ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Antioxidant activity and Estimation of Total Phenolic content of *Momordica balsamina*

Samriti Faujdar[1*], Sumitra Nain[1], A.N.Kalia[2]

1. Banasthali university, Banasthali, Tonk(Rajasthan)-304022

2. ISF College of Pharmacy, Ferozepur-Moga GT Rd, Moga (Punjab)-142001

Manuscript Info

Manuscript History:

Received: 18 December 2013
Final Accepted: 25 January 2014
Published Online: February 2014

Key words:
Total Phenolic , Gallic acid, *Momordica balsamina*

Corresponding Author

Samriti Faujdar

Abstract

In vitro antioxidant activity of methanolic and aqueous extracts of fruit pulp of *Momordica balsamina* was assessed by DPPH free radical scavenging, FRPA, Lipid peroxidation by thiobarbituric acid assay methods. The methanol and the aqueous extract had shown DPPH scavenging activity (IC_{50}=371.29±1.57 µg/ml and IC_{50}=384.22±2.46 µg/ml) respectively when compared with the IC_{50} values of the standards ascorbic acid and rutin (IC_{50}=8.84±0.05 and 3.78±0.153 µg/ml respectively) and IC_{50} values of methanolic (311.07±0.009 µg/ml) and aqueous (386.01±0.006 µg/ml) extracts in lipid peroxidation as compared to BHT (48.89±0.01 µg/ml) µg/ml. The reducing power of the extracts were found to be concentration dependent. The total phenolic content was measured by Folin-Ciocalteu reagent was found to be 2.34%w/w.

INTRODUCTION

Momordica balsamina also known as junglee karela in Hindi, is belonging to family Cucurbitaceae. This plant is most commonly found in the northern parts of India such as Punjab, Haryana and some parts of Rajasthan. *M. balsamina* is fairly common and widespread in Namibia, Botswana, Switzerland and all the provinces of South Africa except the Western Cape. It is also indigenous to tropical Africa and Asia, Arabia, India and Australia. It has been cultivated in gardens in Europe since the 1800's **(**1). Liniment made by infusing the fruit pulp in olive or almond oil is used as an application to chapped hands, burns and haemorrhoids and the mashed fruit is used as a poultice (2). Infusion of this plant is used to cure intestinal and stomach pains. It is believed that this drug is used in the prophylactic relief in diabetes (3). Regular use of *Momordica balsamina* reduces the risk of osteoporosis.(4). There are not so much scientific details are available on this plant, thereby indicating lack of exploration into its pharmaceutical advantages. The present study was carried out to assess the antioxidant activity of this plant using DPPH free radical scavenging, FRPA, lipid peroxidation by thiobarbituric acid assay.

MATERIALS AND METHODS

Plant Material

Dried fruits of *M. balsamina* were procured from the local market of Moga and were identified by Dr. H.B. Singh, Director, Department of Raw Material Herbarium & Museum, National Institute of Science communication and Information Resources (NISCAIR), New Delhi, India (NISCAIR/ RHM 1062/93). The fruits were collected, seeds and fruit pulp of *M. balsamina* were separately shade dried, coarsely powdered and used for the present study.

Preparation of extracts

Momordica balsamina fruit pulp powder was defatted with petroleum ether and then extracted in Soxhlet apparatus with methanol and water and filtered to yield the extracts. The dried methanolic and aqueous extracts were used for the evaluation of antioxidant activity by using 1, 1-diphenyl, 2- picryl hydrazyl (DPPH), radical scavenging activity, lipid peroxidation with thiobarbituric acid and reducing capacity.

Phytochemical screening

Different qualitative tests were performed to determine the presence of various phytoconstituents in plant material.

Determination of total phenolic content

The total phenolic content in fruit pulp of *Momordica balsamina* was determined by using Folin-Ciocalteu's method. Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants. It works by measuring the amount of substance being tested needed to inhibit the oxidation of the reagent. The sample extract dilution was oxidized with Folin-Ciocalteu reagent and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 765nm after 30 min.

Preparation of calibration curve using gallic acid as standard

10mg of standard gallic acid was accurately weighed and dissolved in 100ml distilled water in a volumetric flask (100µg/ml of stock solution). From the above stock solution, 0.5 to 2.5 ml aliquots were pipetted out into 25ml volumetric flasks. 10ml of distilled water and 1.5ml of Folin Ciocalteu reagent were added and diluted according to the label specification to each of the above volumetric flasks. After 5 min., 4ml of 20% sodium carbonate solution was added and volume was made up to 25ml with distilled water. Absorbance was recorded after 30 min. at 765nm and a calibration curve of absorbance *vs* concentration was plotted.

Preparation of test solution

1 g of sample (fruit pulp powder) was added to 15 ml of methanol (50%) and extracted for three times by maceration of 2 hours. Then filtered and make up the volume with methanol (50%) in volumetric flask upto 50 ml. 1 ml aliquot of the sample was taken in a test tube and diluted with 10 ml of distilled water. Then, 1.5 ml Folin Ciocalteu's reagents was added and allowed to incubate at room temperature for 5 min. 4 ml of 20% (w/v) Na_2CO_3 were added, adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature Absorbance of the sample was measured at 765 nm. Three parallel determinations were recorded. Quantification was done on the basis of a standard curve of gallic acid. Results were expressed as µg gallic acid equivalents (GAE) and percentage w/w (5).

In-vitro assays for anti-oxidant activity

Determination of DPPH radical scavenging activity

DPPH• is a stable free radical at room temperature which when accepts an electron or hydrogen radical becomes a stable diamagnetic molecule (6). The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517nm, induced by antioxidants. The absorption maximum of a stable DPPH radical in methanol was at 517nm. On reaction with antioxidant or free radical there is decrease in absorbance of DPPH radical because of scavenging of the radical by hydrogen donation. There is change in color from purple to yellow which is visually noticeable. Hence, DPPH is usually used as a substrate to evaluate the antioxidative property (7). The 0.1 mM solution of DPPH in methanol (22.2 mg in 1000 ml) was freshly prepared. Different concentrations of extract were added at an equal volume to methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. Ascorbic acid and rutin were used as standard controls. Percentage inhibition of DPPH free

radical was calculated based on the control reading, which contain DPPH and distilled water without extract using the following equation:-

DPPH Scavenged (%) = (Acont - Atest) / Acont × 100

Where; Acont is the absorbance of the control reaction and Atest is the absorbance in the presence of the sample of the extracts.

Reducing power assay

The different concentration of the extracts (25-400µg/mL) in 1 mL of deionized water were mixed with phosphate buffer (2.5mL, 0.2M, pH 6.6) and 1% potassium ferricyanide [$K_3Fe(CN)_6$] (2.5mL). The mixture was incubated at 50°C for 20 min. The reaction was stopped by adding trichloroacetic acid (2.5mL, 10%) to the mixture, which was then centrifuged for at 1000 x g for 10 min. The upper layer of solution (2.5mL) was mixed with distilled water (2.5mL) and FeCl3 (0.5mL, 0.1%), and the absorbance was measured at 700nm. Ascorbic acid was taken as a reference (8-9).

Inhibition of lipid peroxidation (%) = (sample OD / blank OD) × 100

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical screening of methanol and aqueous extracts revealed the presence of phenolics, flavonoids, alkaloids, amino acids and carbohydrates.

Total phenolic content

Phenolic constituents are very important in plants because of their scavenging ability due to their hydroxyl groups (10). These substances are known to possess the ability to reduce oxidative damage and act as antioxidants (11). They can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes (12).In addition, it is reported that phenolic substances are associated to play important role in stabilizing lipid peroxidation (13). Total phenolics determined in extract were 2.34% w/w.

DPPH assay

The 90% methanolic and aqueous extracts of the *M. balsamina* fruit pulp showed promising free radical scavenging effect of DPPH in a concentration dependent manner up to a concentration of 80 µg/ml are shown in fig.1 and fig.2. The 90% methanol showed more scavenging activity than the aqueous extracts. Ascorbic acid and rutin were used as the reference standards are shown in fig.3and fig4 respectively. The reduction of alcoholic DPPH by 90% methanol extract was very high and the scavenging ability increased with increasing concentration. The results were expressed as the quantity of extracts required to cause 50% inhibition (IC_{50}) and the results are depicted in table 11. The IC_{50} value for 90% methanol and aqueous extracts were 371.29±1.57 and384.22±2.46 µg/ml respectively. L-Ascorbic acid and rutin were used as the reference standard for antioxidant activity, and the IC_{50} value were 3.78±0.153 and 8.84±0.05 µg/ml.

Fig.1 - Free radical scavenging effect of 90% methanol extract of *M. balsamina* by DPPH

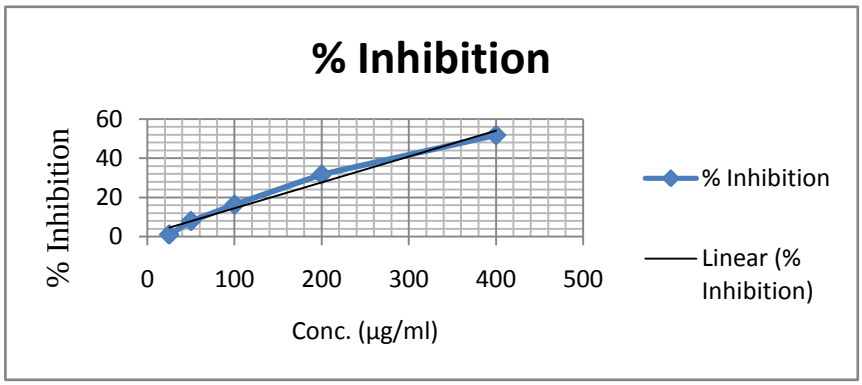


Fig.2 - Free radical scavenging effect of aqueous extract of *M. balsamina* by DPPH

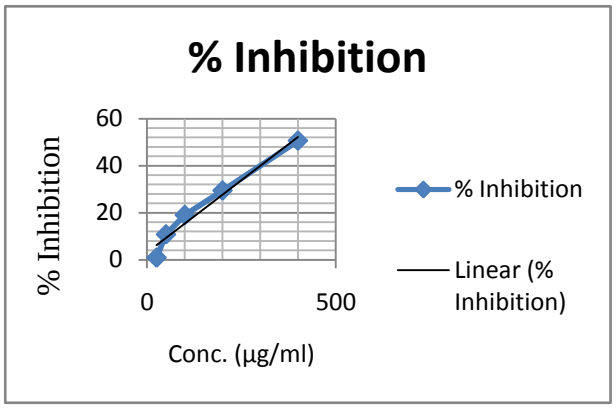


Fig.3 - Free radical scavenging effect of ascorbic acid by DPPH

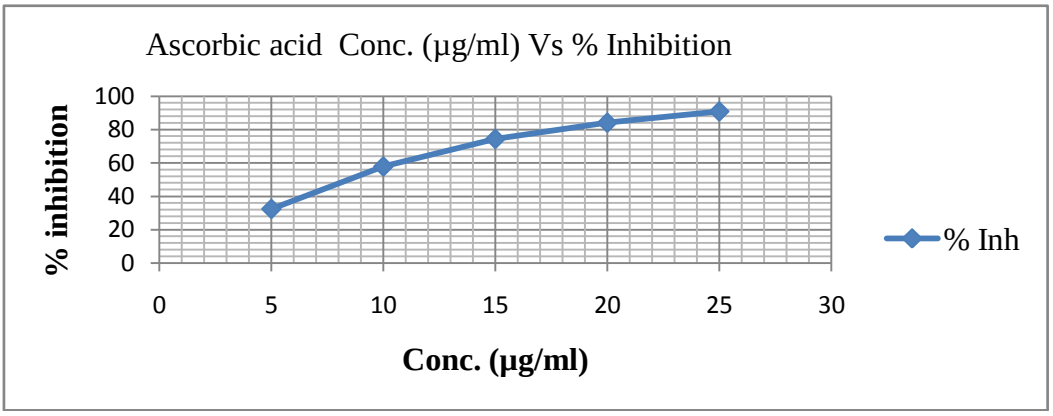
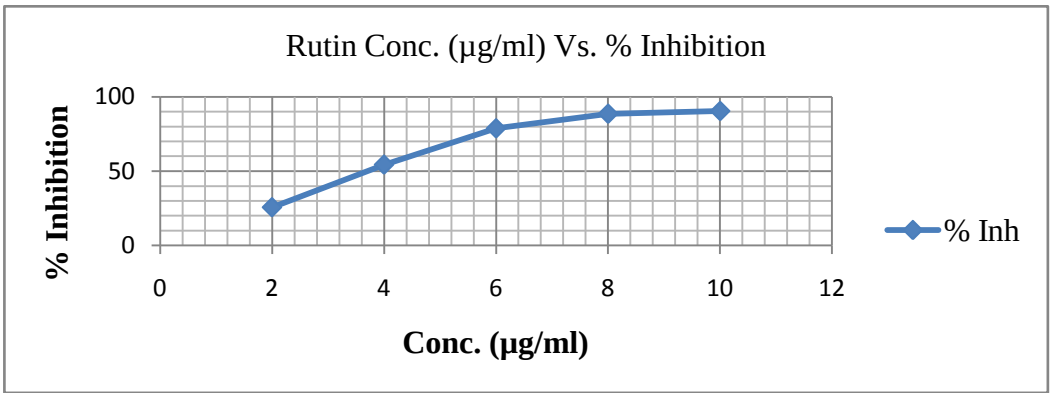


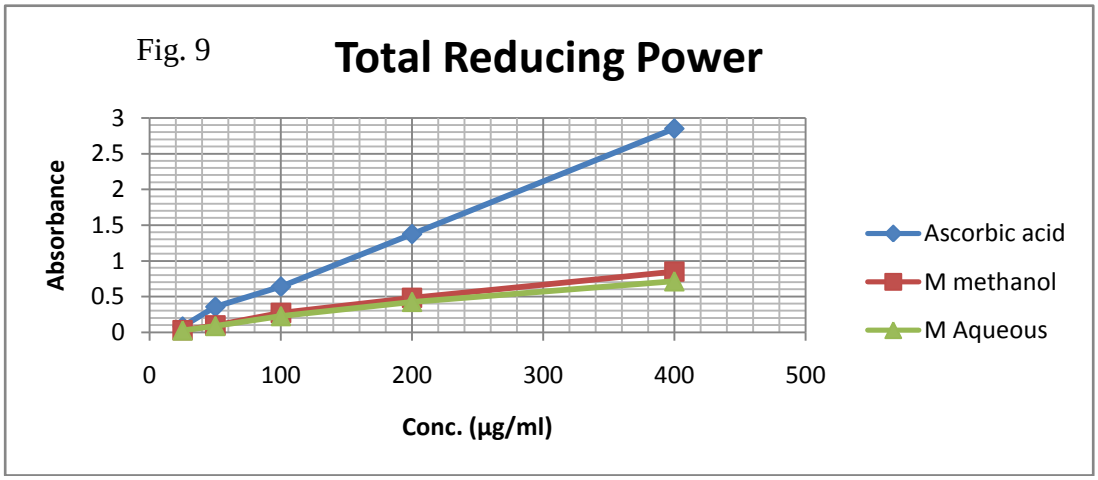
Fig. 4 - Free radical scavenging effect of rutin by DPPH



FRPA method

The reducing power of 90% methanol and aqueous extracts of the *M. balsamina* fruit pulp are shown in fig.5. Ascorbic acid was used as the reference standards. The reducing power of the extracts was found to concentration dependent. The methanol extract showed more reducing power than the aqueous extracts.

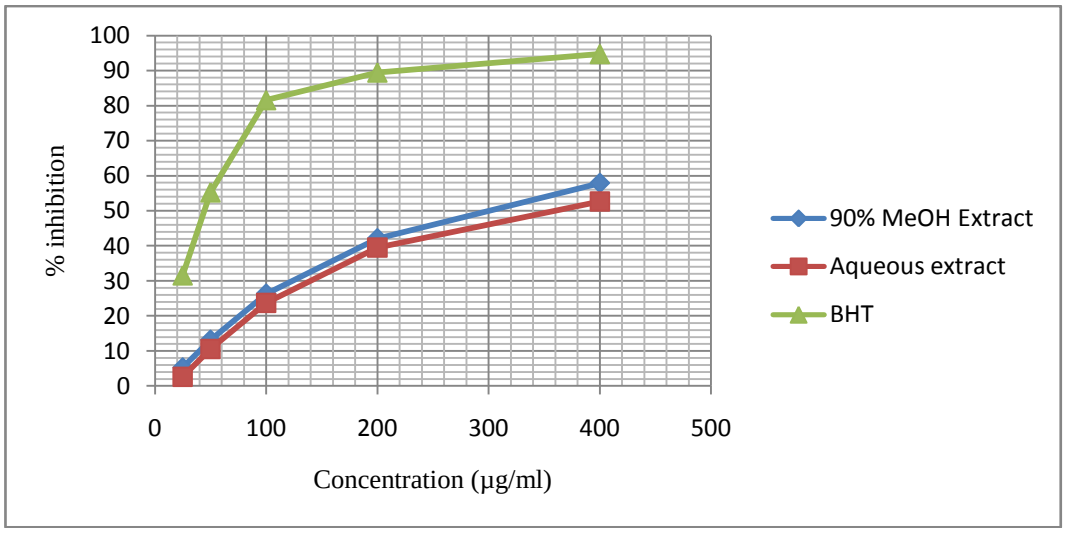
Fig.5 Total reducing power of extracts



TBARS assay

The results of the TBARS assay for 90% methanol and aqueous extracts are given in fig. 6. Both the extracts showed antilipid peroxidation activities, but the activities are not substantial as compared to the butylated hydroxytoluene (BHT).

Fig. 6- Antioxidant index of *M. balsamina* extracts and BHT at different concentration on lipid peroxidation



CONCLUSION

The results of the present study has shown that the methanol and methanolic extracts of *Momordica balsamina* Linn. Fruit pulp possesses antioxidant activity proved via DPPH radical scavenging activity, lipid peroxidation with thiobarbituric acid and reducing power assay. Preliminary phytochemical analysis indicates the presence of polyphenols (tannins, flavonoids) and saponins in methanol and aqueous extracts. Polyphenols like flavonoids and tannins are the well known natural antioxidants (14). So, the antioxidant potential of methanol extract and aqueous extract of *Momordica balsamina* may be due to the presence of polyphenolic content and saponins.

Acknowledgement

The authors are thankful to the administration of ISF college of Pharmacy Moga, Punjab for providing the funds and facilities.

REFERENCES

1. Jeffrey C. Cucurbitaceae. *Flora of Tropical East Africa*. 1967:17-40.
2. Watt JM, Breyer-Brandwijk, Maria G Medicinal and poisonous plants of southern and eastern Africa, Ed. 2, Livingstone, Edinburgh & London, 1962,14-17.
3. Hutchings A, Scott AH, Lewis G, Cunningham AB. Zulu medicinal plants, an inventory. University of Natal Press, Pietermaritzburg, 1996, 243-246.
4. http://herbalafrica.co.za/HerbsMomordica.htm.27th May, 2007.
5. Singleton VL, Orthofer R, Lamuela-Raventos, RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. *Methods Ezymol*. 1999**;** 299:152–178.
6. Soares JR, Dins TCP, Cunha AP, Ameida LM. Antioxidant activity of some extracts of *Thymus zygis*. *Free Rad Res* 1997; 26:469-478.
7. Chang LW, Yen WJ, Huang SC, Duh PD. Antioxidant activity of sesame coat. *Food Chem* 2002; 78:347-354.
8. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. *Jpn J Nutr* 1986; 44:307-315.
9. Jayaprakash GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed extracts on peroxidation models in vitro. *J Agric Food Chem* 2001; 55:1018-1022.
10. Hatano T, Edamatsu R, Mori A, Fujita Y, Yasuhara E. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. *Chem Pharm Bull* 1989; 37:2016-2021.

11. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. *Annu Biochem* 1987; 165:215-219.

12. Lewis NG, Alscher RG, Hess JL. Antioxidants in higher plant. Boca Raton, FL, CRC Press 1993; 135-169.

13. Yen GC, Duh PD, Tsai CL. Relationship between antioxidant activity and maturity of peanut hulls. *J Agri Food Chem* 1993; 41:67-70 .

14. Dreosti IE. Antioxidant polyphenols in tea, cocoa and wine. *Nutrition* 2000 ; 16:692-694.