PHYTOCHEMICAL SCREENING AND IN VITRO ANTIOXIDANT ACTIVITY OF CHLOROFORM EXTRACT OF *URENA SINUATA* (L.)

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ABSTRACT:

The present study was designed to investigate antioxidant properties of chloroform extract of Urena sinuata along with phytochemical study for the presence of phytochemical constituents. Phytochemical analyses were found to be positive for carbohydrates and gum, reducing sugar, alkaloid, steroid, glycoside and flavonoids. Antioxidant potential was evaluated using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assays. In DPPH scavenging method, scavenging of DPPH was observed in different concentrations (20, 40, 60, 80, 100, 200, 400, 800µg/ml). Plant extract found to demonstrate significant scavenging activity which was found to increase with concentration of the extract with IC50 value of 10.64µg/ml while IC50 value of the reference ascorbic acid was 1.61µg/ml.

Keywords: Urena sinuate (L.), Phytochemical, DPPH scavenging method, Antioxidant**.**

INTRODUCTION

Different approaches to drug discovery from plants can be enumerated as: random selection followed by chemical screening, random selection followed by one or more biological assays, follow-up of biological activity reports, follow-up of ethno medicinal (traditional medicine) use of plants, use of appropriate plant parts as such in powdered form or preparation of enriched/standardized extracts (herbal product development), use of a plant product, biologically potent, as a lead for further chemistry, and single new compounds as drugs (Samuelsson,1999). The future of plants as sources of medicinal agents for use in investigation, prevention, and treatment of diseases is very promising (Sofowora,1982). The objective of the later approach is the targeted isolation of new bioactive plant products, i.e. lead substances with novel structures and novel mechanisms of action. *Urena sinuata* L. (Family: *Malvaceae*), locally known as 'Kunjia' in Bangladesh, is a medicinal herb, which has a good reputation in Bangladesh, India and many other countries of the world as a folk medicine for the treatment of a variety of disease such as bronchitis, low back pain, anti-rheumatic, antipyretic etc. The Roots of the plant are considered as emollient, refrigerant and maturant; used as an external application for lumbago (Low back pain). Leaves are prescribed in inflammation of the intestine and the bladder; decoction is given in colic. But till to date, sporadic attempts have been made for the scientific and methodical validation of these traditional claims. The present research suggests that chloroform extract of *Urena sinuata* has significant antioxidant activity. Thus the plant may be a source of effective herbal drug.

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MATERIALS AND METHODS:

Collection and Identification of Plant

The plant *Urena sinuata* was collected from University of Chittagong and identified by Syedul Alam, Research Assistant, Bangladesh Forest Research Institute, Chittagong. A voucher specimen that contains the identification characteristics of the plant was submitted to the herbarium for future reference.

Preparation of Plant Extract

The fresh *Urena sinuata* plant was washed with water immediately after collection. The collected leaves were chopped into small pieces, air dried at room temperature $(25\pm2)^0$C for about 15 days and ground into powder form and stored in an airtight container. 200 gm powder was macerated in 900 ml pure chloroform for 7 days at room temperature with occasional stirring. 7 days later, chloroform extract was filtered off through a cotton plug and finally with a Whatman No. 1 filter paper. The colur of the extract was deep green. The extract was concentrated under reduced pressure within 50-55^0C through rotatory vaccum evaporator (Bibby Sterlin Ltd., England). The concentrated extracts

were collected in a Petri dish and allow to air dry for complete evaporation of chloroform. The whole process was repeated three times and finally, 23.649 gm blackish-green colored, concentrated stem extract was obtained (yield 16.30 % w/w) which was kept in refrigerator at 4^0C (Ghani,2003).

Phytochemical Investigation of *Urena Sinuata*

The freshly prepared crude chloroform extract was qualitatively tested for the presence of chemical constituents. These were identified by characteristic color changes using standard procedures (Ghani,2003; Sofowara,1993; Trease and Evans,1989).

DPPH radical scavenging assay (Brand-William, *et al,*1995; Braca A, *et al,*2001):

The antioxidant activity of *Urena sinuata* chloroform extracts and the standard antioxidant ascorbic acid was assessed on the basis of the radical scavenging effect of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH)-free radical activity with slight modifications. DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can generate stable free radicals in aqueous or methanol solution. With this method it was possible to determine the antiradical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm (Rice-Evans, *et al,*1995). Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form, this molecule has an absorbance at 517 nm which disappears after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.

Experimental Methodology

Urena sinuata chloroform extract with different concentrations (20, 40, 60, 80, 100, 200, 400 and 800µg/ml) were prepared in methanol. Ascorbic acid with different concentrations (20, 40, 60, 80, 100, 200, 400 and 800µg/ml) were prepared in methanol. 0.004% DPPH solution was prepared in methanol. 3 ml of this DPPH solution was mixed with 5 ml of extract solution and standard solution separately. These solution mixtures were kept in dark for 30 min. The degree of DPPH purple decolorization to DPPH yellow indicated the scavenging efficiency of the extract (Suresh *et al*., 2008). The absorbance of DPPH solution (Control solution `A') was measured at 517 nm using UV-Visible Spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan). The absorbance of the mixture was determined at 517 nm using UV-Visible Spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan). Ascorbic acid was served as a positive control. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity.

The (%) scavenging activity against DPPH was calculated using the following equation:

Scavenging activity (%) = [(A - B) / A] x 100

Where,

A was the absorbance of control (DPPH solution without the sample),

B was the absorbance of DPPH solution in the presence of the sample (extract/ ascorbic acid). Then, % scavenging activity or % inhibition was plotted against log concentration and from the graph IC_{50} (Inhibition concentration 50) value was calculated by linear regression analysis (Emran , *et al,*2012).

Standard and test solution preparation

Stock solution of plant extract and ascorbic acid made 5mg/ml. Eight screw cap tubes were labeled as 20, 40, 60, 80, 100, 200, 400 and 800µg/ml.5ml of methanol was taken in each screw cap tube. 20, 40, 60, 80, 100, 200, 400 and 800µl of methanol were discarded (put off) from the screw cap tubes according to label - using micropipette.20, 40, 60, 80, 100, 200, 400 and 800µl stock solution of conc. 5mg/ml were added (put) accordingly – using micropipette.

RESULTS AND DISCUSSION

Phytochemical Screening (Zhang and Guo, 2001; Zhao,C.S. *et al,* 2003; Zhao, G. *et al*,1992) of *Urena sinuata* extract under this study explored the presence of medicinally active secondary metabolites carbohydrate and gum, reducing sugar, alkaloid, steroid, glycoside and flavonoids. This investigation also indicated the absence of tannins and saponins. These findings with their corresponding results are summarized in Table 1.

Table 1: Observation and Result of Phytochemical screening

Secondary metabolite	Name of the test	Observation	Result
Carbohydrate and Gums	Molish test	Red violet ring was produced	++
Reducing sugar	Fehling's solution test	Brick red color ppt.	++
	Benidict's test	Red color ppt.	++
Alkaloids	Dragendroff's test	Orange brown ppt.	++
	Wagner's test	Reddish brown ppt.	++
	Hager's test	Yellowish ppt.	++
	Mayer's test	Yellow color ppt.	++
Steroids	Salkowski reaction	Red color in chloroform layer	++
	Libermann-Burchard reaction	Light green color	++
Glycosides	Salkowski reaction	Red color in chloroform layer	++
	Libermann-Burchard reaction	Light green color	++
Tannins	Ferric chloride test	No black ppt. was present	--
	Potassium dichromate test	No orange ppt.	--
Flavonoids	Hydrochloric acid test	Red color	++
Saponins	Foam test	No foam production	--

N.B. "++" stands for the presence and "--" indicates the absence of secondary metabolites.

DPPH radical scavenging assay

DPPH free radical scavenging method was used for the assay of *Urena sinuata* chloroform extract and the scavenging activity was compared with the standard antioxidant ascorbic acid (Vitamin C). The DPPH free radical scavenging activity of the *Urena sinuata* chloroform extract and ascorbic acid is shown in Table 2 and Table 3; Fig. 1, 2. Both ascorbic acid and *Urena sinuata* chloroform extract showed dose dependent activity. Among the eight different concentrations used in the study (20, 40, 60, 80, 100, 200, 400 and 800µg/ml) ascorbic acid showed 67.78%, 72.89%, 77.82%, 82.75%, 87.15%, 89.26%, 93.13% and 95.95% scavenging activity where highest scavenging activity was 95.95% at concentration 800µg/ml (Table 2). On the other hand, *Urena sinuata* extract showed 46.48%, 62.85%, 68.66%, 78.17%, 80.63%, 82.75%, 84.33% and 87.85% scavenging activity at the above mentioned eight different concentration 800µg/ml (Table 3).

% of scavenging activity or % of inhibition was plotted against log concentration and from the graph IC_{50} (Inhibition concentration 50) value was calculated by linear regression analysis. IC_{50} value of ascorbic acid and *Urena sinuata* chloroform extract

Table 2: DPPH free radical scavenging activity of ascorbic acid (Standard)

Ascorbic acid				IC_{50}
Concentration (µg/ml)	Log Concentration	Absorbance	% Scavenging activity	
Control	-	0.568	-	
20	1.30	0.183	67.78	
40	1.60	0.154	72.89	
60	1.78	0.126	77.82	1.61µg/ml
80	1.90	0.098	82.75	
100	2.00	0.073	87.15	
200	2.30	0.061	89.26	
400	2.60	0.039	93.13	
800	2.90	0.023	95.95	

Table 3: DPPH free radical scavenging activity of *Urena sinuata* chloroform extract

Urena sinuata chloroform extract				IC_{50}
Concentration (µg/ml)	**Log Concentration**	**Absorbance**	**% Scavenging activity**	
Control	-	0.568	-	
20	1.30	0.304	46.48	
40	1.60	0.211	62.85	
60	1.78	0.178	68.66	10.64µg/ml
80	1.90	0.124	78.17	
100	2.00	0.110	80.63	
200	2.30	0.098	82.75	
400	2.60	0.089	84.33	
800	2.90	0.069	87.85	

was found 1.61 and 10.64µg/ml respectively (Table 2 and Fig 1, 2 & 3).The Standard Deviation (SD) of ascorbic acid and *Urena sinuata* chloroform extract was 704.05568 & 278.861. It means that , the greater the SD , the greater is the variation between two observations.

CONCLUSION

A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs" . Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. In the present study, studied on chemical properties was carried out on *Urena sinuata* (Family: *Malvaceae*). Traditional records proved that Malvaceae plants produce diverse classes of pharmacologically active compounds and some of the Malvaceae species used as emollient, refrigerant and maturant; used as an external application for lumbago. Leaves are prescribed in inflammation of the intestine and the bladder; decoction is given in

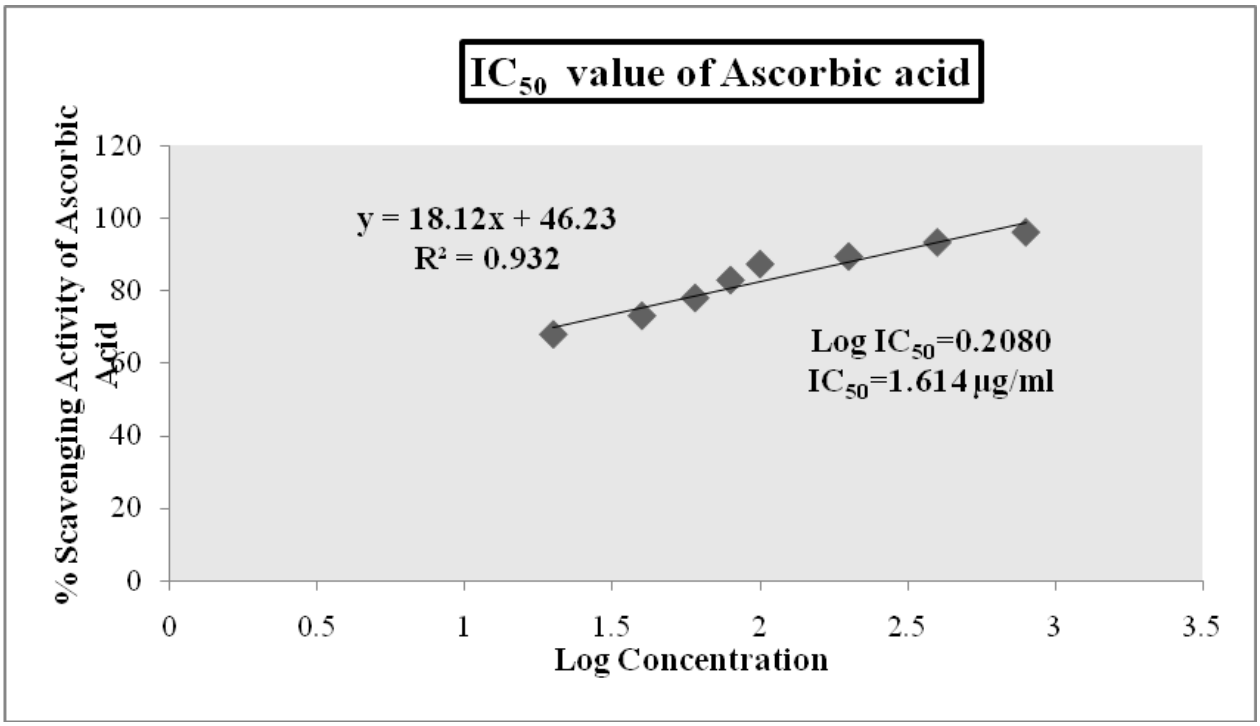

Figure 1: IC_{50} value of ascorbic acid was calculated from above plot linear regression analysis.

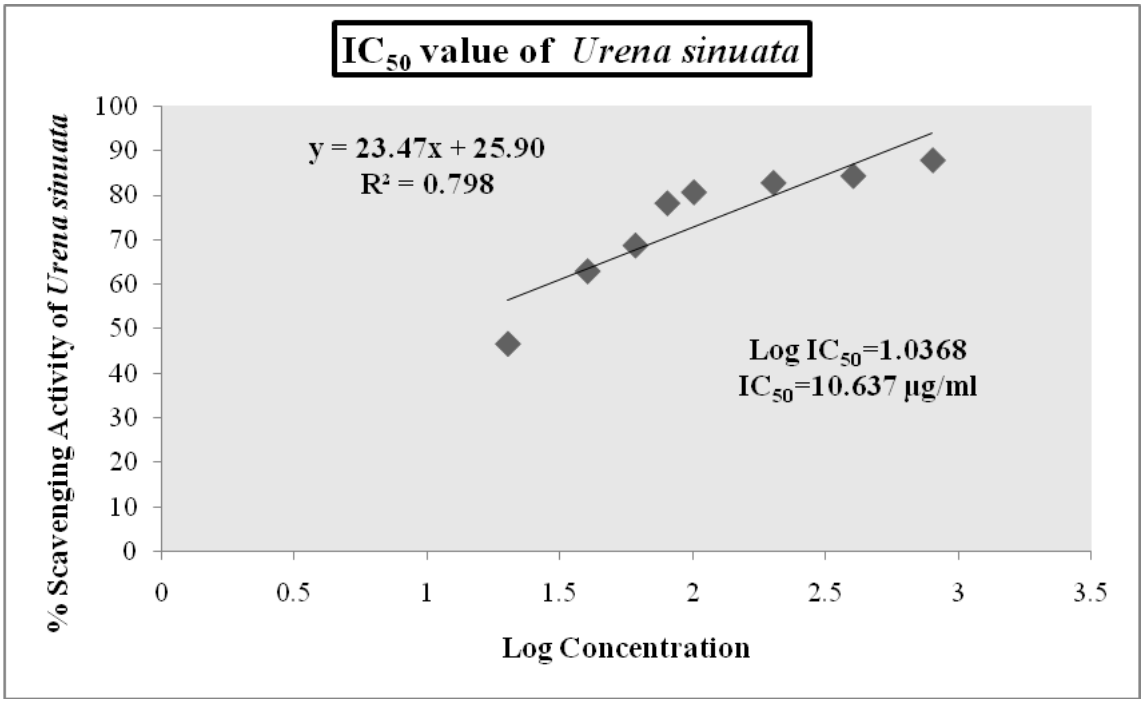

Figure 2: IC_{50} value of *Urena sinuata* chloroform extract was calculated from above plot linear regression analysis.

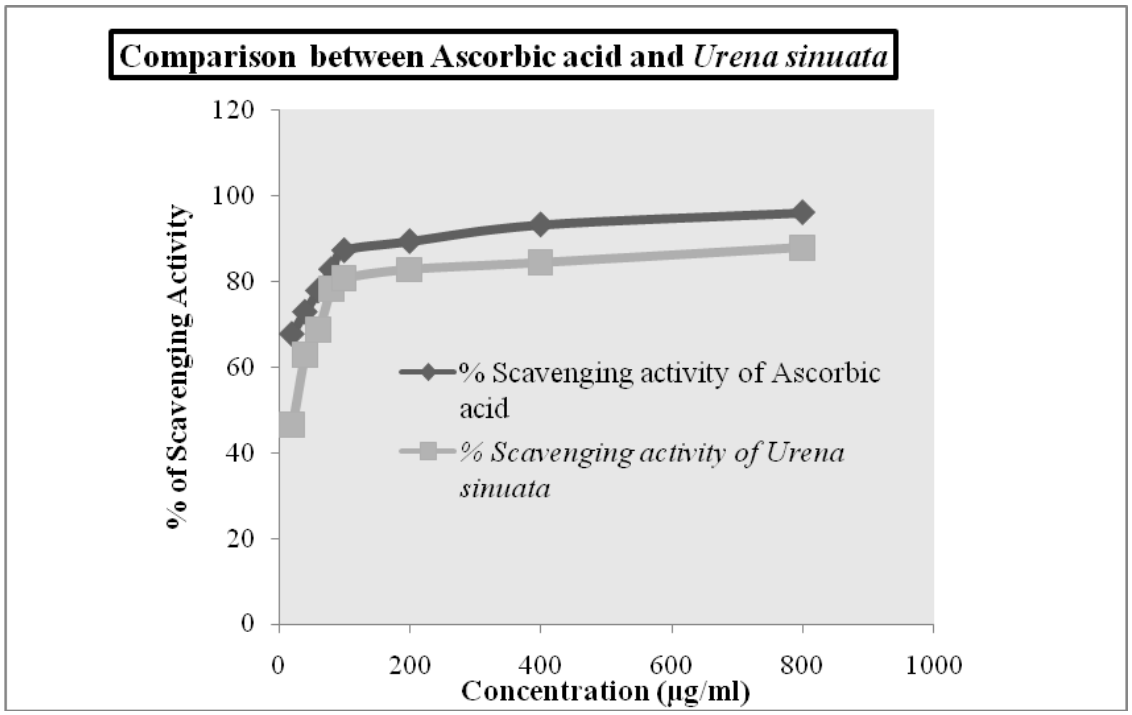

Figure 3: Relative % scavenging activity or % inhibition of standard antioxidant ascorbic acid and *Urena sinuata* chloroform extract.

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colic. Infusion of the flowers is used in bronchitis.
Chemical group tests were analyzed qualitatively on *Urena sinuata* chemical group test showed that carbohydrate and gum, alkaloid, glycosides, flavonoids and reducing sugar were present in the chloroform extract of *Urena sinuata.* The antioxidant study shows that it has significant antioxidant activity.

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