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Antioxidant and Cytotoxic Activities of Methanol Extract of *Urena lobata* (L) Leaves

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The methanol extract of leaves of *Urena lobata* (L) (Malvaceae) has been selected to determine the antioxidant and cytotoxic potentials. The crude extract were tested for *in vitro* free radical scavenging assays such as 1, 1 diphenyl-2-picryl-hydrazyl (DPPH). Total phenolic and flavonoids content of *U. lobata* were analyzed. The sample effectively scavenged free radical at all different concentrations and showed potent antioxidant activity. Finally cytotoxic activity has been assayed using brine shrimp lethality bioassay where vincristine sulphate was used as standard. The antoxidant results were compared with standard antioxidant such as ascorbic acid and quercetin. The whole study shows that the crude methanol leaves-extract has potent antioxidant and cytotoxic activities.

Keyword: *Urena lobata*, Antioxidant, Cytotoxicity and Brine Shrimp.

1. Introduction

The vast majority of complex life on earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species[1]. The reactive oxygen species produced in cells include hydrogen peroxide, hypochlorous acid, and free radicals such as the hydroxyl radical and the superoxide anion[2]. These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins[3]. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms[4,5], while damage to proteins causes enzyme inhibition, denaturation and protein degradation[6] Therefore, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids[3,7]. In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell[1,3] However, reactive oxygen species also have useful cellular functions, such as redox signaling. So, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level[8] Antioxidants are also being investigated as possible treatments for neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic

lateral sclerosis[9,10], and as a way to prevent noise-induced hearing loss[11].

Urena lobata L locally known known as Banokra(Bangladesh) belongs to the family Malvaceae. It is a shrubby perennial, up to 2 m high. Leaves usually broader than long, up to 11.3 cm long, cordate, serrate or toothed, stellately hairy, roundish, angled; lobes generally acute or acuminate, varying in size and numbers. Flowers small, clustered in the axils; corolla 15 mm long, pink. Capsules pubescent, covered with blunt spines. Roots are a popular diuretic; used externally for lumbago and rheumatism. Decoction of stem and root is used in windy colic. The flowers are used as a pectoral and expectorant in dry coughs. Infusion of flowers is used as a gargle for aphthae and sore-throat. Leaves are used in abscess. Seeds contain an enzyme, urease. They also contain pentosans, mucilage and proteins[12]. It is widely distributed in India and Bangladesh. Various studies show that it has antimicrobial[13] antioxidant[14] and antidiarrhoeal[15] effects but according to our knowledge there are no any scientific detailed reports on leaves as cytotoxic activity. So, our present investigation was to design to determine the antioxidant potential along with cytotoxic activity of methanol extract of leaves of *Urena lobata*.

2. Material & Methods

2.1 Chemicals

1-diphenyl, 2-picryl hydrazyl(DPPH) trichloroacetic acid, ferric chloride, and quercetin were obtained from Sigma chemical Co. USA. Ascorbic acid was obtained from SD Fine chem. Ltd., Biosar, India and potassium ferricyanide from May and Backer, Dagenham, UK. Methanol, Folin-ciocalteu reagent, Sodium carbonate and Potassium ferricyanide were purchased from Merck, Germany. All chemicals used were of analytical reagent grade.

2.2 Plant Materials

The fresh leaves of *Urena lobata* were collected from the local area of Chittagong, Bangladesh and was authenticated by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Bangladesh.

2.3 Preparation of Crude Extract

The leaves were dried at room temperature in the shade and away from direct sunlight for 5 days and in hot air oven for 2 days. The dried leaves were then coarsely powdered and extracted keeping 7 days with methanol. The sediments were filtered and filtrates were dried at 40ºC in a water bath. The solvent was completely removed by filtering with Hartman filter paper and obtained dried crude extract kept in a refrigerator which was used for investigation.

2.4 Antioxidant Activity

2.4.1 Dpph Radical Scavenging Assay

DPPH antioxidant assay is based on the ability of 1, 1 diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the change in absorbance and % of scavenging activity is calculated. This method was described by Braca *et al* (2001).[16] According to this method 0.1 ml sample extract was added to 3 ml of a 0.004% methanol solution of DPPH and three different concentrations (125, 250, 500 and 1000µg/ml) were made. At 517 nm Absorbance was taken after 30 min and the percentage inhibition activity was calculated by using the following equation-

$$\% \text{ scavenging activity} = [(A_0-A_1)/A_0] \text{ X100}$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the extract. The inhibition curves were prepared and an IC_{50} value was calculated from the graph.

2.5 Total Phenol Content

To measure total phenol content of plant extract, extract (200µg/ml) was mixed with 400 µl of the Folin-Ciocalteu reagent and 1.5 ml of 20%

sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. The mixture was allowed to stand for 2 hours. Here three different concentration(10, 50, 100 and 200 µg/ml). Thus, phenols are the source of the antioxidants in the Folin assay. Then the absorbance at 760 nm was determined. These data were used to estimate the phenolic contents using a standard curve obtained from various concentration of ascorbic acid. Total phenol content was expressed as mg of ascorbicacid equivalent. This method was described by Singelton *et al* (1999)[17].

2.6 Total Flavonoids Content

Flavonoids are a group of polyphenolic compounds which was determined using a method of Kumaran *et al* (2007)[18] using quercetin as a reference compound. According to this method 1 ml of plant extract in methanol (200µg/ml) was mixed with 1 ml aluminium trichloride in methanol (20 mg/ ml) and a drop of acetic acid, and then diluted with methanol to 25 ml. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 1 ml of plant extract and a drop of acetic acid, and then diluted to 25 ml with ethanol. The total flavonoid content was determined using a standard curve of quercetin (12.5, 25, 50 and 100 µg/ml) and compard with standard quercetin antioxidant.

2.7 Cytotoxicity screening

The Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds using simple zoological organism (Artemia salina) for convenient monitoring for the screening. The eggs of the brine shrimp were collected from an aquarium shop and hatched in artificial seawater (3.8% NaCl solution) for 48 hours to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using method of Meyer *et al* (1982).[19] The test sample extract were prepared by dissolving them in DMSO plus sea water (3.8% NaCl in water) to attain concentrations of 1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200 and 200 µg/ml. A vial containing 50 µl DMSO diluted to 5 ml was used as negative control. Standard vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. The vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted after 24 hours. From the obtained data, the percent (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the

formula: $\% \text{ Mortality} = \frac{N_t}{N_0} \times 100$ Where, Nt = Number of killed nauplii after 24 hours of incubation, N_0 = Number of total nauplii transferred i.e 20.

Then Median lethal concentration(LC_{50}) was then determined.

3. Results

3.1 Antioxidant Screening

DPPH radical scavenging activity

The activity was increased by increasing the concentration of the sample extract. Concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage versus concentration. The results of DPPH radical scavenging assays on plant extracts and ascorbic acid were in the fugure 1. The IC_{50} of the standard ascorbic acid and methanol extract of *Urena lobata* were 150 µg/ml, and 180 µg/ml respectively. So, comparison with the ascorbic acid, it is clear that plant extracts possess potential antiradical activity.

3.2 Total phenol and Flavonoid Content

The total amount phenol and flavonoid which are obtained in separate experiment have been shown in the Table 1.

Table 1: Total amount of phenol, flavonoid content of methanolic extract of leaves

Extract	Total phenol	Total flavonoid
Urena lobata	211.95µg/ml	230.40µg/ml

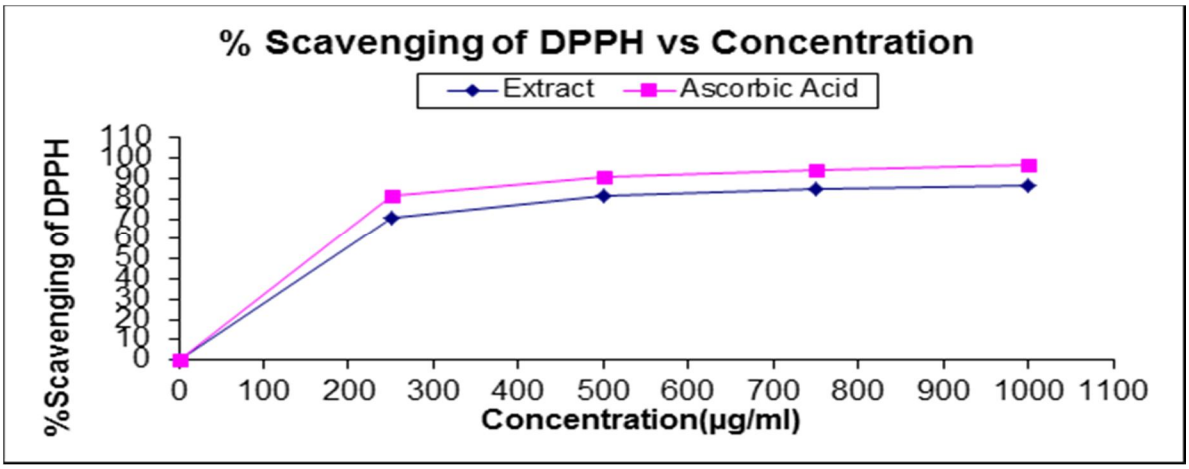

Figure 1: DPPH Radical Scavenging Activity of the Methanolic Extract of *Urena Lobata* Leaves

3.3 Brine Shrimp Lethality Bioassay

The results of brine shrimp lethality bioassay are shown in the table 2. Test samples showed different mortality rate at different concentration. The mortality rate of brine shrimp napulii was found to be increased with the increase with the concentration of the sample. The median lethal concentration (LC_{50}) of methanol of extract*Urena lobata* was 37.50 µg/ml and that forstandard vincristine sulphate was 11.00 µg/ml. No mortality was found in the control group, using DMSO and sea water.

Table 2: Cytotoxicity effects of various concentrations of methanol extract of *Urena Lobata* on the viability of brine shrimp nauplii after 24 hours of incubation

Concentration(µg/ml)	No. of nauplii taken	No. of nauplii dead	% of mortality	LC_{50} µg/ml
1.56	20	0	0	37.50
3.12	20	2	10	
6.25	20	2	10	
12.5	20	3	15	
25	20	5	25	
50	20	15	75	
100	20	18	90	
200	20	20	100	

4. Discussion

Plants have been the traditional source of raw materials for medicine.[20] The trend of using natural products has increased and the active plant extracts are frequently for new drug discoveries.[21] In recent years one of the areas which attracted a great deal of attention is the possible therapeutic potential of antioxidants in controlling degenerative diseases associated with marked oxidative damage. DPPH antioxidant assay is based on the ability of 1, 1 diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to

decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the change in absorbance and % of scavenging activity is calculated. Flavonoids include flavones, flavonols, flavanones, and derivatives and conjugates thereof. In addition, other phenolic and polyphenolic compounds are present in plants such as cinnamic acid derivatives, for example, chlorogenic acid, and isomers of flavones known as isoflavones. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable.

Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. Researchers can either look for cytotoxic compounds, if they are interested in developing a therapeutic that targets rapidly dividing cancer cells, for instance; or they can screen "hits" from initial high-throughput drug screens for unwanted cytotoxic effects before investing in their development as a pharmaceutical.

5. Conclusion

This experiment shows that the methanol extract of leaves of *Urena lobata* possesses strong antioxidant and cytotoxic potential. However, these studies are preliminary in nature and there are plenty of scopes for further studies to examine underlying mechanisms of antioxidant and cytotoxic effects and to carry clinical trial to determine the active compounds responsible for these pharmacological activities which can result in development of a novel compound for drug discovery.

6. References

1. Davies KJ. Oxidative stress: The paradox of aerobic life. Biochemical Society Symposia 1995; 61:1–31.
2. Valko M, Leibfritz D, Moncol J, Cronin M, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology 2007; 39 (1):44–84.
3. Sies H. Oxidative stress: oxidants and antioxidants. Experimental physiology 1997; 82(2):291-295.
4. NakabeppuY, Sakumi K, Sakamoto K, Tsuchimoto D, Tsuzuki T, Nakatsu Y. Mutagenesis and carcinogenesis caused by the oxidation of nucleic acids. Biological Chemistry 2006; 387(4): 373–379.
5. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence". Molecular and Cellular Biochemistry 2004; 266 (1):37–56.
6. Stadtman ER. Protein oxidation and aging. Science 1992; 257(5074):1220–1224.
7. Vertuani S, Angusti A, Manfredini S. The Antioxidants and Pro-Antioxidants Network: An Overview. Current Pharmaceutical Design 2004; 10 (14):1677–94.
8. Rhee SG.CELL SIGNALING: H_2O_2, a Necessary Evil for Cell Signaling. Science 2006; 312 (5782):1882–1883.
9. Di MV, Esposito E. Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Curr Drug Targets CNS Neurol Disord 2003; 2 (2):95–107.
10. Rao A, Balachandran B. Role of oxidative stress and antioxidants in neurodegenerative diseases. Nutr Neurosci 2002; 5(5):291–309.
11. Kopke RD, Jackson RL, Coleman JK, Liu J, Bielefeld EC, Balough BJ. NAC for noise: from the bench top to the clinic. Heart Research 2007; 226 (1–2):114–25.
12. Ghani A. Medicinal Plants of Bangladesh with chemical constituents and uses. Edn 2, Asiatic Society of Bangladesh, Dhaka, 2003.
13. Adeloye OA, Akinpelu A D, Ogundaini OA, Obafemi A. Studies on antimicrobial, antioxidant and phytochemical analysis of *Urena lobata* Leave extract. Journal of Physical and Natural Sciences 2007; 1(2):1-9.
14. Lissy KP, Simon TK, Lathab MS. Antioxidant potential of *Sida retusa*, *Urena lobata* and *Triumfetta rhomboidea*. Anc Sci Life. 2006; 25(3&4):10–15.
15. Arun KY, Vareishang T. Antidiarrheal Activity of Lithocarpus dealbata and *Urena lobata* Extracts: Therapeutic Implications. Pharmaceutical Biology 2007; 45(3):223–229.
16. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia terapotensis*. Journal of Natural Products 2001; 64:892-895.

17. Singelton VR, Orthifer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 1999; 299:152-178.
18. Kumaran A, Karunakaran AJ. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. Food Science and Technology 2007; 40:344-352.
19. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JE, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plants constituents. Journal of Medicinal Plants Research 1982; 45:31-34.
20. Hopking A. The glimpse over the history of Herbalism. Godshaer Herbalist advanced botanical center of medicine 2004.www.godshaer.co.uk/history.htm
21. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research 2010; 4(2):104-111.