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In vitro Free Radical Scavenging and Brine Shrimp Lethality Bioassay of Aqueous Extract of *Ficus racemosa* Seed

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Abstract

The present work was accomplished to explore the free radical scavenging and cytotoxic potential of the freeze-dried aqueous extract of *Ficus racemosa* seed using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), and brine shrimp lethality bioassay method. In both methods, *F. racemosa* showed a significant activity. In case of free radical scavenging activity, it showed a potent radical scavenging activity with IC_{50} value of 22.10 µg/ml. The cytotoxic activity of the extract was moderate having LC_{50} value of 4.04 µg/ml.

Keywords: 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Brine shrimp, Cytotoxicity, *Ficus racemosa*, Moraceae, Radical scavenging.

1. Introduction

In developing countries, especially in rural contexts, people usually turn to traditional healers when in diseased conditions, and plants of ethnobotanical origin are often presented for use. Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents (Roja and Rao, 2000). Thus, plants are considered as one of the most important and interesting subjects that should be explored for the discovery and development of newer and safer drug candidates.

Several members of the genus *Ficus* (family: Moraceae) are being used traditionally in a wide variety of ethnomedical remedies. One of them is *Ficus racemosa* syn. *Ficus glomerata* (Gular; Udumbara) (Kulkarni and Shahida, 2004) is widely distributed all over India, northern Australia, and other parts of Asia.

Ficus racemosa Linn. (Moraceae), commonly known as 'cluster fig', is used widely in Indian folk medicine for the treatment of various diseases, including jaundice, dysentery, diabetes, diarrhea and inflammatory conditions (Anonymous, 1952).

Apart from the usage in traditional medicine, scientific studies indicate that *F. racemosa* possesses various biological effects such as hepatoprotective (Mandal *et al.,* 2003), chemopreventive (Khan and Sultana, 2005), antidiabetic (Rao *et al.,* 2002a), antiinflammatory (Mandal *et al.,* 2000), antipyretic (Rao *et al.,* 2002b), antitussive (Rao *et al*, 2003), and antidiuretic (Ratnasooriya *et al.,* 2003). The bark has also been evaluated for cytotoxic effects using 1BR3, Hep G2, HL-60 cell lines and found to be safe and less toxic than aspirin, a commonly consumed anti-inflammatory drug (Li *et al.,* 2004).

Previously, it was reported that *F. racemosa* stem bark possesses excellent antioxidant properties *in vitro, ex vivo* (Ahmed and Urooj, 2009a) and *in vivo* in streptozotocin-induced diabetic rats (Ahmed and Urooj, 2009b). Besides, *in vitro* antioxidant activity of *F. racemosa* stem bark has also been reported by Veerapur *et al.,* 2007. However, to the best of our knowledge, free radical scavenging and brine shrimp lethality bioassay of *F. racemosa* seeds extract have not been reported previously. The objective of this work was to explore the free radical scavenging and brine shrimp lethality bioassay of the freeze-dried aqueous extract of *F. racemosa* seeds.

2. Materials and Methods

2.1. Collection and Identification of the plant

The fresh seed of *Ficus racemosa* was collected during February 2009 from the area of Purana Palton, Dhaka. The plant was identified by the National Herbarium where a voucher specimen was deposited having the accession number of 34479.

2.2. Drying and Pulverization

The fresh seed was first washed with water to remove adhering dirt and then cut into small pieces, sun dried for 4

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days. After complete drying, the entire portion was pulverized into a coarse powder with the help of a grinding machine and was stored in an airtight container for further use.

2.3. Extraction of Plant Material

Ten grams of powdered seeds were mixed with 1000 ml distilled water, boiled for 10 min and then cooled for 15 min. Thereafter, the aqueous extract was filtered using a Millipore filter (Millipore 0.2mm) to remove particulate matter. The filtrate was then freeze-dried from BCSIR (Bangladesh Council of Scientific and Industrial Research), Dhaka Bangladesh.

2.4. Screening for free radical scavenging activity

Free radical scavenging activities of the seeds of aqueous extract were determined based on their scavenging potential of the stable DPPH free radical in both qualitative and quantitative assay.

i) Qualitative assay: A suitably diluted stock solutions of extracts were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve both polar and non-polar components of the extracts. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved band was observed for 10 minutes and the color changes (yellow on purple background) were noted (Sadhu *et al.*, 2003).

ii) Quantitative assay: The antioxidant activity of the seeds extract of *F. racemosa* was determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay (Hasan *et. al,* 2006; Koleva *et. al,* 2002; Lee *et. al,* 2003). DPPH offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic anti-oxidants (Cao *et al.,* 1997). DPPH solution was prepared in 95% methanol. The crude extracts of *F. racemosa* were mixed with 95% methanol to prepare the stock solution (5 mg/50mL).

The concentration of the sample solutions was 100µg/ml. The test samples were prepared from stock solution by dilution with methanol to attain a concentration of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing *F. racemosa* extract and after 20 min, the absorbance was taken at 517 nm. Ascorbic acid was used as a positive control. The DPPH solution without sample solution was used as control. 95% methanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation:

% DPPH radical scavenging (%) = [1-(As/Ac)] ×100.

where, Ac=absorbance of control, As =absorbance of sample solution.

Then the percentage of inhibition was plotted against respective concentrations used and IC_{50} value was calculated from the graph using Microsoft Excel 2007.

2.5. Brine shrimp lethality bioassay

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds (Meyer *et al,* 1982; Zhao *et al,* 1992). The brine shrimp, *Artemia salina*, was used as a convenient monitor for the screening.

The eggs of the brine shrimp, *A. salina*, were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 hr to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method. The test samples (extract) were prepared by dissolving in DMSO (not more than 50 µl in 5 ml solution) plus sea water (3.8% NaCl in water) to attain log concentrations of – 0.11 µg/ml, 0.19 µg/ml, 0.49 µg/ml, 0.89 µg/ml, 1.09 µg/ml, 1.40 µg/ml, 1.70 µg/ml, 2.00 µg/ml, 2.30 µg/ml and 2.60 µg/ml. A vial containing 50µl DMSO diluted to 5ml was used as a control. Standard vincristine sulfate was used as positive control (Hossain *et al,* 2004; Khan *et a*l, 2008; Nikkon *et al,* 2003). Then matured shrimps were applied to each of all experimental vials and control vial. After 24 hours, the vials were inspected using a magnifying glass and the number of surviving nauplii in each vial were counted. The lethal concentrations of plant extract resulting in 50% mortality of the brine shrimp (LC_{50}) from the 24 h counts and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis (MS Excel version 7); the LC_{50} was derived from the best-fit line obtained.

3. Results

3.1. The result of free radical scavenging activity

i) Qualitative assay: The color changes (yellow on purple background) on the TLC plates were observed due to the bleaching of DPPH by the resolved bands.

ii) Quantitative assay: The aqueous extract of *F. racemosa* seed showed potential free radical scavenging activity having an IC_{50} value of 22.10 µg/ml (Fig 1). On the other hand the free radical scavenging activity of standard (ascorbic acid) was having an IC_{50} value of 15.93 µg/ml.

3.2. The Result of Brine Shrimp Lethality Bioassay

The lethality of the aqueous extracts of *F. racemosa* seed to brine shrimp was determined on *A. salina* after 24 hours of exposure to the test solutions and the positive control, vincristine sulfate by following the procedure of Meyer *et al.,* 1982. The aqueous extract of *F. racemosa* showed potential cytotoxic activity having an LC_{50} value of 4.04 µg/ml in contrast to the LC_{50} value of standard vincristine sulfate of 0.397 µg/ml (Fig 2).

4. Discussion

Antioxidant deficiency and excess free radical production have been implicated in human hypertension in numerous epidemiological, observational and interventional studies. Herbal antioxidants have been gaining prime importance in the antiradical drug discovery due to lesser side effects as reviewed extensively by many authors (Arora *et al.,* 2005, Meenal *et al.,* 2006). Therefore, the antiradical activity of *F. racemosa* seed extract was studied, as the tendencies of electron or hydrogen donation are critical factors in characterizing antioxidant activity that involves free radical scavenging (Hu *et al.,* 2000). The DPPH radical has been widely used to test the potential of compounds as free-radical

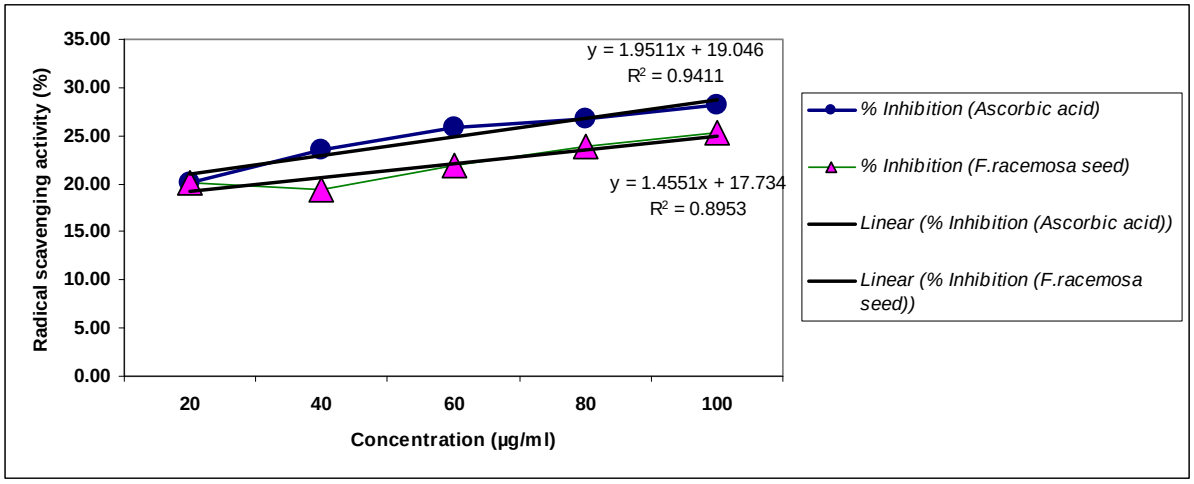

Figure 1: IC $_{50}$ value of aqueous extract of *F. racemosa* seed and ascorbic acid for radical scavenging activity

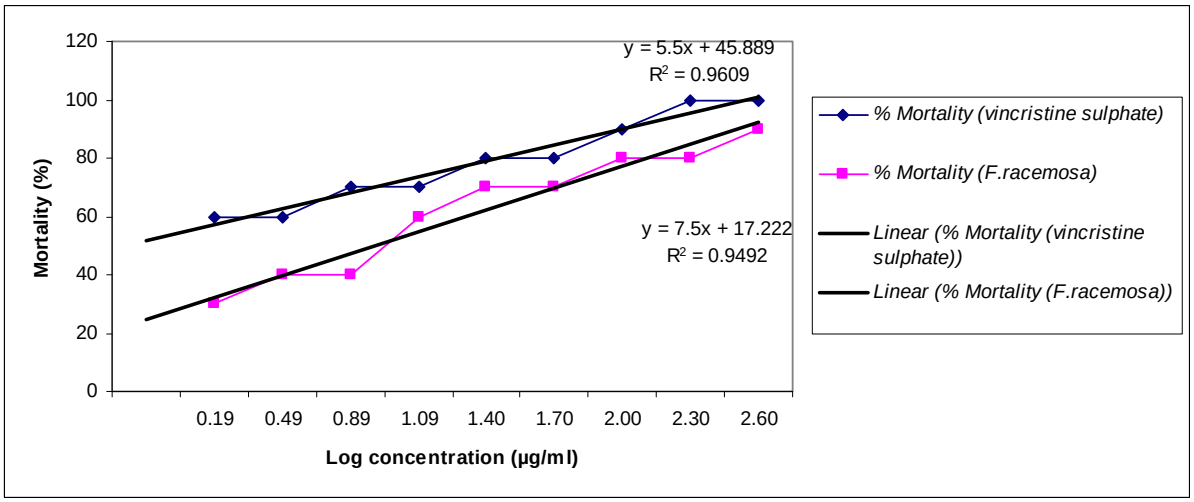

Figure 2: LC $_{50}$ value of aqueous extract of *F. racemosa* seed and vincristine sulfate against the brine shrimp, *Artemia salina*.

scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods (Porto *et al.,* 2000 and Soares *et al.,* 1997). It is a free radical donor that accepts an electron or hydrogen to become a stable diamagnetic molecule.

The present observation that *F. racemosa* seeds extract has free radical scavenging activity can be correlated with other studies done by Ahmed and Urooj, 2009a and Veerapur *et al.,* 2007. This potent antioxidant activity of *F. racemosa* seed may be due to the presence of antioxidant and chemopreventive principles such as, racemosic acid, bergenin, tannins, kaempferol, rutin, bergapten, psoralenes, ficusin, coumarin and phenolic glycosides that have been previously isolated from the bark of this plant (Baruah and Gohain, 1992; Li *et al.,* 2004).

A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in crude extracts is the brine shrimp lethality bioassay (BSLT). The technique is easily mastered, of little cost, and utilizes small amount of test material. 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. It appears that BSLT is predictive of cytotoxicity and pesticidal activity (Ghisalberti, 1993). The result obtained from the brine shrimp lethality bioassay of *F. racemosa* can be used as a guide for the isolation of cytotoxic compounds from the aqueous extract of the seeds of this plant.

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