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Antioxidant, Antibacterial and Cytotoxic Activity of the Methanol Extract of *Urtica Crenulata*

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Abstract

The antioxidant, antibacterial and cytotoxic properties of methanol extract of *Urtica crenulata* (syn: *Laportea crenulata* Gaud) stem has been investigated in the present study. The antioxidant and cytotoxic activity of the *Urtica crenulata* methanol extract were assessed by DPPH free radical scavenging method and brine shrimp lethality bioassay method, respectively. The antibacterial activity of the plant extract (500 µg/disc) was also carried out by disc diffusion technique. Stem extract showed DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging effect compared with ascorbic acid. IC_{50} value of ascorbic acid and stem extract was found 14.72 µg/ml and 1468.9 µg/ml, respectively. In antibacterial experiment, *Urtica crenulata* stem extract showed 8, 14 and 10 mm of diameter of zone inhibition against *Salmonella typhi, Shigella flexneri* and *Shigella sonnei*, respectively and 9 and 8 mm of diameter of zone inhibition against *Bacillus subtilis* and *Bacillus cereus* but no activity was observed against *Staphylococcus aureus*. In brine shrimp lethality assay, the LC_{50} value of the extract was found 104.0 µg/ml, which indicates that the extract has high cytoxic effect. The present study demonstrates that methanol extract of *Urtica crenulata* stem has significant cytotoxic effect. The extract also showed some moderate antibacterial and minimum significant antioxidant effects.

Keywords: Urtica crenulata; Antioxidant; Antibacterial; Cytotoxic; BHT.

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1. Introduction

Urtica crenulata Roxburgh (Syn: *Laportea crenulata*, Gaud) locally known as Agnichutra, is an evergreen shrub [1, 2] that is widely distributed to Bangladesh, India, Srilanka and Malay island [2, 3]. It is 3.7 m tall, branchless spreading and semi woody with elliptic, oblong or obovate-lanceolate rarely rhombic leaf. The shrub contains formic

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acid, mucilage, ammonia, carbonic acid, protein, calcium, phosphorus, iron, magnesium, and beta-carotene, along with vitamins A, C, D, and B complex. Recently a new triterpenoid 2alpha, 3beta, 21beta, 23, 28-penta hydroxyl 12-oleanene and two known compounds, beta-sitosterol and beta-sitosterol 3-beta-D-glucopyranoside have been isolated from the roots of Urtica crenulata Gaud [4].

It is widely used by the tribal communities of Chittagong Hill tracts for ailments of various diseases. The plant is used traditionally for the treatment of bleeding from nose and mouth, excessive acid in the stomach, constipation, weakness, asthma, gout, mumps, whooping cough and chronic fever [2, 4, 5]. The root of the plant is a helpful remedy for ailments of the urinary tract and is said to reduce susceptibility to rheumatic problems and colds. The medicinal use of roots of the *Urtica crenulata (U. crenulata)* and its related species have already been documented and described by various authors [2,3] but pharmacological studies have yet to be performed to evaluate the scientific basis of the use of the plant. In this context, this is a matter of interest to evaluate the pharmacological activity like anti-oxidant, antibacterial and cytotoxic properties of *U. crenulata* stem extract.

Antioxidants are reducing agents, and limit oxidative damage to biological structures by passivating free radicals. Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters show low solubility and have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants [6,7]. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Besides well known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or a nutritional supplements. Also many other plant species have been investigated in the search for novel antioxidants [8-11] but generally there is still a demand to find more information concerning the antioxidant potential of plant species.

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency [12-17]. In this matter of view, development of new antimicrobials from plant sources would be an interesting area of study.

Scientists have been proving that all the natural things are not good for health. Different retrospective studies done over the last 20 years indicated that the incidence of deaths occurring due to exposure to plants (as a proportion of total patients poisoned by traditional plant medicine) was about 1.5% in France, 5% in Belgium, 6.5% in Italy, 7.2% in Switzerland and 6% in Turkey [18]. There is, therefore, a need to have an understanding of the risks posed by herbal medicines so as to ensure that such products

could be used safely. However, study on cytotoxicity of plant extract could give us an idea of the anticancer as well as toxic profile of studied plant extract.

2. Materials and Methods

Collection of plant

The stems of *U. crenulata* were collected from Chittagong Hill Tracts, Bangladesh. The plant was taxonomically identified by Industrial Botany Research Division, Bangladesh Council for Scientific and Industrial Research (BCSIR) Laboratories, Chittagong.

Preparation of plant extract

The fresh stems of *U. crenulata* were washed with distilled water immediately after collection. The collected stems were chopped into small pieces, air dried at room temperature for about 10 days and ground into powder and stored in an airtight container. The resulting stem powder (850g) was extracted in Erlenmeyer flasks with cold MeOH for 7 days at room temperature. The whole extract was combined and evaporated to dryness in vacuo to give 45g (yield 5.3 % w/w) of blackish-green colored stem extract which was kept in refrigerator at 4ºC.

In *vitro* assay for antioxidant activity of plant extract

The antioxidant activity of *U. crenulata* extract was assessed in comparison to standard antioxidant ascorbic acid (Sigma, Germany) on the basis of scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical activity according to established procedure [19. Standard ascorbic acid solution (1ml) and different concentrations (10, 50,100,200,400,600 and 800 µg/ml in methanol) of 1ml of *U. crenulata* solution were mixed with 3 ml of 0.4 mM DPPH solution. The mixtures were kept in dark for 30 minutes to measure the absorbance at 517 nm using UV-Visible Spectrophotometer (Cintra, Australia) and ascorbic acid was used as a positive control. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity.

Assay for antibacterial activity of plant extract

Antibacterial activity of plant extract was determined by disc diffusion method as described by Bauer *et al.* [20]. Three Gram (+) bacteria (*Bacillus subtilis*, Lab ID-BTCC17; *Streptococcus aureus*, Lab ID-BTCC 43, and *Bacillus cereus*, Lab ID-BTCC19) and three Gram (-) bacteria (*Salmonella typhi*, Lab ID-ICDDRB; *Salmonella flexneri*, Lab ID- ICDDRB and *Salmonella sonnei*, Lab ID- ICDDRB) were used for the present study. All the test bacteria were collected from Industrial Microbiology Research Division of BCSIR Laboratories, Chittagong. Dried filter paper discs (4nm in diameter) impregnated

in known amount of test substances (500 µg/discs) were placed on Mueller-Hinton agar medium uniformly seeded with the test organisms. Tetracycline discs (30 µg/disc) soaked in respective solvent were used as positive control. These plates were then kept at low temperature (4ºC) for two to four hours to allow maximum diffusion of compound. The diffusion occurred according to the physical law that controls the diffusion of molecules through agar gel [21]. The plates were then incubated at 37ºC for 24 hours to allow maximum growth of the microorganisms. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganisms giving the clear distinct zone around the disc called "Zone of Inhibition". The antibacterial activity of the test material was determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

Assay for cytotoxicity of plant extract

Cytotoxic activity of plant extract was determined by Brine-Shrimp lethality bioassay as described by Meyer *et al*. [22]. Shrimp eggs were added to the artificial "sea water" (25g salt per liter water) in the larger compartment of an unequally dividend tank which was darkened by covering it with Aluminum foil. The chamber was kept under illumination using a table lamp for 48 h for the eggs to hatch into shrimp larvae. The illuminated compartment attracts shrimp larvae (nauplii) through perforations in the dam. 20 shrimp larvae were added to 5 ml of sea water in 5 test tubes and 1000, 500, 250, 125 and 62.5 µg/ml solution of extracts were added to these test tubes. This procedure repeated three times. A control containing 5 ml of DMSO solvent was used for each solvent. The test tubes were maintained under illumination. After 24 hours have elapsed, survivors were counted with the aid of a 3X magnifying glass. The LC_{50} values were calculated from Probit Chart using computer software "BioStat-2007".

3. Results and Discussions

Assay for antioxidative activity of *U. crenulata* stem extract

Antioxidative activity of *U. crenulata* stem extract was measured by DPPH free radical scavenging method (Table 1) and their scavenging activity was compared with the standard antioxidant ascorbic acid (Fig. 1). Both ascorbic acid and stem extract showed dose dependent activity. However, stem extract showed minimum significant amount of DPPH free radical scavenging effect compared to ascorbic acid. Among seven different concentrations (10 to 800 µg/ml), 800 µg/ml showed the highest (42%) scavenging activity. On the other hand, ascorbic acid showed 40%, 60%, 62%, 70%, 86%, 88% and 88% activity at 10, 25, 50,100, 200, 400, 800 µg/ml, respectively (Table 1). Percent (%) scavenging activity or % inhibition was plotted against log concentration and from the graph IC50 (Inhibition concentration 50) value was calculated by linear regression analysis. IC50 value of ascorbic acid and stem extract was found 14.72 µg/ml and 1468.9 µg/ml, respectively (Table 1).

Assay for antibacterial activity of *U. crenulata* stem extract

Crude extract of *U. crenulata* (500 µg/disc) *showed m*oderate activity against all three Gram (-) bacteria but poor activity was found against Gram (+) bacteria. The zone of inhibition against S*almonella typhi, Shigella flexneri and Shigella sonnei* was 8, 14 and 10 mm, respectively whereas 9 and 8 mm for two Gram (+) bacteria *Bacillus subtilis and Bacillus cereus*, respectively. No activity was observed for *Streptococcus aureus.* On the other hand, standard antibiotic tetracycline (30 µg/disc) showed significant antibacterial activity against all tested Gram (+) and Gram (-) bacteria (Table 2).

Table 1. DPPH free radical scavenging activity of ascorbic acid and *U.crenulata* stem extract.

Test material	Concentration (µg/ml)	% Scavenging activity	IC50 (µg/ml)
Ascorbic acid	10	40	IC50 = 1468.9
	25	60	
	50	62	
	100	70	
	200	86	
	400	88	
	800	88	
Urtica crenulata stem extract	10	10	IC50 = 14.72
	25	20	
	50	32	
	100	35	
	200	38	
	400	39	
	800	42	

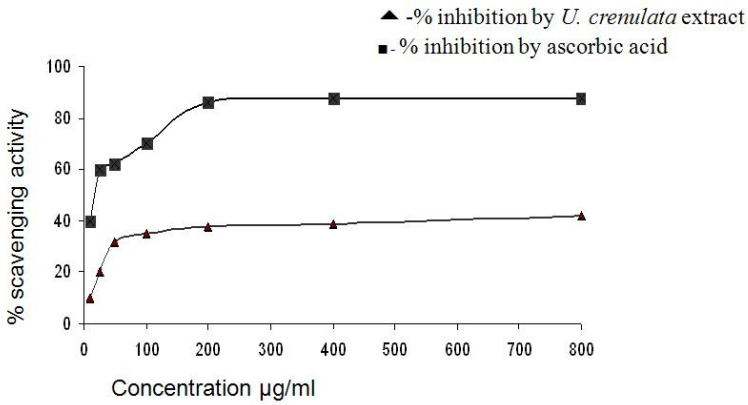

Fig. 1. Comparative % inhibition of DPPH showed by standard antioxidant (ascorbic acid) and *U. crenulata* stem extract.

Table 2. *In vitro* antibacterial activity of *U. crenulata* stem extract.

Bacterial type	Test organism	Diameter of zone of inhibition (mm)	
		U. crenulata stem extract (500 µg/disc)	Tetracycline (30 µg/disc)
Gram (+)	*Bacillus subtilis*	9	25
	Staphylococcus aureus	-	27
	Bacillus cereus	8	28
Gram (-)	*Salmonella typhi*	8	28
	Shigella flexneri	14	26
	Shigella sonnei	10	30

Assay for cytotoxicity of *U. crenulata* stem extract

Brine-Shrimp lethality bioassay for five different concentrations (62.5 to1000µg/ml) of *U. crenulata* extract showed that the percent mortality of brine shrimp was 20%, 70%, 85%, 95%, and 98.75%, respectively at 62.5, 125, 250, 500, 1000 µg/ml (Table 3). From the % lethality of brine shrimp, the probits were calculated for each concentration by using computer program "BioStat-2007". Probits were then plotted against corresponding stem extract log concentration to get LC_{50} (log concentration 50) value through regression analysis. LC_{50} value of stem extract was found 104.0 µg/ml with 95% confidence limit where the lower and upper limits were 72.22 and 135.94 µg/ml (Table 4).

Table 3. Brine Shrimp lethality assay of *U. crenulata* stem extract.

Dose (µg/ml)	Log dose	Total	Alive	Death	%Lethality	Actual %	Probit Y
62.5	1.80	20	16	4	20	20	4.17
125	2.10	20	6	14	70	70	5.51
250	2.40	20	3	17	85	85	6.04
500	2.70	20	1	19	95	95	6.55
1000	3.00	20	0	20	100	98.75	8.12

Table 4. Calculation of LC_{50} value, regression equation and confidence limit by probit analysis.

Log10 [LC_{50}]	LC_{50} (µg/ml)	95% Confidence limit (µg/ml)	Regression equation
2.01	104.0	72.22- 135.94	$Y = - 1.0740 + 2.9800*X$

As we mentioned in the result, that 800 µg/ml of stem extract provided the highest DPPH free radical scavenging effect (42%) compared to ascorbic acid (88%), IC_{50} value

of stem extract and ascorbic acid was found 14.72 µg/ml and 1468.9 µg/ml, respectively (Table 1).

A simple method utilizing the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical has been developed to determine the antioxidant activity of natural products. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric in respect to number of electrons captured. As in this study, the IC_{50} value of *U. crenulata* stem extract (14.72 µg/ml) was found almost 100 times lower than ascorbic acid (1468.9 µg/ml,), it indicates that stem extract does possess minimum significant antioxidative activity.

Antibacterial activity of *U. crenulata* stem extract (500 µg/disc) was studied on three Gram positive and three Gram-negative bacteria by disc diffusion method and compared with the standard antibiotic disc tetracycline (30 µg/disc) (Table 2 and Fig. 2).

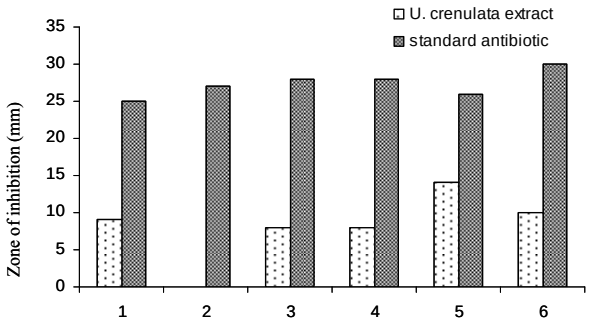

Fig. 2. Comparative antibacterial activity of *U. crenulata* stem extract (500 µg/disc) and tetracycline (30 µg/disc).

U. crenulata stem extract showed 8,12 and 10 mm of diameter of zone inhibition against tested three Gram (-) bacteria like *Samonella typhi, Shigella flexneri and Shigella sonnei*, respectively and 9 & 8 mm of diameter of zone inhibition against tested two Gram (+) bacteria *Bacillus subtilis and Bacillus cereus* but no activity was observed against *Staphylococcus aureus.* On the other hand, standard antibiotic tetracycline (30µg/disc) showed prominent antibacterial activity against all tested Gram (+) and Gram (-) bacteria (Table 2). This results indicate that *Urtica crenulata* stem extract possess certain phytochemicals which have moderate activity against gram negative bacteria but low activity against gram positive bacteria. Our results are consistent with other reports published so far [23].

Brine shrimp lethality assay, used to measure the cytotoxicity of plant extract, is a general bioassay, which is an indicative of cytotoxicity, antibacterial activities, pesticidal effects and various pharmacologic actions [24]. In cytotoxicity, the LC_{50} value of the extract was found very significant (104.0 µg/ml) which indicates that the methanol extract of *U. crenulata* stem has high pharmacological actions [25]. It also indicates that the plant extract, perhaps, possess the potentials to kill cancer cells as well as to kill pests [24]. This finding is also very consistent with previous findings [23]. These activities may lead to conclude the presence of secondary metabolites responsible for showing such biological effects.

Previous phytochemical studies on this plant suggest that *U. crenulata* also possesses steroid, tannins and some flavonoids [4]. Therefore, some of this compound might have the involvement in showing antioxidant, cytotoxic and antimicrobial activities of *U. crenulata* stem extract. However, studies are still needed to evaluate the biological activities and toxicological evaluation of the active constituents of the plant.

4. Conclusion

In summary, pharmacological evaluation of *U. crenulata* stem extract reveals some interesting activities like cytotoxic and antibacterial as well the antioxidant activities of this plant. Since, crude methanol extract of *U. crenulata* showed antibacterial and cytotoxic effect, we assume that different active secondary metabolites are present in its extracts and perhaps some of these compounds may function in a synergistic manner. However, further studies are necessary to elucidate the mechanism lying with this effect. This report may serve as a footstep regarding the biological and pharmacological activities of *U. crenulata* stem.

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