ISSN : 2350-0743

www.ijramr.com

International Journal of Recent Advances in Multidisciplinary Research
Vol. 02, Issue 11, pp.0989-0992, November, 2015

RESEARCH ARTICLE

ANALYSIS OF ANTIOXIDANT POTENTIAL OF *MOMORDICA CHARANTIA* (BITTER GOURD) *IN-VITRO*

[1]Flora-Glad Chizoba Ekezie, *[1]Dr. Jessie Suneetha, W., [1]Uma Maheswari, K., [2]Prasad, T.N.V.K.V. and [1]Anila Kumari, B.

[1] Post Graudate & Research Centre Department of Foods and Nutrition, Professor Jayashankar Telangana State Agricultural University (Formerly part of Acharya N.G. Ranga Agricultural University), Rajendranagar, Hyderabad, 500030, India

[2]Nanotechnology Laboratory, Institute of Frontier Technology, Regional Agricultural Research Station, Acharya N.G. Ranga Agricultural University, Tirupati 517502, India

ARTICLE INFO

Article History:
Received 27th, August 2015
Received in revised form
09th, September 2015
Accepted 25th, October 2015
Published online 30th, November 2015

Keywords:
Antioxidant, DPPH,
Superoxide, Inhibitory concentration,
Bitter gourd, Ethanol.

ABSTRACT

This research investigated the antioxidant potential of *Momordica charantia (*bitter gourd) in various media. The extracts have been assessed for DPPH free radical scavenging and superoxide scavenging effect. In DPPH method, IC_{50} values were 57. 31, 73.16, 90.52, 98.57 and 83.80 µg/ml for ethanol, aqueous, citric acid, Na_2CO_3 and NaCl extracts of bitter gourd respectively. In super oxide scavenging method, IC_{50} values of ethanol, aqueous, citric acid, Na_2CO_3 and NaCl were 76.09, 86.25, 99.91, 96.72 and 102.06 µg/ml respectively. The data obtained in the *in-vitro* models clearly establish the antioxidant potency of bitter gourd extracts with ethanol extract having the best potential and can be used in the development of medicines by isolating the active component(s).

INTRODUCTION

Natural Sources have been used as medicinal agents for thousands of years and a good number of modern drugs have been isolated from them and are now also utilized in traditional medicine. About 64% of the populations still depend on traditional medicine and medicinal plants for their health-care needs in developing countries (Das *et al.,* 2006). The upsurge in the use of plant materials and their potent medicinal agents has been attributed to toxicity and side effects of allopathic medicines (Verma and Singh, 2008). Reports indicated the anti-hyperglycemic (Choudhary *et al*., 2012), anti-migratory (Hsu *et al*., 2012), anti-prolifiretory (Brennan *et al*., 2011) effects of the different extracts of *M. charantia*. This is possibly due to at least three different groups of constituents which include alkaloids, insulin like peptides and a mixture of steroidal sapogenins known as charantin. The yield of the bioactive compounds depends on the extraction media used as substantial liberation of the compounds largely correlates with the nature of the solvent or the media (Anjum *et al*., 2013). It is a common food item of the tropics and is used for the treatment of cancer, diabetes and many ailments in ayurvedic medicine (Bae *et al*., 2008). Free radical reactions in the lipid domain damage the membrane proteins causing alteration and impairment of membrane functions and results in adverse effects on the biological system, which may include atherosclerosis and cancer (Wiseman, 1996; Cook and Samman, 1996). The known antioxidants are likely to exert their effect on lipid peroxidation by scavenging the reactive oxygen species which otherwise would initiate the lipid peroxidation (Tyler, 1975; Halliwell and Gutteridge, 1984). In the present study, the bitter gourd was extracted in different media and the antioxidant activity was analyzed.

Corresponding author: Dr.Jessie Suneetha, W.,
Post Graudate & Research Centre Department of Foods and Nutrition, Professor Jayashankar Telangana State Agricultural University (Formerly part of Acharya N.G. Ranga Agricultural University), Rajendranagar, Hyderabad, 500030, India.

MATERIALS AND METHODS

Sample preparation

1g of dried bitter powder in 100 ml of water, ethanol, citric acid (1M), sodium carbonate (1M) and sodium chloride (1M) solutions was subjected to exhaustive extraction by cold maceration for 72 hours by sealing the conical flasks to avoid evaporation. Later, the slurries were centrifuged at 3000 rpm for 10 minutes and filtered through Whatman No.41 filter paper to obtain clear extracts which were used for antioxidant studies.

Estimation of antioxidant activity

DPPH radical scavenging activity (Brand-William *et al.,* 1995)

From the raw extracts, different concentrations viz. 10, 20, 40, 60, 80 and 100 µg/ml were prepared for antioxidant studies. A positive control of ascorbic acid was also prepared at the same concentrations.

DPPH solution (0.004 %) of 5ml was mixed with the same volume of the various extracts and standard solution respectively. These solution mixtures were kept in dark for 30min and read at absorbance at 517nm using a spectrophotometer. The degree of DPPH purple decolorization to yellow indicated the scavenging efficiency of the extracts. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The scavenging activity against DPPH free radical concentration was calculated using:

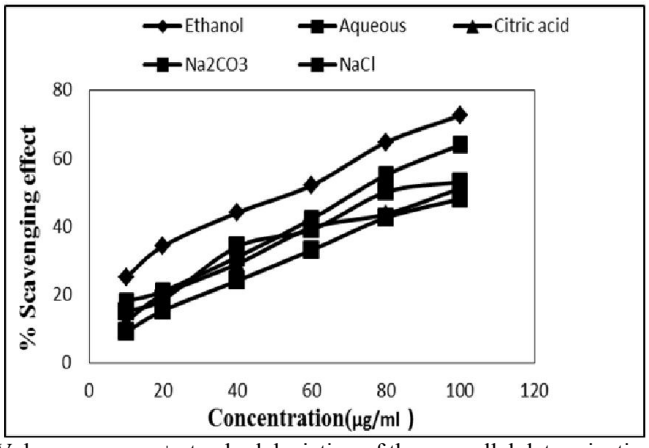
DPPH scavenging (%) = (A – B / A) x 100

Where, A was the absorbance of the control (DPPH solution without the sample), B was the absorbance of DPPH solution in the presence of reaction mixture or standard (extract/ascorbic acid). The antioxidant activity was expressed as $1C_{50}$. All the tests were performed in triplicate and the graph was plotted with the average of the three observations.

Superoxide Scavenging Activity Assay (Nishimiki *et al.*, 1972)

0.1ml of extracts was mixed with 1ml of NBT solution (156uM in 0.1M phosphate buffer, pH 7.4) and 1ml of NADH solution (468uM in 0.1M phosphate buffer, pH 7.4). The reaction was started by adding 100uL of phenazine solution (60uM in 0.1M phosphate buffer, pH 7.4).

The mixture was incubated at room temperature for 5min, and the absorbance was measured at 560nm in spectrophotometer against blank samples. The following formula was used to calculate the percentage inhibition of superoxide anion generation.



Values are mean ± standard deviation of three parallel determinations

Figure 2. Relative percentage scavenging activity by DPPH method

Superoxide anion scavenging activity (%) = $(A_0\text{-}A_1/A_0)$ X 100

Where, A_0 is the absorbance of the negative control consisting of all the reaction agents except the extract; A_1 is the absorbance of reaction mixture or standard of 1% ascorbic acid. The antioxidant activity was expressed as IC_{50}. All the tests were performed in triplicate and the graph was plotted with the average of the three observations.

Statistical Evaluation

Experimental results were mean ± standard deviation of three parallel measurements. Linear regression analysis was used to calculate the IC_{50} value. Data were considered statistically significant only when p value < 0.05 (Snedecor and Cochran, 1983)

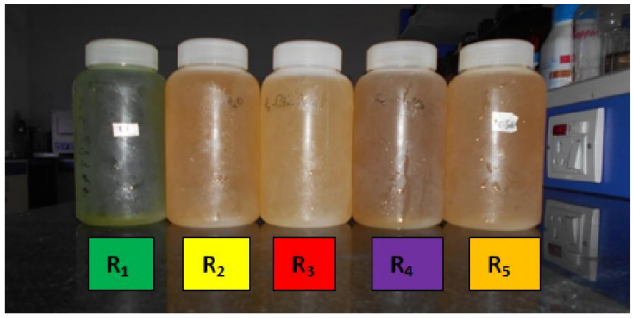
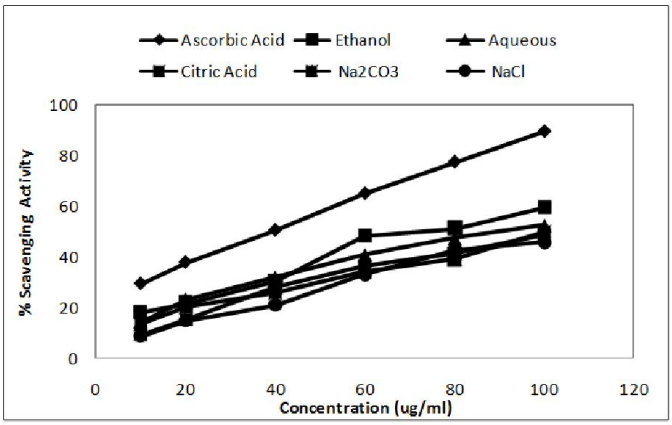


Figure 1. R_1 –Ethanol, R_2 – Aqueous, R_3 – Citric acid, R_4 – Na_2CO_3, R_5 – NaCl extracts

Table 1. IC_{50} values of extracts in 2 antioxidant experimental models

S. No	Sample	Mean IC_{50} (µg/ml)	
		DPPH Radical Scavenging Activity	Superoxide Dismutase Activity
1	Ethanol extract	57.31 ± 0.01^e	76.09 ± 0.032^e
2	Aqueous extract	73.16 ± 0.01^d	86.25 ± 0.040^d
3	Citric acid extract	90.52 ± 0.05^b	99.91 ± 0.12^b
4	Na_2CO_3 extract	98.57 ± 0.06^a	96.72 ± 0.53^c
5	NaCl extract	83.80 ± 0.05^c	102.06 ± 0.16^a



Values are mean ± standard deviation of three parallel determinations

Figure 3. Relative percentage scavenging activity by superoxide scavenging method

RESULTS AND DISCUSSION

Antioxidant activity

Many methods are available to measure the antioxidant capacity of plant materials. Owing to the complexity of the oxidation / anti-oxidation process, no single testing method is capable of providing a comprehensive view of the antioxidant profile of a sample (Parejo *et al.,* 2002). Therefore a multi-method approach is necessary to assess antioxidant activity.

The intensity of the radical scavenging effect is measured by the calculating half-inhibition concentration (IC_{50}), which is the efficient concentration required for decreasing initial free radicals concentration by 50% (Talukder *et al*., 2013). IC_{50} is obtained by interpolation from linear regression analysis of data. In the present study, the antioxidant activity of crude extracts in various media was carried out by *in-vitro* antioxidant models and was studied in relation to ascorbic acid, a known antioxidant because of its ability to impair the formation free radicals in the process of intracellular substance formation throughout the body.

DPPH radicals scavenging activity

Compelling studies indicates that increased consumption of dietary antioxidants or fruits and vegetables with antioxidant properties may contribute to the improvement in quality of life by delaying onset and reducing the risk of degenerative diseases associated with aging. The stable free radical DPPH method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound of plant extracts (Monograph, 2007). Antioxidants react with DPPH, which is a stable free radical and convert it to 2, 2-diphenyl-1-picryl hydrazine. The degree of discoloration indicates the radical scavenging potential of the antioxidant (Singh *et al.,* 1996). The % radical scavenging effect was summarized in Fig.2.

The results obtained indicate that all the samples showed a dose dependent radical scavenging activity. The % scavenging effect of the extracts was observed thus; Ethanol > Aqueous > NaCl > Citric acid > Na_2CO_3 (i.e. 72.54, 63.88, 53.07, 51.14 and 47.97% respectively in the same order). The IC_{50} values were also computed by linear regression. IC_{50} values were 57.31, 73.16, 90.52, 98.57 and 83.80 µg/ml for ascorbic acid, ethanol, aqueous, citric acid, Na_2CO_3 and NaCl extracts respectively.

The antioxidant activity exhibited by the ethanol extract is significant than other extracts due to the presence of substantial amount of phytochemical constituents from the plant material. The mechanism of action by which bitter gourd exhibit antioxidant activity is probably due to the reduction of DPPH radicals, inactivation of free radicals, chelation of metal ions or combinations thereof with phenolic constituents being a major contributor (Zainol *et al.,* 2003).

Superoxide Radical Scavenging Activity

The scavenging activity of the extract against superoxide radical generated in NaOH-alkaline DMSO-NBT system, resulted in the formation of the blue formazan was studied. The generated superoxide remains stable in solution, which reduces nitro blue tetrazolium into formazan dye at room temperature. Superoxide scavenger capable of reacting thus inhibits the formation of a red dye formazan. The inhibition of formazan dye was found to be dose dependent.

The result was also reflected through IC_{50} value viz; 76.09, 86.25, 99.91, 96.72 and 102.06 µg/ml for ethanol, aqueous, citric acid, Na_2CO_3 and NaCl respectively. All the samples were significantly different at 5% level ($p<0.05$). This finding demonstrates that bitter gourd raw extract is capable of non-enzymatically inhibiting the superoxide radical, produced in biological system, which is a precursor of many ROS and shown to be harmful for various cellular components (Jain *et al*., 2009). Other studies have reported the ability of different extracts of fenugreek seeds to scavenge superoxide free radicals and thus making it a potential source of antioxidants (Bukhari *et al*., 2008). Also (Rahman *et al.,* 2012) have also reported that garlic is also an important plant with intense ability to scavenge superoxide acid when extracted in various media.

Conclusion

Bitter gourd extracts in the various media showed antioxidant activity by inhibiting DPPH, and scavenging superoxide due to the phytochemicals present in them. However, the results, stated above showed that the ethanol extract of bitter gourd possessed significant anti-oxidative effects in both models.

REFERENCES

Anjum, F., Shahid, M., Anwer, B. S., Anwar, S. and Latif, S. 2013. Study of quality characteristics and efficacy of extraction solvent/technique on the antioxidant activity of Bitter gourd seed, *J. Food Process. Technol.,* 4, 2

Bae, J. M., Lee, E. J. and Guyatt, G. 2008. Citrus fruit intake and pancreatic cancer risk: A quantitative systematic review. *Pancreas*, 38(2), 168-74.

Brand-William, W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologie.,* 28, 25–30

Brennan, V.C., Wang, C. M. and Yang, W. H. 2011. Bitter melon *(Momordica charantia)* extract suppresses adrenocortical cancer cell proliferation through modulation of the apoptotic pathway, steroidogenesis, and insulin-like growth factor type 1 receptor/RAC-α serine/threonine-protein kinase signaling. *J. Med Food,* 15(4), 325-34.

Bukhari, S. B., Bhanger, M. I. and Memon, S. 2008. Antioxidative activity of extracts from fenugreek seed. *Pakistan Journal of Analytical and Environment Chemistry,* 9(2), 78-83.

Choudhary, S. K., Chhabra, G., Sharma, D., Vashishta, A., Ohri, S. and Dixit, A. 2012. Comprehensive evaluation of anti-hyperglycemic activity of fractionated *Momordica charantia* seed extract in alloxan-induced diabetic rats. Evid Based Complement *Alternat. Med*, 29, 36-50.

Cook, N. C. and Samman, S. 1996. Flavanoids - Chemistry, metabolism, cardio-protective effect and dietary sources *J. Nutr. Biochem.,* 7, 66 – 76.

Das, P. *et al*., 2006. "Screening of anti-helminthic effects of Indian plant extracts: a preliminary report." *J. Altern. Complement. Med*, 12(3), 299-301.

Halliwell, B. and Gutteridge, J.M.C. 1984. Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy, *Lancet* 1, 1396-1397.

Hsu, H. Y., Lin, J. H., Li, C.J., Tsang, S. F., Tsai, C. H., Chyuan, J. H., Chiu, S. J. and Chuang, S. 2012. Anti-migratory effects of the methanol extract from *Momordica charantia* on Human lung adenocarcinoma CL1 cells. Evid Based Complement *Alternat. Med,* 81, 92-96.

Jain, D., Daima, H.K., Kachhwaha, S. and Kothari, S. L. 2009. Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. *Digest journal of nanomaterials and biostructures*, 4(3), 557-563.

Monograph, 2007. *Momordica charantia* (Bitter melon), *Alternative Medicine Review*, 12, 4

Nishimiki, M., Rao, N.A. and Yagi, K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophy. Res. Com*, 46, 849–853.

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Flerlage, N., Burillo, J. and Codina, C. 2002. Comparison between the radical scavenging activity and antioxidant activity of six distilled and no distilled Mediterranean herbs and aromatic plants. *J. Agric. Food Chem.,* 50, 6882-6890.

Rahman, M. M., Fazlic, V. and Saad, N. W. 2012. Antioxidant properties of raw garlic (*Allium satvum*) extracts. *International Food Research Journal*, 19 (2), 589-591.

Singh, R. B., Niaz, M. A., Rastogi, S. S. and Rastogi, S. 1996. Usefulness of Antioxidant vitamins in Suspected Acute Myocardial Infarction. *Am. J. Cardiol.,* 77, 232–236

Snedecor, G. W. and Cochran, W. G. 1983. Statistical Methods, Oxford and IBH publishing Company, New Delhi, 22.

Talukder E U, Aklima J, Emran T B, Islam S, Rahman A and Bhuiyan, R.H, In vitro Antioxidant Potential of Momordica charantia Fruit Extracts. *British Journal of Pharmaceutical Research,* 3(4) (2013) 963-971.

Tyler, D. D. 1975. Role of superoxide radicals in the lipid peroxidation of intracellular membranes. *FEBS let.,* 51, 180-183.

Verma, S. and Singh, S. P. 2008. Current and future status of herbal medicines. *Veterinary World,* 1(11), 347-350.

Wiseman, H. 1996. Dietary influence on membrane function: Importance in protection against oxidative damage and disease, *J. Nutr. Biochem*., **7**, 2-15.

Zainol, M. K., Abdul-Hamid, A., Yusof, S. and Muse, R. 2003. Antioxidative activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* (L.) urban. *Food Chemistry* 49, 5165-5170.

****