Nitric oxide scavenging activity of the methanolic extract of *Elaeocarpus serratus* compared to Ascorbic acid.

Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with superoxides, such as NO_2, N_2O_4, N_3O_4, NO_{3-}, and NO_2 are very reactive. These compounds are responsible for altering the structural and functional behavior of many cellular components. Incubation of solutions of sodium nitroprusside in phosphate buffer saline at 25^O C for 2 hours resulted in linear time-dependent nitrite production, which is reduced by the extract of *Elaeocarpus serratus*.This may be due to the antioxidant principles in the extract, which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. It is to be noted that *Elaeocarpus serratus* have caused a greater inhibition than ascorbic acid which has shown 84.08% inhibition of NO. The IC_{50} value of the extract is 89.325µg/mL which is greater than that of ascorbic acid (47.684µg/mL). (Rice-Evans et al., 1997; Jorgensen et al., 1999). The result of reducing power of the plant extract is shown in Fig 2.

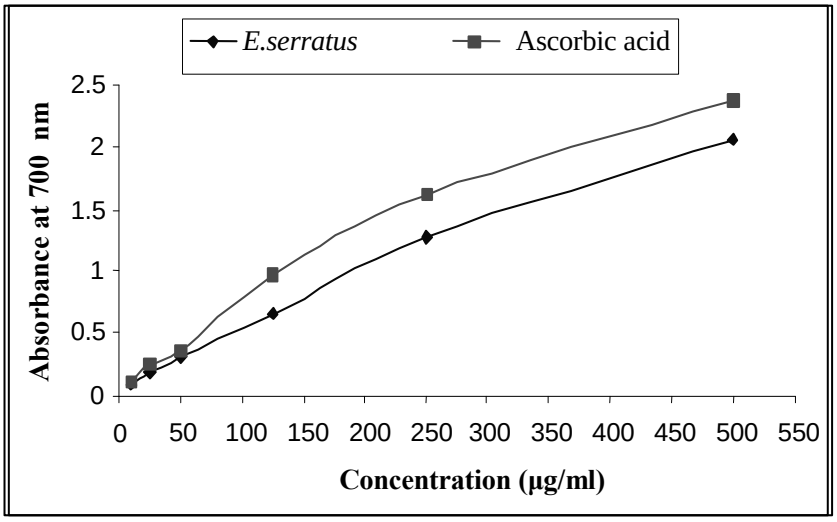

Figure 2: Reducing power of *Elaeocarpus serratus* vs. Ascorbic acid

The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Duh et al., 1999; Gordon, 1990). Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Soares et al., 1997).

CONCLUSION

Based on the results of the present study, it can be concluded that the plant extract possesses remarkable antioxidant and analgesic potential. However, further studies are needed to understand the exact mechanisms of antioxidant and analgesic action and to isolate the compound(s) responsible for such activity.

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