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Evaluation of Antioxidant, Antibacterial, Cytotoxic and Analgesic Potentials of Momordica Charantia

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Abstract

The plant *Momordica charantia*belongs to the family Cucurbitaceae and is commonly known as bitter gourd (English), korolla (Bengali) and used traditionally as a phytomedicine. To provide a scientific basis for the medicinal usage of this plant, the crude methanolic extract of the fruit of *Momordica charantia*was subjected to a number of biological tests including evaluation of peripheral analgesic activity, antioxidant activity, antimicrobial activity and cytotoxicity. The antioxidant activity was evaluated by determining DPPH free radical scavenging capability of the crude methanolic extract. It showed moderate antioxidant activity. The antimicrobial activity of the crude extract was performed by the disc diffusion method where it exhibited moderate antimicrobial activity as compared to ciprofloxacin which was used as a standard. Acetic acid-induced writhing method was used to study the peripheral analgesic activity. Statistical evaluation of the data confirmed that the crude methanolic extract had shown moderate peripheral analgesic activity. The crude extract showed 21.91% (p<0.05) and 32.58% (p<0.01) inhibition at 150 and 300 mg/kg, respectively compared to 62.92% inhibition by standard aceclofenac sodium (50 mg/kg). Moreover, a preliminary toxicological investigation of the crude methanolic extract was performed by brine shrimp lethality bioassay technique. The sample showed significant lethality with LC_{50} value of 4.2679 µg/ml compared to LC_{50} value of 1.1081 µg/ml of the standard vincristine sulphate. Further isolation of the bioactive compounds is required to confirm the biological activities of the fruit extract of *Momordica charantia.*

Keywords: antioxidant, antimicrobial, cytotoxic, analgesic, *Momordica charantia*

Introduction

In the developing countries, traditional medicines are being used in the primary healthcare system. About 80% of the world population depends on medicinal plants for their primary health problems (Leelaprakash G *et al.,* 2011). Plant-derived medicines are gaining increasing popularity over conventional medicines due to minimum side-effects, low cost and easy availability. All these factors intrigue more detailed research on herbal medicines (Modak M *et al.,* 2007). *Momordica charantia,* known as bitter gourd in English and Korolla in Bengali, is a climber belonging to family *Cucurbitaceae* grows in the tropical regions of Asia, Amazon, East Africa and the Caribbean. It has widely been used as a traditional medicine as well as a vegetable for a long time (Leelaprakash G *et al.,* 2011). The fruits of *Momordica charantia* are found to contain glycosides, saponins, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil and free fatty acids. Several phytochemicals are present in it which include momorcharins, momordenol, momordicins, momordicilin, momordicinin, charantin, cryptoxanthin, cucurbitins, goyaglycosides, and goyasaponins. It has been reported that the leaves and fruits are rich in vitamin B (Yuan YR *et al.,* 1999). The plant is found to exert a wide variety of biological properties such as hypoglycemic, anti-ulcerogenic, anti-tumour, antioxidant and anti-microbial effects. As a folk medicine, it is used in the treatment of wounds, peptic ulcers, diabetes, dysmenorrhea, eczema, gout, jaundice, piles, leprosy, psoriasis and management of worms and parasites (Yeşilada E *et al*., 1999). *Momordica charantia* has also been used to

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induce abortion, control birth and aid in childbirth (Beloin N *et al.,* 2005). Present study was designed to evaluate the biological activities of the fruit extract of *Momordica charantia* specifically for the evaluation of peripheral analgesic activity, antioxidant activity, antimicrobial activity and cytotoxicity.

Materials and Methods

Plant material processing and extraction procedure: The unripe, green fruits of *Momordica charantia* was collected from the local market of Mohammadpur, Dhaka in January 2018. The whole fruit was washed, cut into small pieces, sun-dried and ground into a coarse powder. The powdered material was soaked in methanol for 3 days and the whole mixture was filtered off. Finally, the solvent was evaporated using a rotary evaporator to get a crude methanolic extract.

Evaluation of antioxidant activity by DPPH scavenging: The antioxidant activity of the fruit extract was examined using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay (Miliauskas G *et al*., 2004). Ascorbic acid was used as a positive control and various dilutions of ascorbic acid (10-60 µg/ml) were prepared from a stock solution (1mg/ml). Initially, 3 mg of DPPH was dissolved to prepare a solution with a concentration of 20 µg/ml. Next, 2 mg of the plant extract was dissolved in 2 ml methanol to produce a solution having a concentration of 1 mg/ml. Six different concentrations of the extract were prepared by serial dilution (5, 20, 50, 100, 250, 500 µg/ml) and 3.0 ml of a DPPH methanol solution was added to each of them. The mixture was kept in a dark place for 30 minutes and then the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer. Inhibition of free radical DPPH in percentage (I %) was calculated using the following equation:

$$(I\ \%) = (1 - A_{sample}/A_{blank}) \times 100$$

Where, A_{sample} and A_{blank} represent the absorbance of sample and control reaction, respectively.

Evaluation of antibacterial activity: The antimicrobial activity of the extract was determined by disc diffusion technique (Bauer AW *et al*., 1996). Ciprofloxacin (30 µg/disc) disc was used as the standard disc and blank discs were used as a negative control. The sample, control and standard discs were then placed in agar plates pre-inoculated with test bacteria. The plates were then stored at 4 ºC for an hour for ensuring better diffusion followed by incubation at 37 ºC for 24 hours.

Evaluation of peripheral analgesic activity: Peripheral analgesic activity was evaluated by acetic acid-induced writhing method (Ahmed M *et al.,* 2001). Swiss-albino mice (*Musmusculus)* weighing 28-30 grams of either sex, aged 4-5weeks, were obtained from the Animal House of Jahangirnagar University. Methanolic extract of the fruit of *Momordica charantia* was introduced to the experimental mice at a dose of 150 mg/kg and 300 mg/kg of body weight and aceclofenac sodium was used as a standard drug. The number of writhing caused by administration of 0.7% acetic acid was counted following the introduction of the standard and test sample.

Brine shrimp lethality bioassay: Brine shrimp lethality bioassay (Meyer BN *et al*., 1982) was used to aid in the screening and determination of cytotoxicity of the natural products. Vincristine sulphate was used as a positive control and the percentage mortality of *Artemia nauplii* was calculated following exposure to the positive control and the test sample. The median lethal concentration was then determined for vincristine sulphate and the test sample and compared.

Results and discussion

DPPH free radical scavenging activity:

The IC_{50} value of the sample was calculated by plotting %-inhibition against respective concentration (Figure 1) and was found to be 203.9 µg/ml compared to the standard ascorbic acid (IC_{50} = 2.14 µg/ml). Such antioxidant activity may be attributed to phenolic compounds such as gallic acid, catechin, tannic acid present in the fruit.

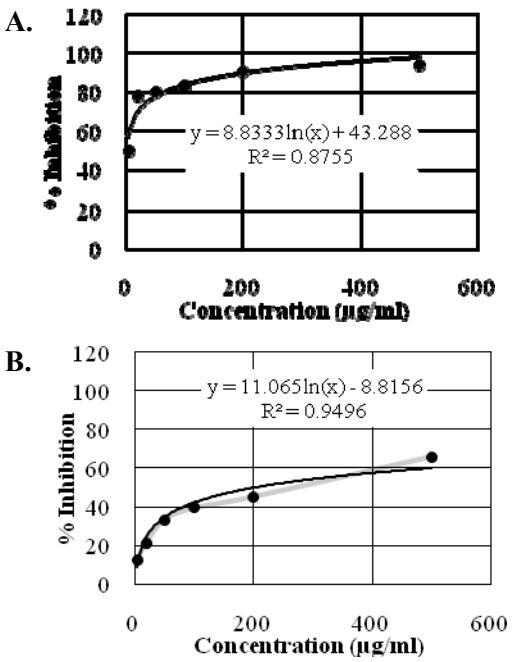

Figure 1. DPPH free radical scavenging effect of ascorbic acid and test sample. A plot of Concentration Vs. % Inhibition and predicted regression line of ascorbic acid (A) and test sample (B) have been shown. IC_{50} values were estimated by extrapolation for 50% inhibition.

Antibacterial activity:

The results shown in Table 1 indicate that the methanolic extract exhibited mild antimicrobial activity against a number of Gram-positive and Gram-negative bacteria as compared to ciprofloxacin. This effect could be associated to the chemical constituents of the extract such as tannin, flavonoid, alkaloid which is known to possess antimicrobial property (Scalbert, 1991).

Table1: Antimicrobial activity of the methanolic extract of the fruit of *Momordica charantia*

Test Microorganisms	Diameter of zone of inhibition (mm)	
	Methanolic extract (400 µg/disc)	Ciprofloxacin (30 µg/disc)
Gram-positive bacteria		
Bacillus subtilis	12	40
Staphylococcus aureus	15	39
Gram-negative bacteria		
Escherichia coli	14	38
Pseudomonas aeruginosa	16	39
Salmonella typhi	15	40

Peripheral analgesic activity:

To investigate the analgesic activity of the extract, Acetic acid-induced writhing method was used. Percentage of writhing and percentage of writhing inhibition were calculated by comparing with the average writhing counts (Mean±SEM) as observed and recorded for the standard group. Compared to the negative control, the test sample showed significant peripheral analgesic activity at 150 and 300 mg/kg body weight (Table 2). The significant pain reduction ability of the methanolic extract of fruit of *Momordica charantia* might be due to the presence of bioactive compounds interfering with prostaglandin synthesis.

Table 2: Peripheral analgesic activity of the methanolic extract of *Momordica charantia*

Sample (Dose in mg/kg)	Writhing Count					Number of Writhing (Mean ± SEM)	Writhing (%)	Inhibition (%)
	M1	M2	M3	M4	M5			
Control	32	34	29	43	40	35.6±2.581	100.00	-
Aceclofenac sodium (50)	15	14	9	16	12	13.2±1.241 ***	37.08	62.92
MFE (150)	28	25	27	30	29	27.8±0.860 *	78.09	21.91
MFE (300)	22	24	23	28	23	24±1.049* *	67.42	32.58

MFE = Methanolic fruit extract, ***P value < 0.001, **P<0.01, *P<0.05

Brine shrimp lethality bioassay:

In order to screen whether the tested extract has any potential to serve as a cytotoxic agent, the bioassay for lethality using brine shrimp was designed. Vincristine Sulphate (VS) was used as a positive control. Compared with the negative control, VS (positive control) gave significant mortality and the LC_{50} value of the test sample was compared to this positive control. First, the %mortality was plotted against the logarithmic value of the respective concentration (0.781 to 400 µg/ml), both for the positive control (VS) and the test extract. Next, the LC_{50} values were calculated by extrapolation from the respective graph (Figure 2) for 50% mortality. The LC_{50} value of the test sample was found to be 4.27 µg/ml, whereas the positive control, VS had a value of 1.1µg/ml. The results obtained in this study reveal that the test sample showed toxicity on the brine shrimp larvae.

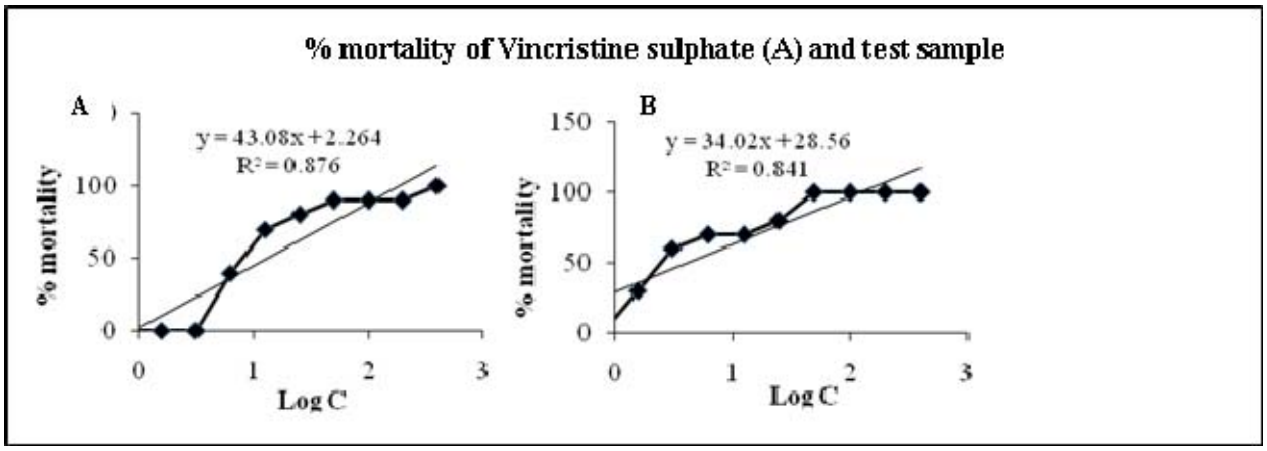

Figure 2: Assessment of cytotoxic activity. Effects of vincristine sulphate (A) and test sample (B) on brine shrimp nauplii have been compared. LC_{50} values were estimated by extrapolation from the 50% mortality for both standard and test extract.

Conclusion

From the beginning of human civilization medicinal plants are contributing to our health care system. Herbal medicine is paving the pathway for novel and efficacious treatments by integrating empirical and scientific data. The present study focuses on the biological activity of the fruit of *Momordica charantia*. Although this plant has been reported to be used traditionally for its various biological effects including analgesic, antimicrobial, antioxidant as well as antitumor effects, there is no scientific study to claim such effects based on scientific evidence. In this study, by using the methanolic extract of the fruit of *Momordica charantia*, we demonstrated moderate antioxidant and antimicrobial activity. It has also exhibited significant peripheral analgesic activity, and cytotoxicity. However, more scientific experiments are warranted for investigation of active principle of the fruit of *Momordica charantia* as part of future drug development.

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