Kaniz Fatima Urmi et al /J. Pharm. Sci. & Res. Vol.4(12), 2012, 2004 - 2008

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Antioxidant Activity and Brine Shrimp Lethality Bioassay of Different Parts of the Plant *Calotropis gigantea* R.Br

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Abstract:
The present study reports the antioxidant activity and brine shrimp lethality bioassay of leaf and root of *Calotropis gigantea.* For determining the antioxidant activity, DPPH radical scavenging and NO scavenging capacity were measured. Methanol extract of root was found have highest scavenging activity with IC_{50} value of 2.13 µg/ml which is followed by *n*-hexane fraction with IC_{50} value of 3.80 µg/ml. The radical scavenging activities of leaf fractions were lower than that of the fractions of root. The IC_{50} value of standard ascorbic acid was 33.77 µg/ml. Incase of NO scavenging activity, methanol of extract of leaf was found to have highest scavenging activity with IC_{50} value of 0.97 µg/ml than the methanol extract of root. *n*-hexane fraction of root (IC_{50} value of 1.06 µg/ml) was found to have more activity than *n*-hexane fraction of leaf (IC_{50} value of 1.16 µg/ml). The IC_{50} value of standard ascorbic acid was 71.06 µg/ml. Incase of brine shrimp lethality bioassay, the highest cytotoxic activity was observed for methanol extract of root with LC_{50} value of 0.56 µg/ml followed by ethyl acetate fractions (LC_{50} value of 0.95 µg/ml) and *n*-hexane fraction (LC_{50} value of 1.36 µg/ml). The lowest activity was observed for *n*-hexane fraction of leaf (LC_{50} value 2.42 of 1.36 µg/ml). Thus from present study, it can be deduced that leaf and root of *C. gigantea* may be a good source of potent antioxidant and cytotoxic agent.

Key words: DPPH, NO, *Calotropis gigantea*, brine shrimp.

INTRODUCTION:

Antioxidants are compounds that play important role in preventing or delaying the onset of major degenerative diseases. Physiologically, these compounds scavenge the free radicals. Free radicals are highly unstable and reactive species which are capable of damaging molecules such as DNA, proteins and carbohydrates. The body is under constant attack from these free radicals formed as a consequence of the body's normal metabolic activities [1]. A potent scavenger of these species may act as a possible preventive for free radical-mediated diseases [2]. In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule [3]. It has been found from the recent studies that a number of plant products including polyphenolic substances (e.g. flavonoids and tannins) and various plant or herb extracts exert antioxidant actions [4-8].

Calotropis gigantea belonging to the family Asclepiadaceae known as milkweed, is a laticiferous shrub widely distributed in Bangladesh, India, Burma, Pakistan and sub Himalayan tract [9]. The roots and leaves of *Calotropis gigantea* has been reported to be used traditionally for treatment of abdominal tumors, boils, syphilis, leprosy, skin diseases, piles, wounds, rheumatism, insect-bites, ulceration and elephantiasis [10]. Different parts of this plant have been reported to exhibit multiple therapeutic properties like anti-inflammatory, analgesic, anticonvulsant, anxiolytic, sedative, antidiarrhoeal and antipyretic [11-14].

The present study was designed for determining the antioxidant activity and brine shrimp lethality of leaf and root part of *Calotropis gigantea* R.Br.

MATERIALS AND METHODS:

Plant materials:

The fresh leaf and root of the plant *Calotropis gigantea R.Br* were collected from the area of Mirpur in Dhaka during the month of February, 2011. *Calotropis gigantea R.Br* was taxonomically identified by The National Herberium. The accession number of *Calotropis gigantea R.Br* is 35546.

Extraction of plant material:

The powdered 150g of leaf and root part of *Calotropis gigantea R.Br* was extracted three times with methanol in a flat bottom glass container, through occasional shaking and stirring for 7 days. The extracts were then filtered through filter paper. The filtrates were concentrated at 40ºC under reduce pressure.

Solvent-solvent partitioning of methanolic extracts:

Partitioning with *n*-hexane:

The concentrated methanolic extracts of *Calotropis gigantea R.Br* 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 40^0 C.

Partitioning with ethyl acetate:

The concentrated methanolic extracts of *Calotropis gigantea R.Br* was 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 40^0 C.

Tests for Antioxidant Activity:

DPPH radical scavenging activity:

The free radical scavenging activity of the extract, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Braca *et al.*, 2001 [15]. 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 $[(A_0–A_1)/A_0]$ x100, 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.

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 [16]. 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.

Brine Shrimp lethality Bio-assay

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds [17-18]. 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.

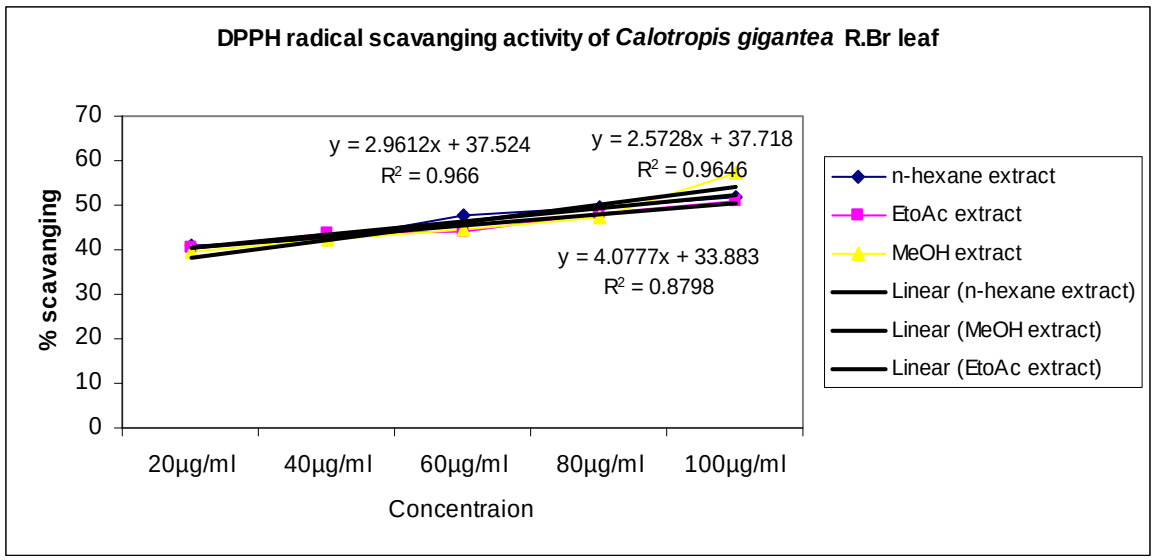
4.0 mg of each sample was dissolved in 200µl of DMSO. A series of solutions of lower concentrations were prepared by serial dilution with DMSO. From each of these test solutions 100 µl were added to the pre marked glass test tubes containing 5 ml of sea water and 10 shrimp nauplii. So, the final concentration of samples in the test tubes was 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:

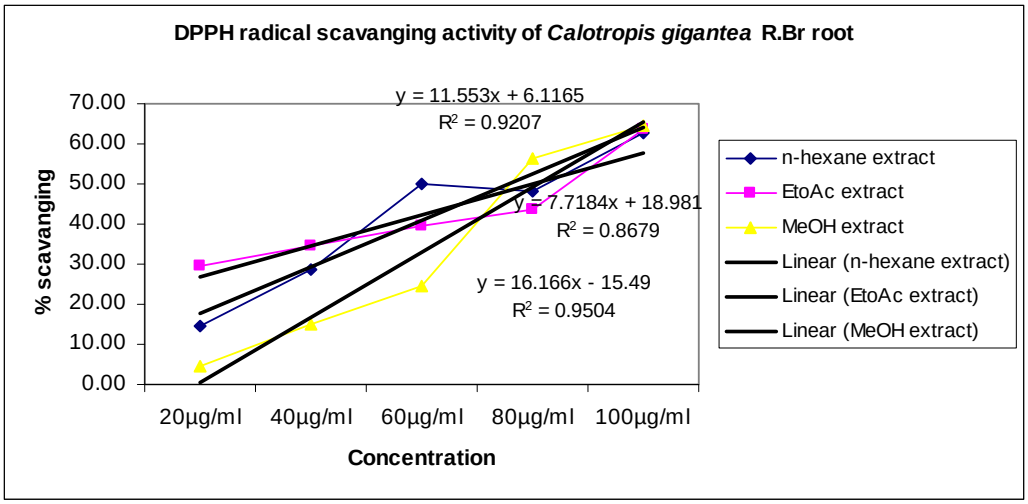
Antioxidant activity:

Incase of DPPH radical scavenging activity, methanol extract of root was found have highest activity with IC_{50} value of 2.13 µg/ml which is followed by *n*-hexane fraction of root with IC_{50} value of 3.80 µg/ml. The radical scavenging activities of leaf fractions were lower than that of the fractions of root. The IC_{50} value of standard ascorbic acid was 33.77 µg/ml (Graph 1 and 2). For NO scavenging activity, the reverse trend of result was observed. The methanol of extract of leaf was found to have highest scavenging activity with IC_{50} value of 0.97 µg/ml than the methanol extract of root. *n*-hexane fraction of root (IC_{50} value of 1.06 µg/ml) was found to have more activity than *n*-hexane fraction of leaf (IC_{50} value of 1.16 µg/ml). The IC_{50} value of standard ascorbic acid was 71.06 µg/ml (Graph 3 and 4).

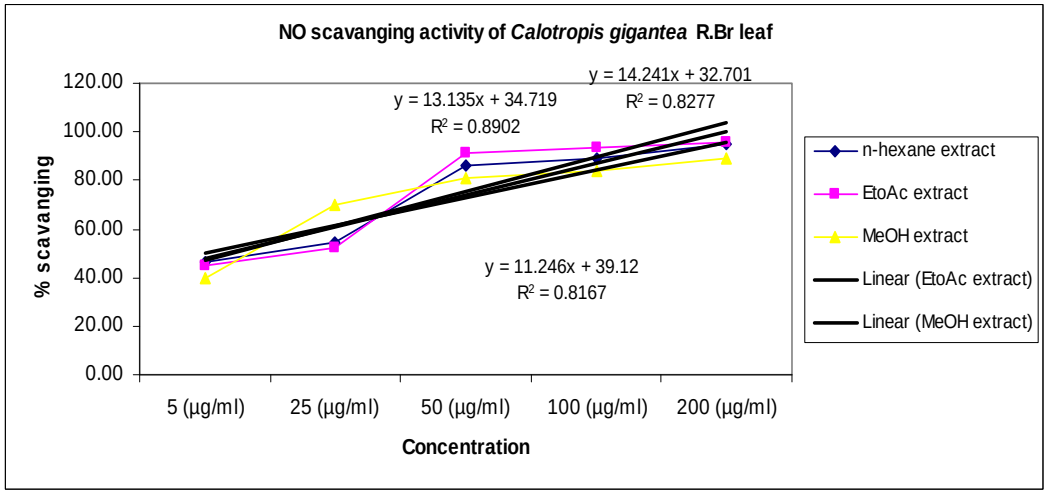
Graph 1: DPPH radical scavenging activity of *Calotropis gigantea* leaf



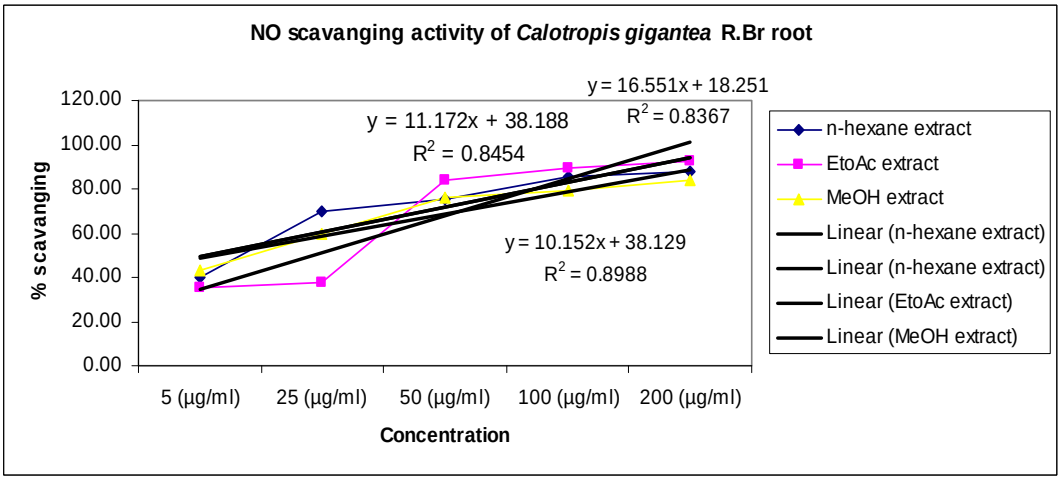
Graph 2: DPPH radical scavenging activity of *Calotropis gigantea* root



Graph 3: NO scavenging activity of *Calotropis gigantea* leaf



Graph 4: NO scavenging activity of *Calotropis gigantea* root.



In a study conducted by Rathod, *et al.*, 2009 [19], it has been reported that administration of *C. gigantea* leaf and flower extracts significantly improved the level of superoxide dismutase (SOD) and catalase (CAT) in STZ-induced diabetic rats.

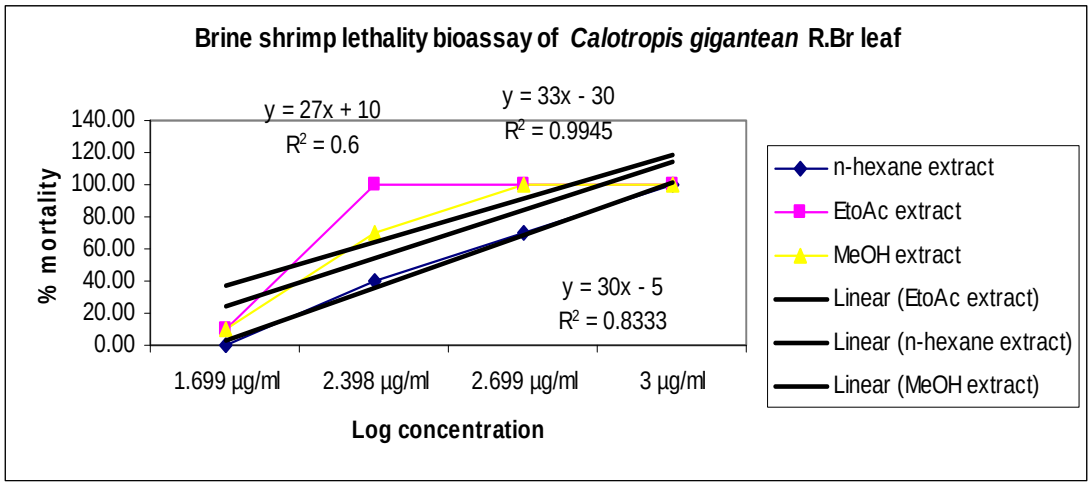
The SOD and CAT are the two major scavenging enzymes that remove free radicals *in vivo* [20]. A decreased activity of these enzymes can lead to an excess availability of superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), which in turn generate hydroxyl radicals ($^{\cdot}OH$), resulting in initiation and propagation of lipid per oxidation (LPO). The

SOD can catalyze dismutation of ($O_2^{\cdot-}$) into H_2O_2, which is then deactivated to H_2O by catalase or SOD works in parallel with selenium-dependent glutathione peroxidase, which plays an important role in the reduction of H_2O_2 in the presence of reduced glutathione forming oxidized glutathione, and it protects cell protein and cell membranes against oxidative stress[20,21]. The observed free radical scavenging activity of *C. gigantea* may be due to increase the level of SOD and CAT.

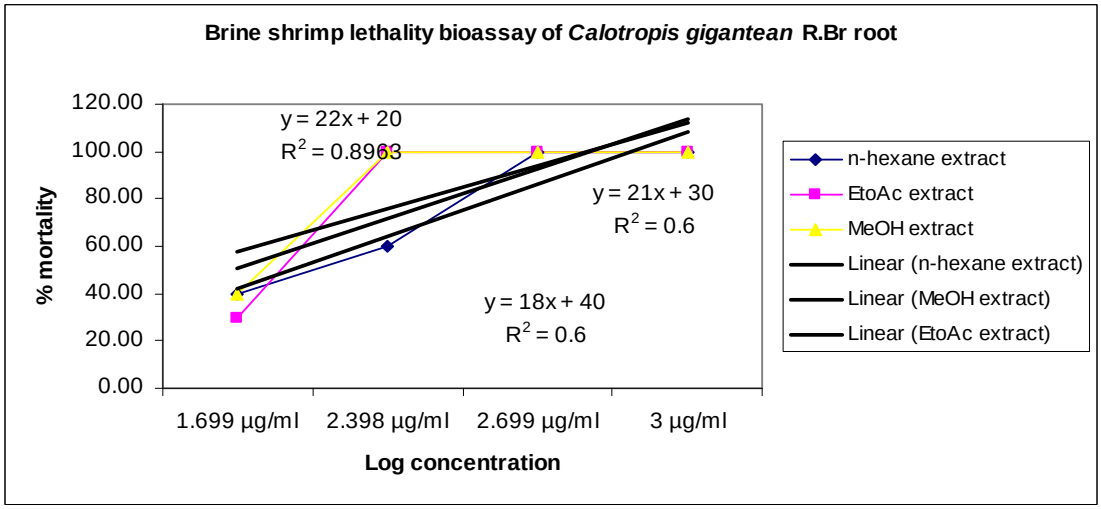
Brine shrimp lethality bio-assay:

All the fractions showed potent cytotoxic activity with LC_{50} value ranging from 0.56 to 2.42 µg/ml. The highest cytotoxic activity was observed for methanol extract of root with LC_{50} value of 0.56 µg/ml followed by ethyl acetate fractions (LC_{50} value of 0.95 µg/ml) and *n*-hexane fraction (LC_{50} value of 1.36 µg/ml). The lowest activity was observed for n-hexane fraction of leaf (LC_{50} value 2.42 of 1.36 µg/ml) (Graph 5 and 6). The observed cytotxic activity of this plant may be due to the compound anhydrosophoradiol- 3-acetate or other compounds as well. It has been found from the previous research that anhydrosophoradiol- 3-acetate exhibited potent cytotoxicity against A549, SK-OV-3, SK-MEL-2, MES-SA and HCT-15 tumour cell lines [22].

Graph 5: Brine shrimp lethality bioassay of *Calotropis gigantea* leaf.



Graph 6: Brine shrimp lethality bioassay of *Calotropis gigantea* root.



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