In vitro antioxidant potential of *Momordica charantia* fruit extracts

Md. Ehsan Uddin Talukder[1], Jannatul Aklima[1], Talha Bin Emran[2], Sayedul Islam[1], Atiar Rahman[1] and Robiul Hasan Bhuiyan[1*]

[1]*Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong-4331, Bangladesh*

[2]*Department of Pharmacy, BGC Trust University Bangladesh*

Running Title: Antioxidative effects of Momordica charantia

Corresponding Author:* ***Robiul Hasan Bhuiyan**, Assistant Professor, Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong-4331, Bangladesh. E-mail: jannattulaklima@yahoo.com, biochemistrobi79@gmail.com. Tel: +88-01816280401.*

ABSTRACT

This research investigated the antioxidant potential of *Momordica charantia* fruit extracts in ethanol and ethyl acetate. The extracts have been assessed for DPPH free radical scavenging effect, $FeCl_3$ reducing power and superoxide scavenging effect. In DPPH method IC_{50} value of ascorbic acid, ethanol and ethyl acetate extract were found 2.19 µg/ml, 111.87 µg/ml and 157.03 µg/ml respectively. In power reducing method, IC_{50} value of ascorbic acid ethanol and ethyl acetate extract were found 50 µg/ml, 931.63 µg/ml and 754.86 µg/ml respectively. In super oxide scavenging method, IC_{50} value of curcumin , ethyl acetate and ethanol extract were found 29.51 µg/ml, 331.26 µg/ml and 489.77 µg/ml respectively. The results of all three *in vitro* antioxidant assays exhibited that *M. charantia* possess relatively moderate antioxidant property than standards. The data obtained in the *in vitro* models clearly establish the antioxidant potency of the fruits extracts.

Keywords: *Antioxidant, Ethanol, Ethyl acetate, Petroleum ether, Momordica charantia, DPPH*.

INTRODUCTION

Oxidative stress is among the major causative factors in induction of any chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [1]. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems [2]. The most effective path to eliminate and diminish the action of such free radicals, which cause the oxidative stress, is antioxidative defense mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective. They are known to inhibit lipid peroxidation, scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions.

Fruits have many health beneficiary functions. Recent research has confirmed that consumption of fruits and vegetables can reduce the risk of stroke and cancer [3, 4, 15, and 21] as well as inflammation and problems caused by aging [1]. This risk reduction is related to the presence of antioxidative agents in fruits. They fight free radicals by supplying them the electron they lack, and thus neutralize them. There are various types of antioxidants that our body needs to operate optimally. Different antioxidants scavenge different free radicals, some work directly, while others work indirectly as catalysts to boost our own body's production of antioxidants. Therefore, we need a multitude of vitamins, minerals and enzymes to operate proficiently, so we need a wide range of antioxidants [12, 13].

Momordica charantia L. (Cucurbitaceae), locally known as tit korla, has important role as a source of carbohydrates, proteins, vitamins, minerals and other nutrients in human diet, which are necessary for maintaining proper health [18]. *M. charantia* fruit is also very important economic source of proteins, minerals, and calories of vitamins, essential for human nutrition [19]. Researchers reported the antihyperglycemic [8], anti-migratory [14], anti-prolifiretory [6] effects of the different extract and compounds of *M. charantia*. As a part of our continuous work on medicinally important plants, we report here the antioxidative effects of ethanol and ethyl acetate extracts of *M. charantia* in reducing power model, DPPH free radical scavenging model and superoxide scavenging model.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals used were of analytical grade. Ascorbic acid, nitro blue tetrazolium (NBT), trichloroacetic acid, 2, 2-diphenyl-1-picryl hydrazyl free radical (DPPH) were purchased from Sigma-Aldrich, (Germany). Ethyl acetate (98%) and absolute ethanol (99.5%) were also procured from Sigma (India).

Collection of plant material

M. charantia fruits were collected from Chittagong region. Foreign materials of the fruits were removed, dried in the sunlight for four consecutive days and crushed into fine powder. The powder was dried at 40°C for 4 h by electric oven.

Preparation of extract

The powder of dried fruit was socked in ethanol and ethyl acetate in separate conical flask for 12 days with 3 days interval at room temperature (28 ± 2°C) with occasional shaking and stirring. The conical flasks were sealed to avoid evaporation. After that the contents were filtered and the filtrate was evaporated to dryness with rotary evaporator (RE 200, Bibby sterling, UK) under reduced pressure at 45°C. The blackish green crude extract was preserved at 4°C until further use.

Antioxidative assay by *in vitro* methods

Free radical scavenging activity assay

The antioxidative effect of the fruit of *M. charantia* ethanol and ethyl acetate extract was assessed by the established method of Brand-William *et al.*,1995 [5] with slight modifications. Briefly, the extracts (20, 40, 60, 80, 100, 200, 400, 800 µg/ml) were prepared in ethanol and ethyl acetate. Positive control ascorbic acid solution was made with the concentration between 1-100 µg/ml. DPPH solution (0.004%) was prepared in ethanol and 5 ml of this solution was mixed with the same volume of extract and standard solution separately. These solution mixtures were kept in dark for 30 min to read absorbance at 517 nm using a spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan). The degree of DPPH purple decolorization to DPPH yellow indicated the scavenging efficiency of the extract. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The scavenging activity against DPPH was calculated using the equation.

Percent of scavenging activity = [(A-B)/A] X 100, where, A was the absorbance of control (DPPH solution without the sample), B was the absorbance of DPPH solution in the presence of the sample (extract/ascorbic acid). The control (ascorbic acid) was conducted in the same manner, except that distilled water was added instead of sample.

Reducing power assay

The reducing power of the fruit extracts was determined according to Oyaizu ,1986 [17]. A 1.0 ml of extract solution (100, 500, 1000, 2000, 5000 µg/ml) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%, w/w), and then mixture was incubated at 50°C for 20 min. After incubation at 50°C for 20 min, the solutions were mixed with 2.5 ml of 10% (w/w) trichloro-acetic acid and then centrifugation at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The resulting solution was read at 700 nm. Increased absorbance of the reaction mixture indicated increasing reducing power.

Super oxide scavenging activity assay

In this method, superoxide radical is generated by the addition of sodium hydroxide to air saturated dimethyl sulfoxide (DMSO). The generated superoxide remains stable in solution, which reduces nitro blue tetrazolium (NBT) into formazan dye at room temperature and that can be measured at 560 nm. Briefly, to the reaction mixture containing 1 ml of alkaline DMSO (1 ml DMSO containing 5 mM NaOH in 0.1 ml water) and 0.3 ml of the extract in freshly distilled DMSO at various concentrations, added 0.1 ml of NBT (1 mg/ml) to give a final volume of 1.4 ml. The absorbance was measured at 560 nm. The percentage of super oxide radical scavenging by the extracts and standard compounds were calculated as follows:

$$\% \text{ superoxide scavenging activity} = \frac{\text{Test absorbance - control}}{\text{Test absorbance}} \times 100$$

RESULT

DPPH radical scavenging assay

The radical scavenging effect of ethanol and ethyl acetate extract was summarized in Figure 1. Results showed that both the extracts showed a dose dependent radical scavenging effect.

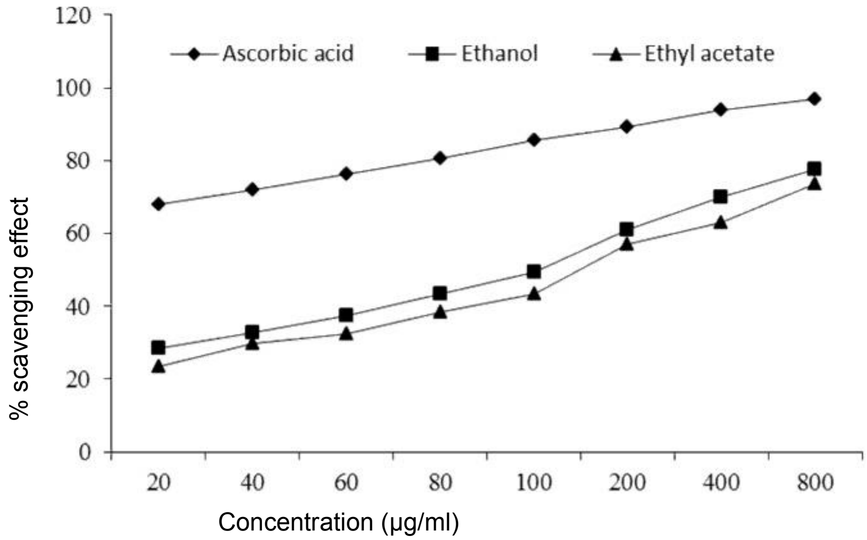

Figure 1: Relative percentage of scavenging activity for standard and *M. charantia* extracts by DPPH method

The most prominent scavenging effect of ethanol and ethyl acetate extracts were 77.65% and 73.75% at a concentration of 800 µg/ml which were comparable to the highest activity (96.86) of ascorbic acid. The inhibition concentration (IC_{50}) of the extract was determined by plotting a graph of scavenging activity against the log concentration. The IC_{50} value of ascorbic acid, ethanol and ethyl acetate extracts was found 2.19 µg/ml, 111.87 µg/ml and 157.03 µg/ml respectively (Table 1).

Reducing power by $FeCl_3$

Results showed that the reducing power of the extracts increased with the concentrations. The extracts showed a dose dependent effect in reducing power measurement. Ethanol and ethyl acetate extracts showed the highest reducing power 70.51% and 70.83%, respectively, which were higher than that of ascorbic acid (34.91%) (Figure 2).

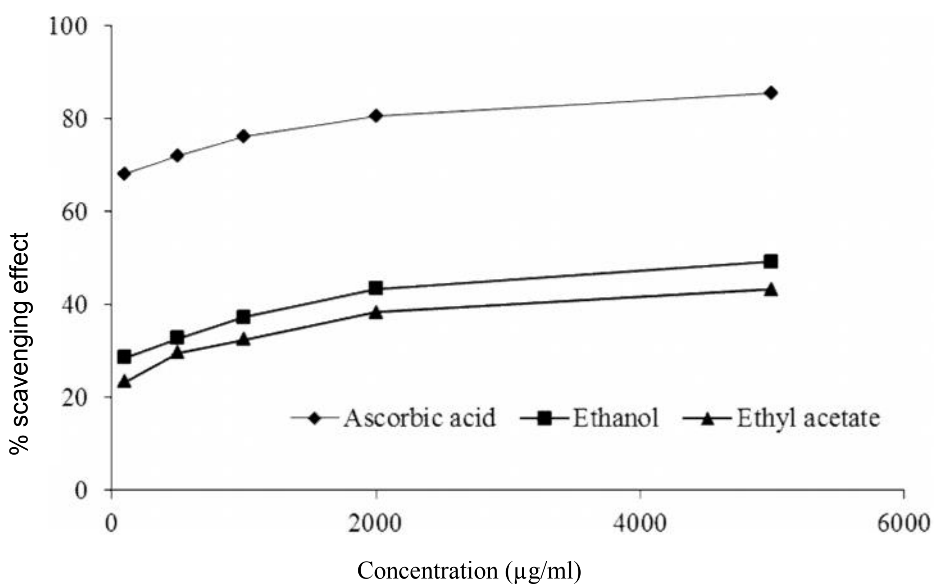

Figure 2. Relative percentage of scavenging activity for two solvents by reducing power method

The percentage (%) of reducing power or % of inhibition was plotted against log concentration and from the graph IC_{50} value was calculated by linear regression analysis. IC_{50} value of ascorbic acid, ethanol and ethyl acetate extract were found 50 μg/ml, 931.63 μg/ml and 754.86 μg/ml, respectively (Table 1).

Super oxide scavenging activity by alkaline DMSO method

Super oxide free radical was formed by alkaline DMSO which reacted with NBT to produce colored diformazan. The ethanol and ethyl acetate displayed a dose dependent activity in inhibiting the superoxide radicals. The best scavenging effect was shown 71.18% for ethanol and 71.58% for ethyl acetate extract. These promising scavenging effects of ethanol and ethyl acetate extracts were stronger than the reference agent curcumin (Figure 3).

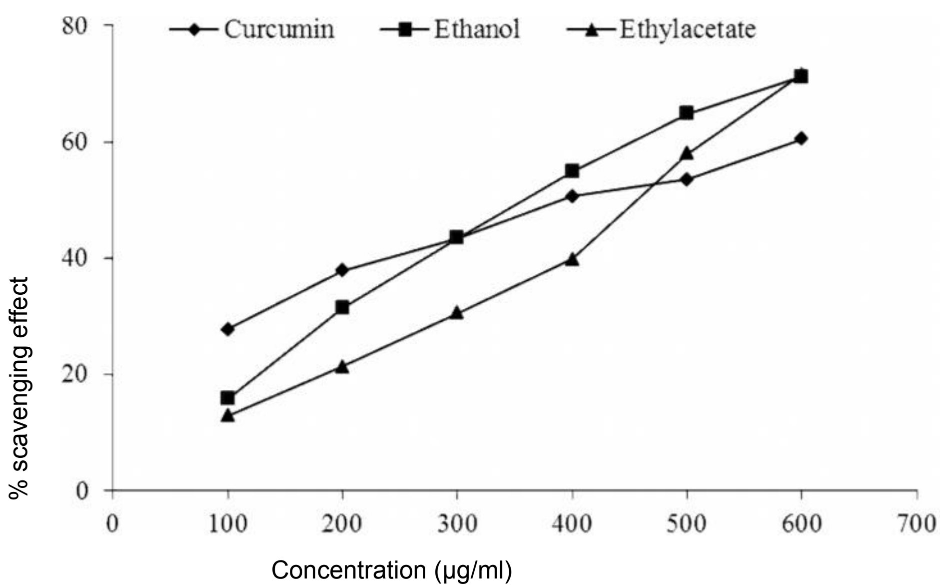

Figure 3 . Superoxide scavenging activity of *M. charantia*.

Scavenging activity (%) was plotted against log concentration and from the graph IC_{50} was calculated by linear regression analysis. IC_{50} value of curcumin, *M. charantia* ethanol and ethyl acetate extract was found 29.51, 489.77 μg/ml and 331.26 μg/ml, respectively (Table 1).

Table 1. IC_{50} Values of the extracts in different experimental models

Antioxidative models	Standard/ Samples	IC_{50} Values (µg/ml)
DPPH Free radical scavenging effect	Ascorbic acid	2.19
	Ethanol	111.87
	Ethyl acetate	157.03
Reducing effect	Ascorbic acid	50
	Ethanol	931.63
	Ethyl acetate	754.86
Superoxide scavenging effect	Curcumin	29.51
	Ethanol	489.77
	Ethyl acetate	331.26

DISCUSSION

However, plants of high antioxidative effects can be pivotal sources of such uses [10]. In the present study, the antioxidative activity, in terms of the scavenging of the radical DPPH of the ethanolic and ethyl acetate extracts of *M. charantia* was determined and compared with ascorbic acid, the reference antioxidative agent. The proton-radical scavenging action has been known as an important mechanism of antioxidation. DPPH was used to determine the proton-radical scavenging action of the extracts, since it possesses a proton free radical and shows a characteristic absorption at 517 nm. The purple color of the DPPH solution rapidly turned into yellow once it encounters proton-radical scavengers.

The intensity of the radical scavenging effect is measured by the calculated half-inhibition concentration (IC_{50}), the efficient concentration required for decreasing initial DPPH concentration by 50%. IC_{50} was obtained by interpolation from linear regression analysis of data shown the IC_{50} values were 111.87 µg/ml for ethanol extract was and 157.03 µg/ml for ethyl acetate extract suggesting that ethanol extract had the stronger antioxidative potential of the extracts. However, both the scavenging effects were biologically important because the cutoff value for antioxidative power is 1000µg/ml. Extracts or chemical agents with the values higher than this are not effective as antioxidants. Ascorbic acid is used as reference standard because ascorbic acid impairs the formation of free radicals in the process of intracellular substance formation throughout the body.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing power of *M. charantia* ethanol and ethyl acetate extract along with that of ascorbic acid at concentrations between 5-50 µg/ml showed that high absorbance indicates high reducing power. The reducing power of the plant extract was increased as the amount of extract concentration increases. This is because the presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. In our study, the reducing power of extract was lower than that of ascorbic acid.

The scavenging activity of the extract against superoxide radical generated in NaOH-alkaline DMSO-NBT system, resulting in the formation of the blue formazan was studied in this research. The generated superoxide remains stable in solution, which reduces nitro blue tetrazolium into formazan dye at room temperature. Superoxide scavenger capable of reacting inhibits the formation of a red dye formazan. The inhibition of formazan formation by the extract was reflected through the IC_{50} value for ethanol and ethyl acetate extract, 489.77 µg/ml and 331.26 µg/ml respectively , which was significantly ($p < 0.05$) different compared to that of curcumin, 29.51 µg/ml. This finding demonstrates that *M. charantia* fruit extract is capable of non-enzymatically inhibiting the superoxide radical, produced in biological system, which is a precursor of many ROS and is shown to be harmful for various cellular components. Although the enzyme superoxide dismutase possessed in aerobic and anaerobic organisms can catalyze the breakdown of superoxide radical.

CONCLUSION

The results stated above showed that the ethanolic extract of *M. charantia* possessed noteworthy antioxidative effects in all the models. Whatever the solvent for extraction, the antioxidative effect of *M. charantia* evidenced that it could be a very good source of natural medicines on standard formulation.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of competing interest.

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