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Preliminary antioxidant, antibacterial and cytotoxic activities of *Momordica charantia* Linn leaf

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SUMMARY

We undertook the present study to evaluate different pharmacological as antioxidant, antibacterial and cytotoxic activities of the crude ethanolic extract of *Momordica charantia* (Family: Cucurbitaceae) leaves. The antioxidant property of the extract was assessed by 1, 1-diphenyl-2-picryl hydrazyl free radical scavenging assay. The extract showed antioxidant activity where IC_{50} was about ~500 µg/ml and IC_{50} was about ~10 µg/ml for standard drug ascorbic acid. The extract showed a broad spectrum of antibacterial activity against all the tested gram positive and gram-negative bacteria where *Staphylococcus epidermidis, Staphylococcus aureus, Salmonella typhi* and *Shigella dysenteriae* were prominent. And the zones of inhibition were ranging from 8 - 15 mm for all the tested bacteria. Its cytotoxic property was evaluated by brine shrimp lethality bioassay. The extract showed significant lethality and the LC_{50} value was 20 µg/ml.

Key words: *Momordica charantia*; Antioxidant activity; 1, 1-diphenyl-2-picryl hydrazyl; Antibacterial activity; Cytotoxic activity

INTRODUCTION

Momordica charantia Linn (Family: Cucurbitaceae) locally known as 'Usta' (Beng) is an annual herb cultivated extensively as a vegetable crop throughout the Bangladesh (Gani, 2003). Studies have shown its efficacy as anthelmintic, purgative, antimalarial and in eczema, gout, jaundice, abdominal pain, leprosy, pneumonia, psoriasis, rheumatism, fever, scabies and in various cancers (Grover *et al*., 2004). Vincine, mycose, momordicoside A and momordicoside B from unmatured fruits (Xie *et al*., 1998) and cucurbitane-type triterpenoids from fruits (Kimura *et al*., 2005) have been identified. It has shown scavenging of NO (Jagetia *et al*., 2004) and antioxidant activity in STZ-induced diabetic rats (Babu *et al*., 2004). Leaf extracts were found to be larvicidal (Prabakar *et al*., 2004) and were able to reverse the MDR phenotype, which is consistent with an increase in intracellular accumulation of the drug (Limtrakul *et al*., 2004); momordicins from it were anthelmintic but not antiviral (Beloin *et al*., 2005). It induces both intestinal and also systemic anti-inflammatory responses as diet (Manabe *et al*., 2003). Considering traditional uses and researches done before on it, we intended to investigate antioxidant, antibacterial on some others bacteria and cytotoxic activities.

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MATERIALS AND METHODS

Plant material and extraction

The leaves were collected from Khulna University campus in November, 2005 and identified by Bangladesh National Herbarium where a voucher

specimen had been submitted for future reference (Voucher specimen no. DACB-14632). The leaves were dried for 21 days without the direct contact of sunrays. Then the dried leaves were finally ground and about 400 g were extracted by maceration over 20 days with 1,200 ml of 80% ethanol. The extract was filtered off and the solvent was evaporated at room temperature with the help of an electric fan to get the dried extract (approx. yield value 6.3%). The crude extract was subjected to preliminary phytochemical screening for the detection of major chemical groups (Evans, 1989). The extract showed the presence of alkaloids, reducing sugars, gums, steroids, tannins, flavonoids etc. In each test 10% (w/v) solution of the extract in solvent was taken unless otherwise mentioned in individual test. This extract was used for pharmacological screening.

Antioxidant activity

Free radical scavenging or antioxidant activity of the extract was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical.

Qualitative assay

Suitably diluted stock solution was spotted on pre-coated Silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow on purple background) were noted (Sadhu *et al*., 2003).

Quantitative assay

Quantitative assay was performed on the basis of the modified method of Gupta *et al*. (2003). Stock solution (10 mg/ml) of the plant extract was prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 μg/ml. Diluted solutions (2 ml) were added to 2ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm for each concentration and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} was calculated. The experiment was performed in duplicate and average absorbance was noted for each concentration. Ascorbic acid was used as positive control (Gupta *et al*., 2003).

Antibacterial activity

Antibacterial activity of the crude extract was determined by disk diffusion method (Bauer *et al*., 1966; Ahmed *et al*., 2001).

Preparation of disks: Three types of disk were used for antibacterial screening.

Sample disks: Sterile filter paper disks (5mm in diameter) were taken in a Petri dish. 6 μl of sample solution (prepared by dissolving 1 g of the extract in 10 ml of methanol) of the desired concentration (100 μg/μl) was applied on the disks with the help of a micropipette in an aseptic condition. These disks were left for few minutes in aseptic condition for complete removal of solvent.

Standard disks: In this investigation standard amikacin disks (30 μg/disk, Oxoid, UK) were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by sample.

Blank disks: These were used as negative control. 6 μl of methanol was applied on the sterile filter paper disk with the help of a micropipette and left for few minutes for complete removal of solvent.

Preparation of media: 14 g of dried Nutrient Agar Media (Oxoid, UK) was dissolved in 500 ml of distilled water and a clear medium was obtained by thorough shaking and heating in a water bath. The media was then sterilized in an autoclave at a temperature of 121°C and pressure of 15 lbs/sq-inch for 20 min.

Table 1. Antioxidant activity of *Momordica charantia* leaf

Sample	Concentration (μg/ml)	Inhibition (%)	IC_{50} (μg/ml)
Et. extract of *Momordica charantia* (leaf)	1	0.5	~ 500
	5	2	
	10	4	
	50	9	
	100	17	
	500	55	
Ascorbic acid	1	17	~ 10
	5	30	
	10	52	
	50	96	
	100	97	
	500	97	

Selection of the test organisms: Following bacteria were used as test organisms for the antibacterial activity test (Table 2).

Preparation of the seeded test plates: 16 ml of the sterilized medium was poured to each (sterilized) test tube aseptically, under laminar air hood. Each of the test organisms were transferred from the subculture to the test tube with the help of the sterilized inoculating loop at 45°C under laminar air hood. The test tubes were shaken by rotation to get a uniform suspension of organisms. The bacterial suspensions were immediately transferred to the sterile Petri dishes and then were rotated several times first clockwise and then anticlockwise to assure homogeneous distribution of the test organisms to give a uniform layer of depth of approximately 4mm. After the medium became cooled to room temperature, it was stored in a refrigerator (4°C) for 2 h. All of the three disks (sample, standard and blank) were then placed in the seeded test plates using sterile transfer loop for antibacterial screening. The plates were then kept at 4 - 8°C facilitating maximum diffusion. The plates are then kept in an incubator at 37°C for 12 - 18 h to allow the growth of bacteria. The experiment was carried out more than twice and the mean of the reading was recorded.

Cytotoxic activity

Brine Shrimp: The investigation was done on *Artemia salina* (Brine shrimp). One spoon of cyst were hatched for 48 h in saline water, prepared by dissolving 20 g pure NaCl and 18 gm normal edible NaCl into 1L water. The cyst became nauplii.

Lethality Bioassay: Solution of different concentrations was prepared with the extract by using Dimethyl Sulfoxide (DMSO) as solvent. Eight test tubes were used, in each test tube 10 shrimps were taken and solution of different concentrations applied on it. Finally volume of liquid was adjusted by saline

Table 2. Antibacterial activity of *Momordica charantia* leaf

Bacteria	Zone of inhibition (mm)	
	Methanol extract (600 μg/disk)	Amikacin (30 μg/disk)
Gram Positive		
Staphylococcus aureus	13	24
Staphylococcus epidermidis	13	29
Gram Negative		
Shigella dysenteriae	15	28
Shigella flexneri	9	24
Shigella sonnei	8	28
Shigella boydei	12	25
Streptococcus pyogenase	11	28
Proteus spp	11	28
Salmonella typhi	13	32

(-): No inhibition

Table 3. Result of brine shrimp lethality bioassay of *Momordica charantia* leaf

Sample	Conc. of extract (μg/ml)	Number of shrimps taken	Number of shrimps alive	Number of shrimps died	Mortality (%)
Ethanolic extract of *Momordica charantia* (leaf)	5	10	8	2	20
	10	10	7	3	30
	20	10	5	5	50
	40	10	0	10	100
	80	10	0	10	100
	160	10	0	10	100
Chloramphenicol	200 μg/ml	10	0	10	100

water. The test tubes were kept for 24 h. For blank control, a test tube with saline water was kept for observation with 10 shrimps under the same condition with the test sample. For positive control, in another test tube 10 shrimps were taken with saline water. A known drug chloramphenicol as standard was introduced in the test tube with a concentration of 200 μg/ml (Table 3). The percent of mortality of the brine shrimp nauplii was calculated for every concentration to determine LC_{50} (lethal concentration).

RESULTS

In the preliminary phytochemical screening the extract showed the presence of alkaloids, reducing sugars, gums, steroids, tannins, flavonoids etc. Antioxidant activity of the extract was performed on the basis of the modified method of Gupta *et al*. (2003). The extract showed comparable antioxidant activity (IC_{50} about ~500 μg/ml) against DPPH free radical to that of standard drug ascorbic acid (IC_{50} about ~10 μg/ml) (Table 1). The extract showed antibacterial activity against the entire tested gram positive and gram-negative bacteria (Table 2); where *Staphylococcus epidermidis, Staphylococcus aureus, Salmonella typhi* and *Shigella dysenteriae* were prominent and the zones of inhibition were ranging from 13 - 15 mm. The extract showed a mild activity against others where the zones of inhibition were ranges from 8 - 12 mm compare to standard antibiotic Amikacine. The bark extract was found to show lethal activity against the brine shrimp nauplii and LC_{50} was found at 20 μg/ml (Table 3).

DISCUSSION

Both *in vitro* and *in vivo* systems have been therefore developed to evaluate antioxidant activity of drugs that are useful in free radical-induced different types of diseases. In this study % inhibition of free radical scavenging activity was increased with the increase concentrations of both crude extract and ascorbic acid (Fig. 1). The result might partially support the traditional uses of it for different tumours. Further studies as lipid per-oxidation inhibition, xanthin oxidase inhibition, erythrocytic membrane stability and other could be performed. The extract also showed antibacterial activity against all the tested bacteria where activity was prominent against some bacteria and activity was mild against others. Some of them might be the causative agents of different diseases

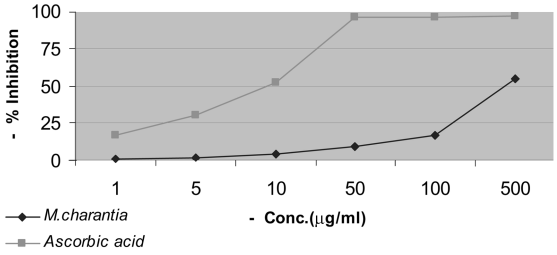

Fig. 1. Graphical representation of % inhibition of DPPH vs. concentration.

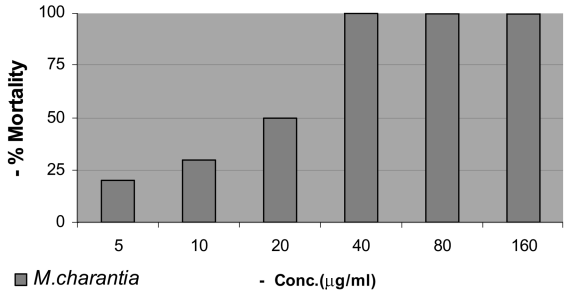

Fig. 2. Graphical representation of lethality bioassay.

for which plant gets different traditional uses. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor etc. activity of the compounds (Meyer *et al.,* 1982). The extract was found to show significant lethality to Brine Shrimp nauplii. The rate of mortality was found to increase with the increase of concentrations that indicate a linear relationship between them (Fig. 2). Further investigations using carcinoma cell line or others are suggested for it.

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