British Journal of Pharmaceutical Research
4(7): 778-786, 2014

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Antioxidant and Cytotoxic Activity of Crude Flavonoid Fraction from the Fruits of Hybrid Variety of *Momordica charantia* (Bitter Gourd)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RB and MSI designed the study and protocol; authors RB, SS, MEUT performed the experiments; author KC wrote the first draft of the manuscript. Authors MSI and CMMH managed the analyses of the study. Author CMMH managed the literature searches. Authors RB and KC performed statistical analysis. Author MSI revised manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 1st October 2013
Accepted 19th January 2014
Published 7th February 2014

ABSTRACT

Aims: The study was conducted to evaluate the antioxidant and cytotoxic properties of crude flavonoid fraction from hybrid variety of *Momordica charantia (L.*) (Bitter gourd) fruit.
Place and Duration of Study: The study was carried out in 2011 in the Department of Biochemistry and Molecular Biology, University of Chittagong, Bangladesh.
Methodology: In vitro assay for Antioxidant activity test was determined by means of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, using ascorbic acid as standard. The Brine shrimp lethality test was used to assess the cytotoxicity of the extract with Gallic acid as positive control. Data were analysed by statistical software BIOSTAT 2009 and Excel.
Results: The fractionated crude flavonoid of *Momordica charantia (L.)* fruits showed

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moderate antioxidant activity with IC_{50} value of 75.21 µg/ml. Compared to Gallic acid (with LC_{50} of 4.40 µg/ ml), fractionated flavonoid demonstrated a significant cytotoxic activity (having LC_{50} of 12.38 µg/ml) which indicates that fractionated flavonoid is promisingly cytotoxic.

Conclusion: The study indicates the moderate antioxidant and potent cytotoxic activities of flavonoid fractionated from *Momordica charantia (L.)* fruits which can be used as a source of pharmacological references although specific flavonoids are responsible for such actions are unknown.

Keywords: Flavonoid; Momordica charantia l; bitter gourd; cytotoxicity; antioxidant.

ABBREVIATIONS

DPPH= 2, 2-Diphenyl-1-picrylhydrazyl; CU= University of Chittagong; AIDS= Acquired immunodeficiency syndrome.

1. INTRODUCTION

Momordica charantia L. (Bitter gourd) also known as Balsam pear or Karela, is a tropical vegetable; it is a common food in South Asian cuisine and has been used extensively in folk medicine. The fruit of the plant is widely used as a vegetable as well as a medicine in ayurvedic and unani system for the treatment of many diseases. The fruits and leaves of *M. charantia L.* are useful in piles, leprosy, jaundice, diabetes, snake-bite and it is found to have vermifuge and antioxidant properties [1]. The earlier reports showed that the plant also has anti-malarial, antiplasmodial properties [2,3] and insecticidal activity against mustard saw fly [4]. These activities of *M. Charantia L.* may be due to a combination of different biologically active constituents rather than any single compound, being the most interesting the alkaloids, the steroids, the flavonoids and the triterpenoids. The presence of these secondary metabolites was confirmed by the preliminary phytochemical analysis [5]. In medicine, natural products have been subjected to variety of tests and clinical trials for possible utilization in the management of varied diseases such as cancer, AIDS etc. Crude extracts that have shown promising activity are further fractionated and the bioactive compounds isolated and purified to determine their structures and or have the structures modified to achieve set goals of efficacy and minimum toxicity [6,7].

Antioxidants are inhibitors of the process of oxidation which have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants are found in dietary sources like fruits, vegetables and tea, etc. Current research on antioxidant action focuses on phenolic compounds such as flavonoids [8]. Due to widespread distribution of flavonoids, their variety and their relatively low toxicity compared to other active plant compounds, animals, humans, ingest significant quantities in their diet. Preliminary research indicates that flavonoids may modify allergens, viruses, and carcinogens, and so may be biological "response modifiers". In vitro studies show that flavonoids also have anti-allergic, anti-inflammatory [9], antimicrobial [10], anticancer [11], antioxidant [12] and antidiarrheal activities [13]. So, consumption of foods rich in flavonoids is important for long-term health benefits. Identification and incorporation of flavonoid rich foods into the diet is considered essential in this context. As a result, the exploration of some potential flavonoids from certain sources has received considerable attention in the development of new drugs. In this study we made an attempt to evaluate the antioxidant and

cytotoxic properties of crude flavonoid fractionated from a hybrid variety of *Momordica charantia L.* (Bitter gourd) fruit.

2. MATERIALS AND METHODS

2.1 Sample Collection, Identification and Extraction

Hybrid variety of *Momordica charantia L.*, "GOJNEE" collected from the local market of Chittagong, a district of Bangladesh. The study was carried out in 2011 in the Department of Biochemistry and Molecular Biology, University of Chittagong (CU), Bangladesh. The variety was identified by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, CU. The seeds, fruits and vegetables were separated and the fruit part (pericarp) was air-dried for 5 days, cut into pieces and grinded to powder with an electrical grinder. The powdered material was then stored in air-tight container in a fridge at 25^0C. Flavonoids were extracted and determined by the methods developed by Boham and Kocipaiabyazan [14] described by Kwada and Tella [15]. 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was then filtered using Whatman (125 mm) filter paper. The filtrate was later transferred into crucible and evaporated to dryness over a water bath and weighed to a constant weight. The weight is flavonoids. For further confirmation preliminary phytochemical tests were used [16,17]. The fruit contains 2.01 % of flavonoid.

2.2 *In vitro* Assay for Antioxidant Activity

The antioxidant activity of fractionated flavonoid from *M. charantia L.* fruit was tested on the basis of its effect on stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging effect according to established procedure [18] with slight modification using Ascorbic acid (BDH, England) as a positive control.

Ascorbic acid solution (5ml) and different concentrations of crude flavonoid (20, 40, 60, 80, 100, 200, 400 and 800mg/ml in methanol) solutions (5ml) were mixed with 3 ml of 0.4 mM (0.004%) DPPH (Sigma,USA) solution. The mixtures were kept in dark for 30 minutes to measure the absorbance at 517 nm using UV-Visible Spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan). 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 is absorbance of control (DPPH solution without the sample), B is the absorbance of DPPH solution in the presence of the sample (crude flavonoid/ Ascorbic acid).

The scavenging activity (%) or % inhibition was then plotted against log concentration and from the graph IC_{50} (Inhibition concentration 50) value was calculated by linear regression analysis with Microsoft office Excel 2007.

2.3 *In vitro* Assay for Cytotoxic Activity

Brine shrimp lethality bioassay was performed to investigate the cytotoxicity of crude flavonoid fractionated from *Momordica charantia L.* fruit. The cytotoxicity assay was

performed on brine shrimp nauplii [19,20]. Brine shrimp (*Artemia salina*) eggs were collected from the Institute of Marine Science and Fisheries, CU, Bangladesh and hatched in artificial seawater (38 g sea salt pure NaCl was weighed, dissolved in 1 litre of distilled water adjusted to pH 8.5 using 1N NaOH and was filtered off to get clear solution) in the larger compartment of an unequally divided tank which was darkened by covering it with Aluminium foil for 48 hours to mature shrimp called "nauplii" with continuous aeration. After hatching, active nauplii free from egg shells, were collected from brighter portion of the hatching chamber. The test samples (crude flavonoid) were prepared by dissolving them in DMSO (not more than 50 µl in 5 ml solution) plus sea water to attain concentrations of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, 100µg/ml, 250µg/ml, and 500µg/ml, in triplicates. A vial containing 30µl DMSO (Sigma, USA) diluted to 5ml was used as a negative control. Standard Gallic acid (Sigma, USA) was used as positive control [21]. Then matured shrimps were applied to each of all experimental vials and control vials. All test tubes were maintained at room temperature for 24 hours. After 24 hours have elapsed, survivors were counted with the aid of a 3X magnifying glass. From the % lethality of brine shrimp, the probits (probability unit) were calculated for each concentration by using "BioStat-2009" software. Probits were then plotted against corresponding log concentration of crude flavonoid to get LC_{50} (lethal concentration 50) value through regression analysis.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity

DPPH radical scavenging is considered a good in-vitro model widely used to assess antioxidant efficacy within a very short time [22]. In its radical form, DPPH changes colour on reduction by an antioxidant compound or a radical species to become a stable diamagnetic molecule resulting in the colour change from purple to yellow, which could be taken as an indication of the hydrogen donating ability of the tested samples [23,24].

The result of DPPH free radical scavenging activity of the crude flavonoid extract from *M. charantia* fruit and Ascorbic acid. Both Ascorbic acid and crude flavonoid showed dose dependent activity (Fig. 1). The activity increased as the concentration increased for both extract and standard. The increased formation of free radicals was associated with the increase in lipid peroxidation. One of the important roles of antioxidants is to inhibit the chain reaction of lipid peroxidation [25]. Among the eight different concentrations used in the study, Ascorbic acid showed highest scavenging activity of 97.39 % at concentration 800µg/ml. On the other hand, highest scavenging activity of crude flavonoid was 78.86% at concentration 800µg/ml. IC_{50} value of Ascorbic acid and crude flavonoid fractionated from *M. charantia* fruit were found 1.13 and 75.21 µg/ml. The obtained IC_{50} value for crude flavonoid and ascorbic acid indicate that the efficiency to neutralize free radicals of crude flavonoid is lower than the ascorbic acid (Fig. 1). Different research suggests that most of the plant extracts showing antioxidant activity are due to the presence of phenolic compounds [26,27].

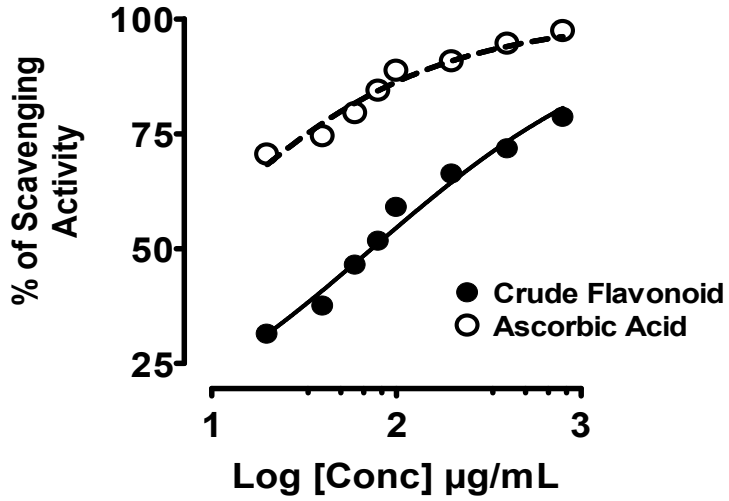


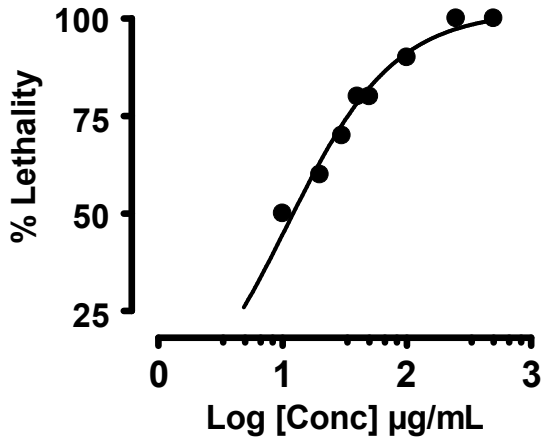
Fig. 1. Comparative % scavenging activities of crude flavonoid and Ascorbic acid
IC_{50} of Ascorbic acid 1.13µg/ml
IC_{50} of crude flavonoid fractionated 75.21µg/ml

Phenolic natural compounds such as flavonoids possess antioxidant activity due to their redox properties which allow them to act as reducing agents and singlet oxygen quencher. In addition, they have metal chelating potentials [28]. Several studies reported that methanolic extract of *M. charantia L.* showed DPPH scavenging activities (IC_{50}) of 306-562 µg/ml [29,30]. It can be clearly seen that the scavenging activity of crude flavonoid in this study is much higher than the methanolic extract reported previously. One explanation for this great difference in antioxidant activity was the flavonoid concentration. Because recent studies have shown that, fruit and vegetable phenols and polyphenols such as flavonoids are one of the major groups that indicate a large spectrum of biological activities that are principally ascribed to their antioxidant property. They prevent free radical damage and lipid peroxidation [31,32]. Moreover, flavonoids including flavanols, flavones, and isoflavones, in general, had stronger antioxidant activities than phenolic acids, including benzoic and cinnamic acid derivatives [33].

3.2 Cytotoxic Activity

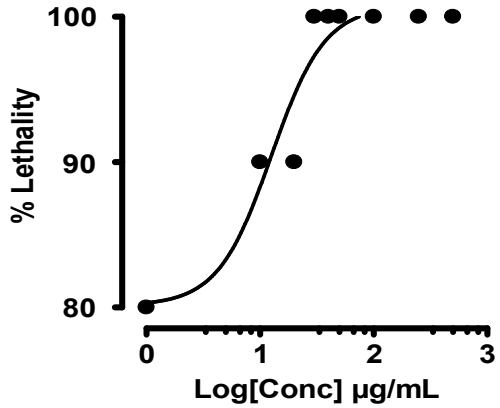
Determining the LC_{50} value of crude flavonoid from *M. charantia* fruit.

Brine shrimp bioassay results showed that percentage lethality of brine shrimp at ten different concentrations (10 to 500µg/ml) of crude flavonoid fractionated from *M. charantia* fruit. Standard Gallic acid and crude flavonoid both showed lethality in a dose dependent manner. More specifically, crude flavonoid showed 50, 60, 70, 80, 80, 90, 100 and 100% mortality of brine shrimp at 10, 20, 30, 40, 50, 100, 250 and 500µg/ml concentrations respectively. LC_{50} value of crude flavonoid was found 12.38 µg/ml, with 95% confidence limit where the lower and upper limits were 2.536 and 21.6193 µg/ml respectively (Fig. 2). The Percentage (%) of Lethality of Gallic acid. The LC_{50} value of Gallic acid was 4.40µg/ml (Fig. 3).



LC_{50} = 12.38 µg/ml

Fig. 2. Determination of LC_{50} value of crude flavonoid from *M. charantia* fruit



LC_{50} = 4.40µg/ml

Fig. 3. Regression line for determining the LC_{50} value of Gallic acid (positive control).

In comparison with the positive control (Gallic acid), the cytotoxicity exhibited by crude flavonoid is considerably high. This clearly indicates the presence of potent bioactive principles in this crude flavonoid which might be useful as antiproliferative, antitumor,

pesticidal and other bioactive agents [34]. So, these cytotoxic samples may have clinical and therapeutic potentials in the most life threatening diseases like cancer. Kaur *et al* [35] reported that phenolics such as Gallic acid and flavonoids extract have capacity to inhibit cancer cell proliferation which provides evidence for cytotoxic effect of extract. As the cytotoxicity is an indicator of wide range of pharmacological activities [36,37], further studies on the crude flavonoid extract of *M. charantia* fruit are required to investigate for such activities.

4. CONCLUSION

The results of the study demonstrate that the crude flavonoid of *Momordica charantia* fruit exhibits moderate antioxidant and potent cytotoxic effect in experimental models which supports the claims by traditional medicine practitioners. On the basis of the results, it can be used as a source of pharmacological references although specific flavonoids responsible for such actions are unknown. Further investigations aimed at isolating the individual bioactive compound(s) present in the crude flavonoid fraction of the *M. charantia* fruit are required for possible therapeutic utilization.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Subodh Kumar Sarkar, Department of Biochemistry & Biotechnology, University of Science & Technology, Chittagong (USTC) for his help during the execution of the experiment. Thanks are also due to Dr. Sheikh Bokhtear Uddin, Department of Botany, CU for identification of collected samples.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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