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In vitro Antidiabetic, Antibacterial and Antioxidant Activity of the Alcoholic Extracts of *Momordica charantia* L Fruits

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Abstract

Objective: The aim of the study was to assess *in vitro* antidiabetic, antibacterial and antioxidant activity of alcoholic extracts of *Momordica charantia* L Fruits. **Method:** *Momordica charantia* L Fruits extracts were prepared by soaking in methanol and ethanol. The extracts were used for evaluating their antidiabetic, antibacterial and antioxidant activity using dinitrosalicylic acid method, disc diffusion and DPPH assay respectively. The results were compared with standard compounds. **Results:** The ethanolic extract of *Momordica charantia* L Fruits showed maximum α-amylase inhibition (77±0.23 % of glucose release inhibition at 2.0 mg/mL) and DPPH radicals scavenging (IC_{90};769.88 mg/mL) activity at prescribed concentrations dependent manner. Methanolic extract showed nearly similar activity. The ethanolic and methanolic extracts also exhibited a significant extent of inhibition on the growth of bacteria. **Conclusion:** *Momordica charantia* L Fruits can be further studied to isolate the phytocompounds responsible for the antidiabetic, antibacterial and antioxidant activity.

Keywords
Free radical, glucose release, bitter gourd, enzyme inhibition

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INTRODUCTION

Bitter gourd or *Momordica charantia* (MC) L has been reported for its antidiabetic and antioxidant activity (1,2) and has been used as a traditional remedy in Africa, India, England, and Sri Lanka (3). There are several promising bioactive phytochemicals, polypeptide K (4), which is well known for regulating glucose uptake (5,6). The alfa-bonds of polysaccharides is hydrolyzed by α-amylase (EC 3.2.1.1), that is found in saliva, pancreatic juice and

many other tissues (7). Inhibition of α-amylase activity disrupting the hydrolysis of polysaccharide and reducing the blood glucose level. Well known α-amylase inhibitor is acarbose, which controls blood glucose level (8). Herbs or plant extracts show the potential anti-α-amylase role *in vitro* and *in vivo* investigations (9–14). On the other hand, it has been reported that methanolic, ethanolic, and aqueous extracts of MC showed antimicrobial potential against *E. coli, Shigella dysenterae*, and *Mycobacterium tuberculosis*(14). Kubola J and Siriamornoun S have been reported that the water extract of bitter gourd has dose-dependent response with diverse antioxidant methods (15). The objectives of this study were to screen the antidiabetic, antibacterial and antioxidant activity through *in vitro* investigations.

MATERIALS AND METHODS

Plant materials

Fruits of *Momordica charantia* L (FMC) were collected from local market, Dhaka and authenticated by local agriculture officer, Bangladesh. Fruit parts were shed-dried, powdered and stored in an airtight container for further analysis.

Extraction

The powder of fruits of *Momordica charantia* L was extracted with methanol (10:1 v/w) and ethanol (10:1 v/w) at room temperature for 7 days. Extracts were filtered and residues were extracted again for five days and were filtered. Filtrates were combined and concentrated by using a rotary evaporator and lyophilized to obtain solid crude extracts. Crude extracts of FMC were used for antioxidant, antidiabetic, and antibacterial activity through *in vitro* investigations.

In vitro antibacterial activity study

Traditionally, antibacterial activity has been carried out by disc diffusion method (16). In this experiment, bacterial strains *Stapylococcus aureus*, *Escherichia coli*, *S. paratyphi,* and *S. dysentery* were obtained from the Department of Pharmacy, Primeasia University, Bangladesh and were used for antibacterial activity test. FMC extracts were tested with prescribed concentrations (100 µg/ml). The selected bacterial strain was seeded over medium with 10^6 cells/mL(17). After 24 hrs of incubation at 37^0C, the zone of inhibition was measured in mm.

In vitro antioxidant activity

Antioxidant activity of the FMC extract was carried out using DPPH radical scavenging assay(18). This experiment was done with triplicate reaction mixture which composed of 50 µL of each FMC extract and 5.0 mL of DPPH solution (0.04% w/v in ethanol) trailed by 80% ethanol and 80 % methanol for corresponding samples as a blank preparation. BHT was used as the positive control. After 30 minutes of incubation, absorbance of each test tube was taken at 517 nm and free radical scavenging effect was determined by the following formula;

DPPH scavenging effect (%) = (Ao-As)/Ao x 100

Where, Ao was the absorbance of control and as was the absorbance of selected sample.

In vitro antidiabetic activity

Four different concentrations (0.5, 1, 1.5, and 2 mg/mL) of MC fruit extracts and acarbose (standard compound) were for this investigation, which was carried out in vitro by measuring the inhibition of α-amylase activity on the hydrolysis of starch. Enzyme, α-amylase, solution (0.5 mg/mL) was prepared in phosphate buffer saline (0.2 M PBS, pH-6.9) with 0.006 M NaCl and starch solution (1% starch in PBS was prepared. 1 mL of freshly prepared α-amylase solution and FMC extracts and acarbose were added in corresponding test tube and was allowed to incubate for 10 minutes at room temperature. 0.5 mL starch (1%) solution was added in each test tube and was incubated for 10 minutes at room temperature. To stop reaction, 1 mL of DNSA was added and finally was incubated in a boiling water bath for 5 minutes and was cooled. Reaction mixtures were diluted by adding 10 mL of PBS. The absorbance of each test tube was measured at 540 nm and the % inhibition of α-amylase activity was calculated by using the following formula;

% inhibition of α-amylase activity = [(Ac-As)/Ac] *100

Where, Ac and As were absorbance of control and samples(19).

RESULTS

Figure 1 shows the antibacterial activity *in vitro* investigation against *S. aureus, E. coli, S. paratyphi,* and *S. dysentery.* Methanolic extract showed the better antimicrobial activity in compared to ethanolic extract of *Momordica charantia* L fruits. Growth of *E. coli* was inhibited intensively by methanolic extract whereas ethanolic extract showed competitive activity ($p<0.05$).

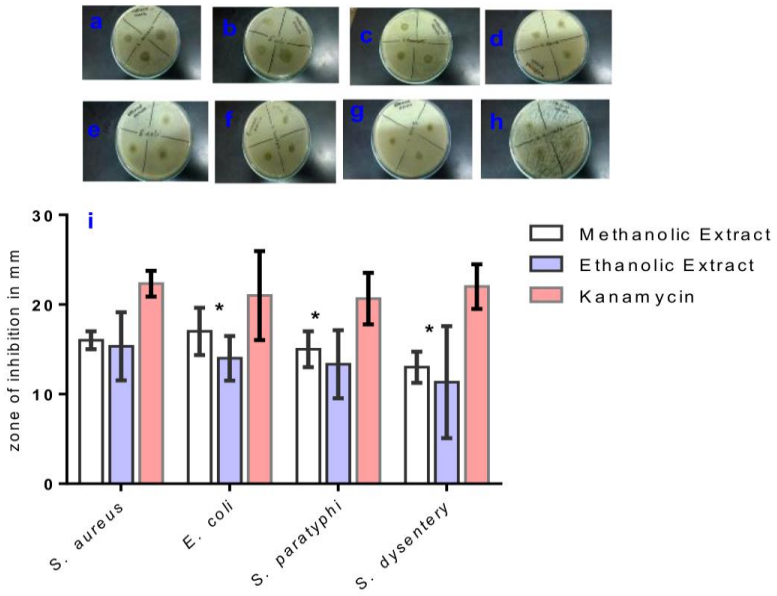

Figure 1. Antibacterial activity of FMC extracts. Effects of methanolic extract on the growth of a) *S. aureus*, b) *E. coli*, c) *S. paratyphi* and d) S. dysentery and effects of ethanolic extract on the growth of e) *E. coli*, f) *S. paratyphi, g)* S. dysentery and h) *S. aureus*. i) shows zone of inhibition with 95 % CI (*p<0.05).

Crude extracts of the fruits of *Momordica charantia* L exhibit potential dose-dependent antioxidant activity through *in vitro* inhibition of DPPH free radicals' formation, as shown in figure 2.

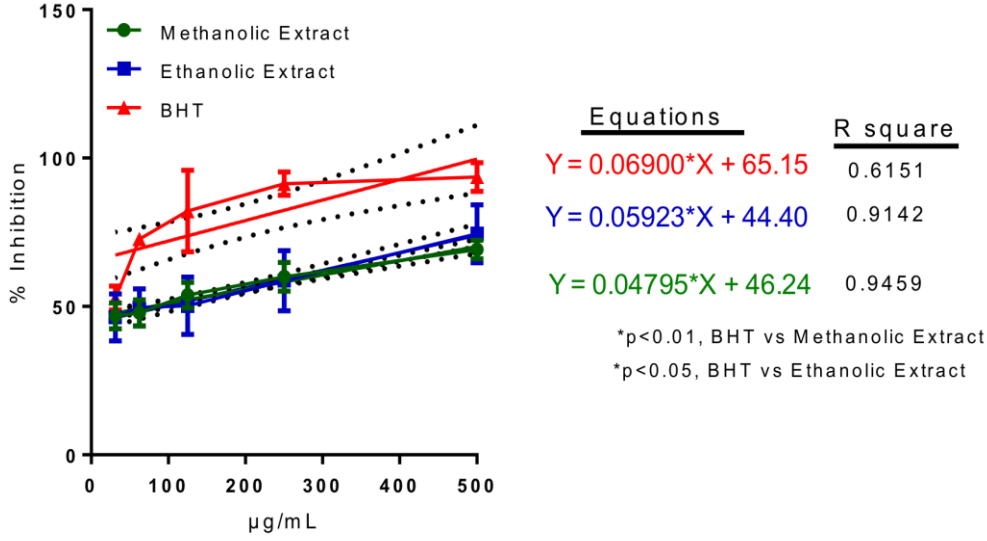

Figure 2. Inhibition of DPPH free radicals' formation by methanolic and ethanolic extracts of FMC in compared to BHT, positive control.

At 500 µg/mL, extracts of FMC showed about 20 % higher DPPH radical scavenging activity in compared to the initial dose (31.25 µg/mL). The antioxidant activities were increased gradually when concentrations of extract were increased steadily. IC_{90} of methanolic and ethanolic extracts of FMC were 912.61 µg/mL and 769.88 µg/mL respectively.

The dose-dependent antidiabetic role of FMC against the inhibition of glucose release from starch by α-Amylase are shown in figure 3.

At highest dose, 2 mg/mL, the ethanolic extract showed around 77±0.23 % of glucose release inhibition. Methanolic extract of FMC also showed similar pattern of activity.

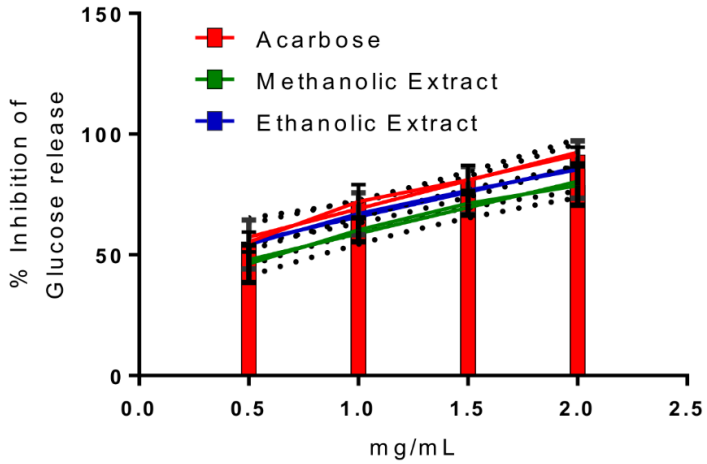

Figure 3. Effect of alcoholic FMC extracts on the release of glucose from starch.

DISCUSSION

The present study was designed to determine the effect of FMC extracts on antioxidant, antibacterial and antidiabetic activity through *in vitro* analysis. The results of this study showed that FMC has significant role in aforementioned biological activities. Surprisingly, no significant differences were found in ethanolic and methanolic extracts of FMC while in comparison with positive control, FMC extracts showed less potency with prescribed concentrations. This finding confirms the association between extraction methods /concentrations of FMC extract and their biological activities.

This study investigated the antibacterial activity of FMC against *Stapylococcus aureus, Escherichia coli, S. paratyphi,* and *S. dysentery*. The methanolic extract showed the intensive bactericidal role in compared to ethanolic extract of FMC. It is promising to compare this figure with found by de Lucenafilho JHS (2015) who found that results found for *P. euruginosa, P. mirabilis, P. rettgeri* and *S. aureus* bacteria were highlighted, given the clinical importance of each one of them(20). DPPH free radical scavenging assay was used to measure the antioxidant potential of FMC. The ethanolic extract showed significant level of free radical scavenging ($p<0.05$), in comparison to methanolic extract. This finding showed that FMC solution reduced free radicals resulting diminishes cellular damage. Results found in DPPH assay were supported by several previous studies(21,22). Yadav M *et al* (2010) have reported that FMC showed significant hypoglycemic effect(23). In this study we have found similar hypoglycemic activity of FMC. From previous studies, it can be confirmed that FMC exhibits antidiabetic activity due to the presence of polyphenols, flavonoids and other phytoconstituents which show a glucose lowering activity(24). Here, we found that Acarbose was a more effective antidiabetic agent than that FMC extracts. Ethanolic extract showed the highest effect on the glucose release from starch. Due the lack of evidence on the antidiabetic effects of FMC, further studies are required to valid this finding.

CONCLUSION

The main goal of the current study was to determine the antioxidant antibacterial and antidiabetic activity of FMC extracts. The study revealed that the ethanolic extracts of FMC exhibited highest antioxidant and antidiabetic activity while methanolic extract of FMC showed highest antibacterial activity in suggested doses. It is suggested that the association of phytocompounds is investigated in future studies.

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