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EVALUATION OF ANTIOXIDANT, ANTIBACTERIAL AND PHYTOCHEMICAL SCREENING OF *MOMORDICA CHARANTIA* FRUIT EXTRACT

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ABSTRACT

The present study is the continuation of a program aimed at investigation of antioxidant, antimicrobial and photochemical properties of *Momordica charantia* (Karela) extract to justify the traditional claim endowed upon this herbal drug in Ayurveda. The In vitro antioxidant of methanolic extracts of *Momordica charantia* (Karela) extract. The antioxidant activity determinate by DPPH assay. The *Momordica charantia* (Karela) fruit extract have dose depended antioxidant activity. The antimicrobial activity was evaluated according to the disk diffusion method by using Gram positive; *B. subtilius, S. aureus* and *S. epidermidis* and Gram negative; *E. coli, S. flexneri, P. aeruginosa* bacteria. This study shows that methanolic extracts of *Momordica charantia* (Karela) inhibit the growth of microorganisms dose dependently. Phytochemical screening of the extracts showed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids. The presence of flavonoids and glycosides as major constituents of the plant extract that are commonly known to posses antimicrobial activity. These results confirm the antioxidant and antibacterial activity of *Momordica charantia* (Karela) and support the traditional use of the plant in therapy of bacterial infection.

Key words: Antioxidant, Phytochemicals, Antibacterial, Disk Diffusion Method, Bacteria, *Momordica charantia.*

INTRODUCTION

Momordica charantia is a medicinal plant indigenous to China. This plant has many medicinally important active components. Previously the fruit and seeds extract of *Momordica charantia* have been used in China for centuries as an anti-viral, antitumor and also as an immunopotentiating agents (Li *et al.*, 1977). Earlier worker have reported that, several proteins have been isolated from the seed extracts of *Momordica charantia* (Barbieri *et al.*, 1980) and these proteins belong to the family of single chain ribosome-inactivating proteins.

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They inhibit in vitro translation of eukaryotic cells by catalytic inactivation of the 60S ribosomal subunit. It was also reported that, these proteins were also found to inhibit the multiplication of several viruses like herpes simplex virus-1 (HSV-1) and poliovirus 1 in Hep-2 cells (Foa-Thomasi et al., 1982). Fruit extracts of Momordica charantia have been shown to possess in vivo antitumor activity and immune-enhancement ability. These extract also inhibited the formation of prostate adeno-carcinoma in rats (Flatcuer *et al.*, 1980) and lymphoma in mice (Jilka *et al.*, 1983). *Momordica charantia* (*M. charantia*), also known as bitter melon, karela, balsam pear, or bitter gourd, is a popular plant used for the treating of diabetes-related conditions amongst the indigenous populations of Asia, South America, India, the Caribbean and East Africa (Cefalu and Wang, 2008). Its fruit has a distinguishing bitter taste, which is more pronounced as it ripens, hence the name

bitter melon or bitter gourd. Biochemical and animal model experiments have produced abundant data and hypotheses accounting for the anti-diabetic effects of *M. charantia*. In comparison, clinical studies with human subjects are sparse and low quality in design (Cousens, 2008). Therefore the present study have been undertaken to assess antioxidant, antimicrobial and photochemical properties of *Momordica charantia* (Karela) extract of Momordica charantia extract.

MATERIALS AND METHODS

Reagent and authentic samples – The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany).

Microorganisms

The test organisms included the grampositive bacteria *Bacillus cereus, Staphylococcus aureu and gramnegative* bacteria *Klebsiella pneumoniae,, Escherichia coli), Pseudomonas pseudoalcaligenes.* All the bacterial strains were obtained from National Chemical Laboratory (NCL), Pune, India.

Preparation of extract

Dried powdered of *Momordica charantia* (10 g) were extracted by continuous mixing in 100 ml 50% methanol, 24 h at room temperature. After filtration, methanol was evaporated until only water remained through evaporation on water bath at 60-70 0c temperature. The dried powder was kept in air tied box.

Antioxidant Activity

The OH- radical scavenging activity of *Momordica charantia* fruit extract (10–100 ug/ml) was determined according to the deoxyribose method of Halliwell, *et. al*., (1987) in the presence of 100 lM EDTA. FeCl3, H2O and ascorbic acid were prepared in degassed H2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 lM EDTA, 1 mM H2O2, 100 lM L- ascorbic acid, 100 lM FeCl3, H2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Follow in incubation at 38º C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture which was then heated in a boiling water bath for 15 min. Once samples were cooled, the absorbances were read at 532 nm.

Antibacterial Assay

Antibacterial activity of *Momordica charantia* extract was determined by agar disk diffusion method (Nair and Chanda, 2005) at four different concentrations i.e., 100 mg/ml, 75mg/ml, 50 mg/ml and 25 mg/ml. Muller Hinton Agar was prepared according to the manufacturer's instructions. The medium was sterilized by autoclaving at 121^{o} C for 15 minutes at 15 psi pressure and was used for tests. Sterile molten cool (45^{o} C) agar was poured aseptically into sterile petridishes (15 ml each) and the plates were allowed to solidify at room temperature in sterile condition. After solidification and drying, the plates were seeded with appropriate micro organisms by streaking evenly on to the surface of the medium with a sterile cotton swab or pouring the appropriate microorganism on the surface of dry agar plate present in peptone broth. Care was taken for the even distribution of culture all over the plate. The inoculums were allowed to dry for 5 minutes. The discs of 6 mm diameter were prepared from Whatmann filter paper No. 1 and were sterilized. The discs were then impregnated with the extracts, solvent DMSO and Gram positive (TE- Tetracycline, OF- Ofloxacin, AZ- Azithromycin & PC- Piperacillin) and Gram negative Fu - Nitrofurantoin, GM - Gentamicin, CX - Cefotaxime , NF –Norfloxaci,(5 µg/disc) were used as standard. Sterile Whatmann No 1 filter paper with different test concentrations ranging from 100, 75, 50, 25 mg/ml/disc were placed on to the agar with flamed forceps and gently pressed down to ensure contact along with the diluted extract, one appropriate control dry disc also placed at the center. Then the plates were incubated below 37^{o}C for 24 hrs to allow perfusion of drugs being tested.

Preliminary Phytochemical Screening

Phytochemical screening was carried out for henna leaf samples using the method adopted by Crombie *et. al*., (1990). Photochemical screening tests of methanolic extracts were carried out for leaf of *Lawsonia inermis* constituents. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavanoids, saponin, tannins using standard procedures (Trease and Evans., 1989; Harborne, 1993).

Detection of Glycosides:

Borntrager's test: Another portion of the extract was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides. Ammonia layer acquires pink color, showing the presence of glycosides.

Test for Phytosterol

Chloroform test: 0.5 gm of extract was treated with 10 ml chloroform and filtered. The filtrate was used to test the presence of phytosterols and triterpenoids. The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification has taken place. The mixture was diluted and extracted with ether. The residue was dissolved in few drops of dilute acetic acid,

10 ml of acetic anhydride followed by few drops of concentrated sulphuric acid. Appearance of bluish green color shows the presence of phytosterol.

Tests for steroidal compounds

Salkowski's Test - 2.5g extracts were dissolved in 12 ml chloroform in a test tube. Concentrated sulfuric acid was added on the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicated the presence of steroid ring (i.e., the aglycone portion of the glycoside).

Tests for Saponin

Froth Test: 1.5g extracts were dissolved in 3ml of distilled water for about 30 seconds. The test tube was stoppered and shaken vigorously for about 120 seconds The test tube was allowed to stand in a vertical position and observed over 30 minutes period of time. If a "honey comb" froth above the surface of liquid persists after 30 minutes the sample is suspected to contain saponin.

Test for Tannins

Ferric chloride Test- A portion of the extracts were dissolved in water. The solution was clarified by filtration; 20% ferric chloride solution was added to the clear filtrate. This was observed for a change in colour to bluish black.

Tests for Flavanoids

Tests for free flavanoids- 5ml of ethyl acetate was added to a solution of 0.5g of the extract in water. The mixture was shaken, allowed to settle, and inspected for the production of yellow colour in the organic layer, which is taken as positive for free flavanoids.

RESULTS

Our study shows that antioxidant activity of *Momordica charantia* extract using DPPH assay. The antioxidant activities of *Momordica charantia* extract scavenge OH' radical was assessed using the DPPH assay. Extent of hydroxyl radical scavenged was determined by the decrease in intensity of pink coloured chromophore at 532 nm wavelength. The dose dependent inhibition at different concentration of *Momordica charantia* extracts ranging from 10 to 100 µg/ml. The results are summarized to Table 1.

The results confirmed the presence of glycosides, phytosterol, steroids, saponin, Tennins and Flavonoids in extracts of the plant. The phytochemical analysis results of *Momordica charantia* were reported in Table 2.

Another set of experiment **t**he extracts of *Momordica charantia* had a concentration dependent antibacterial activity with more sensitivity for gram negative bacteria than gram positive bacteria used in the study. The results of antibacterial activity are reported in Table 3 and 4.

Table 1. Antioxidant activity of *Momordica charantia* extract

Concentration (µg/ml)	% inhibition (TBRAS)	
	Ascorbic acid	*Momordica charantia*
10	21.10	12.30
20	38.50	21.20
30	46.50	38.10
40	58.70	41.89
50	65.20	46.10
60	70.10	58.20
70	78.20	65.78
80	82.40	69.30
90	87.40	72.15
100	91.20	78.50
Control OD at 532 nm – 0. 280		

Table 2. Phytochemical Screening of solvent extracts of *Momordica charantia*

S. No.	Name of Tests	Tests/Reagents	Level*
1	Glycosides	Borntrager's	+
2	Phytosterol	chloroform	-
3	Steroidal	*Salkowski's Test*	+
4	Saponins	Froth test	+
5	Tennins	Ferric chloride test	+
6	Flavonoids	Test for free flavanois	+

*Here, + : presence, - : absence.

Table 3. Effect of *Momordica charantia* Leaves extract in antimicrobial activity

Test sample concentration in (mg/ml)	Name of the Microorganism (Inhibition zone in mm)					
	Gram Positive			Gram Negative		
	B. subtilius	*S. aureus*	*S. epidermidis*	*E. coli*	*Sh. flxineri*	*Ps. aeriuoginosa*
100	08.0	09.0	08.9	14.20	08.20	09.0
50	07.0	06.0	08.0	10.10	07.50	6.50
75	08.20	07.20	05.90	09.20	09.00	08.00
25	07.00	08.0	07.50	07.00	06.10	07.20

Table 4. Standard Antibiotics

S. NO.	Gram Positive/ Nagative	Name of the Organisms	Zone of Inhibition(In mm)			
			Different concentrations of Antibiotic (5µg/disc)			
			TE	**OF**	**AZ**	**PC**
1	**Gram Positive**	*B.subtilius*	17	15	14	11
		S.epidermidis	15	17	19	16
		S. aureus	12	14	17	12
			Fu	**GM**	**CX**	**NF**
2	**Gram Negative**	*E. coli*	11	12	9	14
		Ps.aeriuoginosa	16	14	19	17
		Sh. flxineri	16	16	14	18

TE = Tetracycline, OF = Ofloxacin, AZ = Azithromycin, PC = Piperacillin Fu = Nitrofurantoin, GM = Gentamicin, CX = Cefotaxime , NF =Norfloxacin.

DISSCUSION

Bitter melons are seldom mixed with other vegetables due to the strong bitter taste, although this can be moderated to some extent by salting and then washing the cut melon before use. It is also a popular vegetable in Indian cooking, where it is often prepared with potatoes and served with yogurt on the side to offset the bitterness, or used in sabji. According to the results of present study, fruit of *Momordica charantia* extract have some active chemical components which have antioxidant and antimicrobial activities.

The ability of *Momordica charantia* extracts to scavenge OH ' radical was assessed using the Fenton reaction assay. The *Momordica charantia* extract was found to have antioxidant activity and it was compared with ascorbic acid as a positive control.

The antibacterial activity was expressed at varying degrees with the activity being both strain and dose dependent. Six bacteria's were used for antibacterial studies. The methanolic extract of *Momordica charantia* was active against six different bacteria's. Four concentrations of the extract were used (100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml). It is estimated that if an inhibition is obtained by 25 mg/ml-100 mg/ml) of test solution, the extract can be considered worthy for further investigations. Plants showing significant activity may be due to the presence of glycosides, phytosterol, steroids, saponin, tennins and flavonoids.

Plants showing significant activity may be due to the presence of glycosides, phytosterol, steroids, saponin, tennins and flavonoids.. Among the various microorganisms, the methanolic extract *Momordica charantia* was more active against *E. coli*. Further evaluation needs to be carried out on *Momordica charantia* in order to explore the concealed areas and their practical clinical applications, which can be used for the welfare of the mankind.

The mechanisms by which *Momordica charantia* extract may reduce the risk of cancer may not solely be via reduction of mutation; but may involve the antioxidant activity, immune systems enhancing properties and high levels of tannins present in garlic which help reduce incidence of tumors (Chung et. al, 1998). Hence, the consumption of this fruit spice may actually be giving protection to human body against mutation of cells and cancer.

REFERENCES

Barbieri L, Zamboni M, Lorenzoni E, Montanaro L, Sperti S and Stirpe F. *Biochem J*, 186, 1980, 443-452.

Cefalu WT, Ye J, Wang ZQ. Efficacy of dietary supplementation with botanicals on carbohydrate metabolism in humans. *Endocr Metab Immune Disord Drug Targets*, 8, 2008, 78–81.

Chung KY, Wong TY, Wei CI, Wang Huang YW & Lin Y. Tannins and human health: a review. Critical review. *Food Science Nutrition*, 38(6), 1998, 421-64.

Cousens G. There is a cure for diabetes: the tree of life 21 day program. California: North Atlantic Books, 2008, 191–192.

Crombie L, WML Crombie and DA Whiting. Alkaloids of Khat (Catha edulis). *Alkaloids*, 39, 1990, 139-164.

Fletcuer MA, Caldwell K, Clafflin A & Malinin T. *Proc Fed Am Soc Exp Biol*, 39, 1980, 414.

Foa-Tomasi L, Campadelli-Fiume G, Barbieri L & Stirpe F. *Arch Virol*, 71, 1982, 322-332.

Halliwell B, Gutteridge JMC and Aruoma OL. The deoxyribose method: A simple ‘‘test-tube’’ assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry*, 165, 1987, 215–219.

Harborne JB. Phytochemical method, 3rd Edition, Chapman and Hall, London, 1993, 135-203.

Jilka C, Strifler B, Fortner GW, Hays EF & Takemoto DJ. *Cancer res*, 43, 1983, 5151-5155.

Li SC. 1596. Pen Ts'ao Kang Mu (Chinese Pharmaceutical Compendium) reprinted by People's Medical Publishing House, Beijing. 1977.

Trease GE and WC Evans. Pharmacognosy. 14th Edition, Brown Publication. 1989.