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COMPARATIVE ANTIOXIDANT ACTIVITY AND BRINE SHRIMP LETHALITY BIOASSAY OF DIFFERENT PARTS OF THE PLANT- *ACACIA AURICULIFORMIS*

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ABSTRACT

The present study was undertaken to evaluate and compare the antioxidant activity and brine shrimp lethality bioassay of different parts (leaf and bark) of the plant *Acacia auriculiformis.* DPPH radical scavenging and NO scavenging capacity were measured for the determination of antioxidant activity. Ethyl acetate fraction of bark was found to possess highest DPPH radical scavenging activity with IC_{50} value of 7.80 µg/ml followed by methanol extract of leaf (IC_{50} value of 7.95 µg/ml). The IC_{50} value of standard ascorbic acid was 33.77 µg/ml. Incase of NO scavenging activity, *n*-hexane fraction of root found to have highest scavenging activity with IC_{50} value of 1.75 µg/ml followed by ethyl acetate fraction of leaf (IC_{50} value of 3.35 µg/ml). The IC_{50} value of standard ascorbic acid was 71.06 µg/ml. When compared with other fractions, the methanol fraction of leaf and bark had highest cytotoxic activity with LC_{50} value of 0.55 and 0.79 µg/ml respectively. This was followed by ethyl acetate fraction of leaf with LC_{50} value of 0.95 µg/ml. The standard vincristine sulfate had LC_{50} value of 0.52 µg/ml. Thus, this study suggests that leaf and bark of *A. auriculiformis* may be a potential source of antioxidant and cytotoxic agents.

INTRODUCTION: An efficient defense system protects us from oxidative stress induced by ROS, however, capacity of the defense system is affected by age, diet, health status of individual [1]. To keep a proper equilibrium between ROS and defense system components, there is a need to provide antioxidants as part of diet [2]. Antioxidants also play important role in preventing oxidative deterioration of food and indirectly eliminating radicals from it [3]. Synthetic antioxidants are often used in foods to prevent oxidative degradation. But due to safety and health effect, using of synthetic antioxidants in foods is negatively perceived by consumers [4, 5]. That is why potential sources of natural antioxidant have been searched in different types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, tree barks, roots, spices and herbs [6].

Keeping this in mind, the present study was designed to reinvestigate and compare the antioxidant activity of different parts of the plant *Acacia auriculiformis.* At the same time, to the best of our knowledge, this is the first report on brine shrimp lethality bioassay of this plant.

Acacia auriculiformis belonging to the family Fabaceae is a vigorously growing, deciduous or evergreen tree, possibly attaining 30 m height is locally known as Akasai, Akasia and Akasmony. It is reported to be rich in methylglucuronic acid, glucuronic acid, galactose, arabinose, and rhamnose [7]. It has also been reported to possess central nervous system – depressant, spermicidal and filaricidal activities due to the presence of tannins and triterpenoid saponins [8, 9, 10].

MATERIALS AND METHODS:

Plant materials: The fresh leaf and bark of the plant *Acacia auriculiformis* were collected from the area of Mohakhali in Dhaka during the month of April, 2011 and taxonomically identified by the National Herberium, Mirpur, Dhaka having accession no. 35576.

Drying and Pulverization: The fresh leaf and bark part of the plant *Acacia auriculiformis were* washed with water to remove adhering dirt and then cut into small pieces and finally sun dried for 7 days. After complete drying, the entire portions were pulverized into a coarse powder with help of a grinding machine and were stored in an airtight container for further use.

Extraction of Plant Material: The powdered 150g of leaf and root part of *A. auriculiformis* was extracted three times with methanol in a flat bottom flask, through occasional shaking and stirring for 7 days. The extracts were then filtered through filter paper. The filtrates were concentrated at 40^{o}C under reduced pressure.

Solvent-solvent partitioning of Methanolic Extracts:

1. **Partitioning with n-hexane:** The concentrated methanolic extracts of *A. auriculiformis* was made slurry with water. The slurry was taken in a separating funnel and few ml of n-hexane (100 ml) was added. The funnel was shaken vigorously and allowed to stand for a few minutes. The n-hexane fraction (upper layer) was collected. The process was repeated three times. The n-hexane fractions of different parts of the plants were the evaporated using rotary evaporator at 40^{o}C.

2. **Partitioning with ethyl acetate:** The concentrated methanolic extracts of *A. auriculiformis* were made slurry with water. The slurry was taken in a separating funnel and few ml of ethyl acetate (100 ml) was added. The funnel was shaken vigorously and allowed to stand for a few minutes. The ethyl acetate fraction (lower layer) was collected. The process was repeated three times. The ethyl acetate fractions of different parts of the plants were evaporated using rotary evaporator at 40^{o}C.

Tests for Antioxidant Activity:

1. **DPPH Radical Scavenging Activity:** The free radical scavenging activity was based on the scavenging activity of the stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical, determined by the method described by Braca *et al.,* 2001 [11]. Plant extract (0.1 ml) was added to 3 ml of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition activity was calculated from $[(A_0–A_1)/A_0]$ x 100, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. The inhibition curves were prepared and IC_{50} values were calculated.

2. **Nitric Oxide Scavenging Assay:** Nitric oxide radical scavenging was estimated on the basis of Griess Illosvoy reaction using method followed by Govindarajan *et al.,* 2003 [12]. In this investigation, Griess-Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5 %). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and plant extract (5 to 250 µg/ml) or standard solution (ascorbic acid, 0.5 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink coloured chromophore formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions.

3. **Brine Shrimp Lethality Bio-assay:** Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds [13, 14]. Here, simple

zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of brine shrimp (*Artemia salina* Leach) were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in a tank at a temperature around 37 °C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. 4.0 mg of each sample was dissolved in 200µl of DMSO. A series of solutions of lower concentrations were prepared by serial dilution with DMSO.

From each of these test solutions 100 µl were added to the pre marked glass test tubes containing 5 ml of sea water and 10 shrimp nauplii. So, the final concentrations of samples in the test tubes were 50µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml respectively. With the help of a pasteur pipette 20 living nauplii were put to each of the vials. After 24 h, the vials were observed and the number of nauplii survived in each vial was counted with the help of magnifying glass. From this, the percentage of lethality of brine shrimp nauplii was calculated for each concentration of the extract. Vincristine sulphate was used as standard cytotoxic agent.

RESULTS AND DISCUSSION:

Antioxidant activity: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical has been widely used to test the free radical scavenging ability of various natural products [15] and has been accepted as a model compound for free radicals originating in lipids [16, 17]. **Fig 1 and 2** depict the DPPH radical scavenging activity of the leaf and root of *A. auriculiformis.* Ethyl acetate fraction of bark found having highest radical scavenging activity with IC_{50} value of 7.80 µg/ml which followed by methanol extract of leaf (IC_{50} value of 7.95 µg/ml). The IC_{50} value of standard ascorbic acid was 33.77 µg/ml.

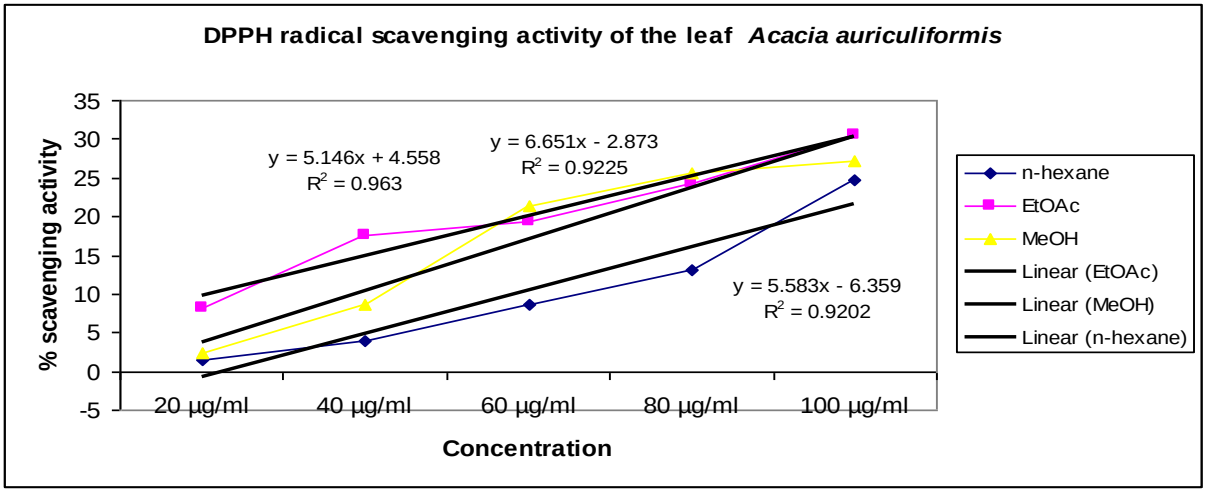

FIG 1: DPPH RADICAL SCAVENGING ACTIVITY OF THE LEAF OF *A. AURICULIFORMIS*

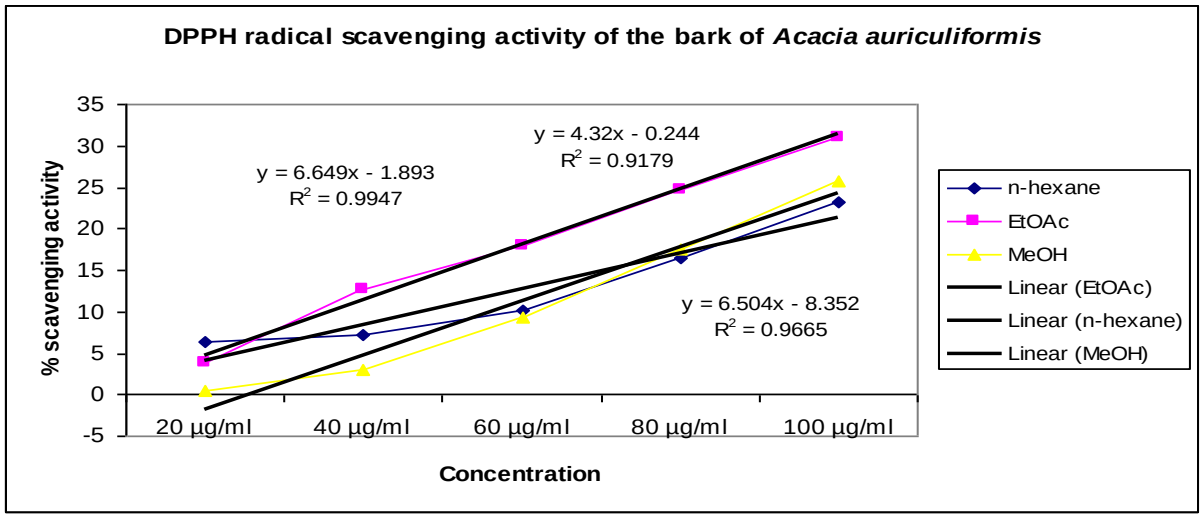

FIG 2: DPPH RADICAL SCAVENGING ACTIVITY OF THE BARK OF *A. AURICULIFORMIS*

Fig. 3 and 4 depict the NO scavenging activity of the leaf and root of *A. auriculiformis.* The n-hexane fraction of root found to have highest scavenging activity with IC_{50} value of 1.75 µg/ml which is followed by ethyl acetate fraction of leaf (IC_{50} value of 3.35 µg/ml). The IC_{50} value of the methanol extract of leaf and bark was 5.86 and 5.77 µg/ml respectively. The IC_{50} value of standard ascorbic acid was 71.06 µg/ml.

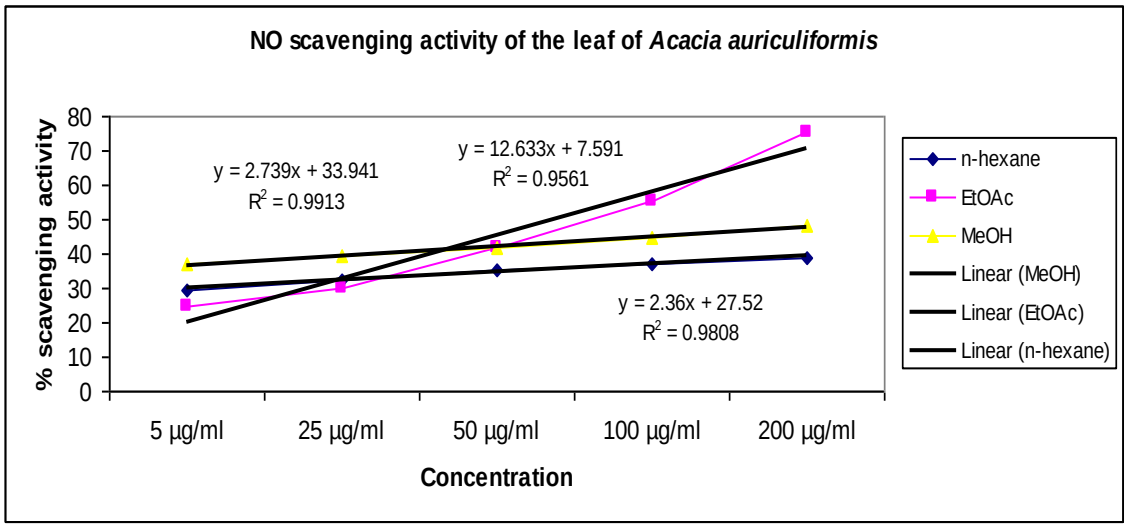

FIG. 3: NO SCAVENGING ACTIVITY OF THE LEAF OF *A. AURICULIFORMIS*

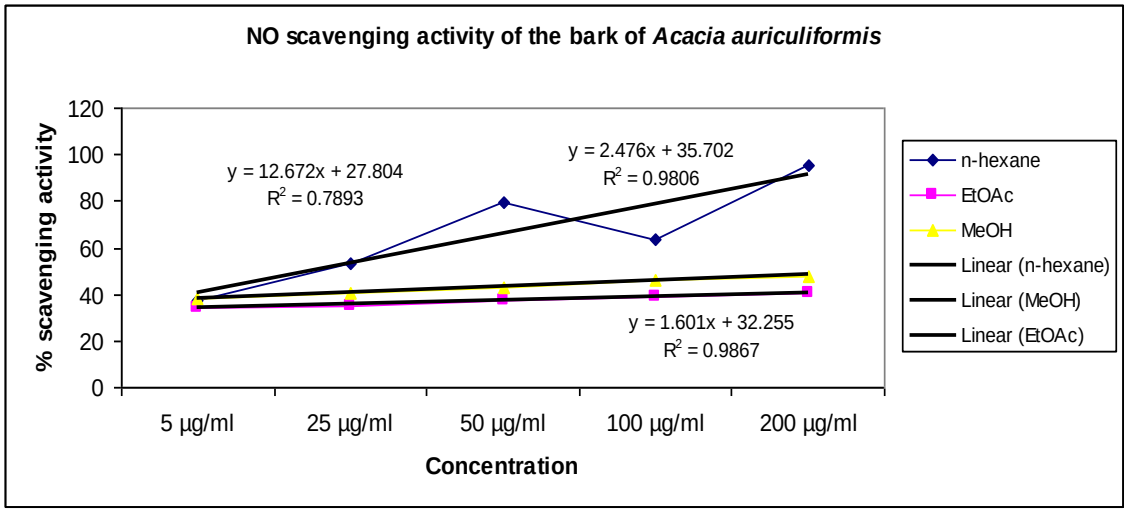

FIG 4: NO SCAVENGING ACTIVITY OF THE BARK OF *A. AURICULIFORMIS*

In a previous study [18] (Singh *et al.,* 2007), it has been observed that the antioxidant potential generally was more with the extracts obtained by decreasing order of polarity. This is congruent with NO scavenging activity for the root of present study. In other cases, the results were not similar.

Brine Shrimp Lethality Bioassay: Fig. 5 and 6 depict the brine shrimp lethality bioassay of the leaf and root of *A. auriculiformis.* For both leaf and bark, the methanol fraction had highest cytotoxic activity with LC_{50} value of 0.55 and 0.79 µg/ml respectively. It was followed by ethyl acetate fraction of leaf with LC_{50} value of 0.95 µg/ml. The standard Vincristine sulfate had LC_{50} value of 0.52 µg/ml.

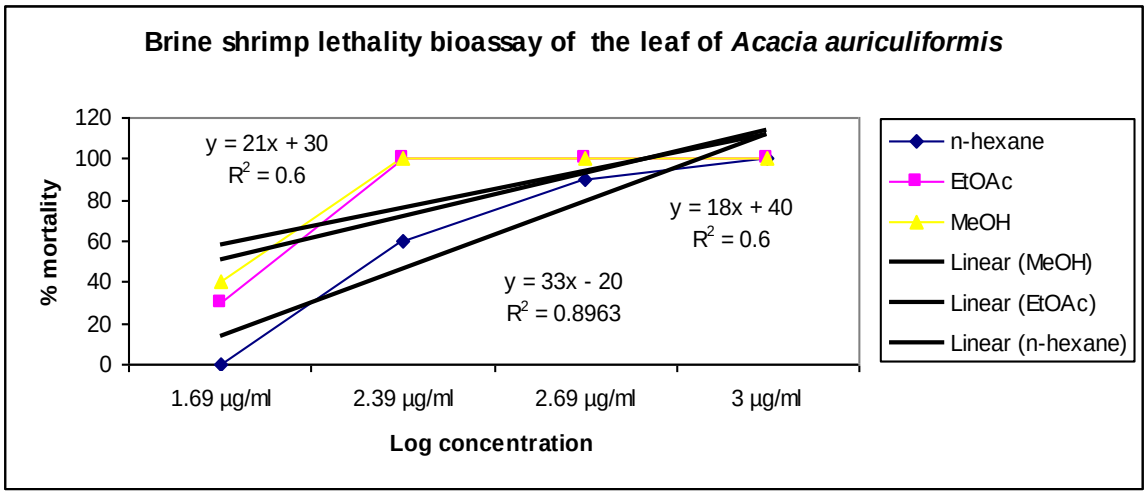

FIG. 5: BRINE SHRIMP LETHALITY BIOASSAY OF THE LEAF OF *A. AURICULIFORMIS*

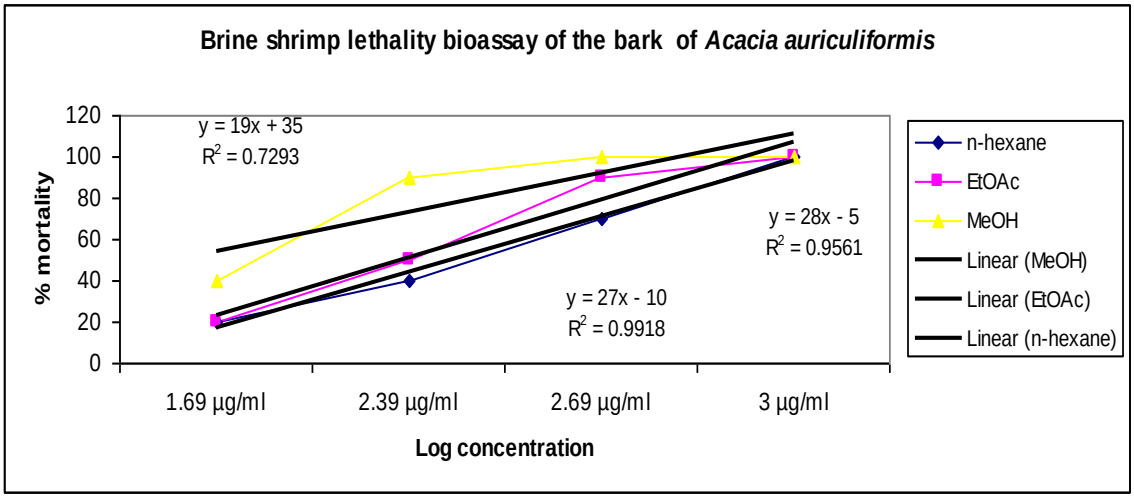

FIG. 6: BRINE SHRIMP LETHALITY BIOASSAY OF THE BARK OF *A. AURICULIFORMIS*

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