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IN VITRO ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF MOMORDICA CHARANTIA LEAVES

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ABSTRACT

The aim of the present study was to investigate the *in vitro* antimicrobial and antioxidant activity of aqueous and methanol extracts of *Momordica charantia* leaves. In preliminary phytochemical analysis we observed glycosides, alkaloids, phytosterols, saponins, phenolic compounds, proteins, fats and fixed oils and flavonoids, and thin layer chromatography was also performed. Antimicrobial activity was evaluated for *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus subtilis* by using stokes disc diffusion and well diffusion methods. Methanolic plant extract showed a maximum zone of inhibition in *E.coli* by disc method but in well diffusion method *Bacilli* and *Klebsiella* showed maximum inhibitory activity. The antioxidant activity of the plant extract was also determined by DPPH and ABTS methods using ascorbic acid and gallic acid as standards respectively. IC50 values were also calculated.

Keywords: Antioxidants, Antimicrobial agents, TLC, DPPH.

INTRODUCTION

Plants are integral part of human civilization. Medicinal plants are also been relied upon by over 80% of the world population for their basic health care needs.¹ *Momordica charantia* is a medicinal plant belonging to the family *cucurbitaceae*, found in tropical and subtropical regions of the world such as India, Asia, South America, and widely used as food and medicine.² It is commonly known as Bitter gourd in English, Paakharkaai in Tamil, Karela in Hindi and Bengali, kakarakaya in Telugu and hagalakayi in Kannada.³ It is a slender climbing annual vine with long-stalked leaves and yellow, solitary male and female flowers borne in the leaf axils. The Latin name *Momordica* means “to

bite” referring to the jagged edges of the leaves, which appear as if they have bitten.⁴

Momordica charantia is known to contain compounds such as momorchanins, momordenol, momordicilin, momordicius, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitns, cucurbitacins, cucuritanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acid, gentisic acid, goyaglycosides, goyasaponins, and multiflorenol.^{5,6}

The fruit and leaves contain alkaloids, glycoside, saponin like substances, rennin an aromatic volatile oil mucilage. Bitter gourd shows a significant antimicrobial activity and is of great use in medicine for treatment of many diseases

such as piles, leprosy, jaundice; diabetes and snake bite. Its fruits and leaves have been shown to exhibit various biological activities including anti-diabetic, anti-rheumatic, anti-ulcer, anti-inflammatory and anti-tumor.^{7,8} An antimicrobial is a compound that kills or inhibits the growth of microbes such as bacteria (antibacterial activity), fungi (antifungal activity), viruses (antiviral activity) or parasites (anti parasitic activity)

A leaf tea is used to treat diabetes, to expel intestinal gas, promote menstruation, and as antiviral agent against measles and hepatitis viruses.^{9,10} Its antimicrobial properties were investigated against *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis* using stokes disc diffusion sensitivity technique and well diffusion methods.

Free radicals play an important role in development of tissue role and pathological events in living organisms.^{11,12} There are evidences that explain that increased uptake of fruits and vegetables reduce the risk of cancer.^{13,14} This is attributed by antioxidants presents in fruits and vegetables.¹⁵⁻¹⁷ Asli *et al.* reported antioxidants and chemoprotective action of *Momordica charantia* fruit extracts.¹⁸ The present study was carried out to evaluate the antimicrobial efficacy and antioxidant activity of methanol and aqueous extract of *Momordica charantia* which helps in the development of new, novel drugs.

MATERIALS AND METHODS

Collection of Plant Materials

The study period was from January 2011 to March 2011. The leaves of *Momordica charantia* was collected from agriculture farm located in kalkere near Bangalore, India. The fresh leaves were washed thoroughly with tap water to remove dust particles then washed with sterile distilled water. The leaves were again washed with mild detergent tween 20. Leaves are then surface sterilized with calcium hypochlorite. Then the leaves were dried, powdered, stored in

an air tight containers and was used for the study.

Extraction Procedure

Aqueous and methanol are used as solvents for extraction of the plant materials. The air dried and fine powdered leaves were extracted with water and methanol using soxhlet extraction apparatus according to soxhlet method where materials are extracted by repeated percolation which lasts about 6-8 hours under reflux in a specialized glassware. The aqueous and methanolic extract obtained was used for study.

Chemicals

All chemicals and reagents used were of analytical grade and obtained from sigma chemical company and used without further purification.

Phytochemical screening¹⁹⁻²¹

Small quantity of aqueous and methanolic extracts of *Momordica charantia* was dissolved and used for detection of phytochemicals such as glycosides, phytosterols, proteins, alkaloids, flavonoids, tannins, saponins, fats & fixed oils, gums and mucilages.

Test for glycosides

The extract was hydrolyzed with HCL for few hours on hot water bath and the hydrolysate was subjected to Fehling's, Benedict's, Barfoed's tests and the result was recorded.

Test for alkaloids

Presence of alkaloids was tested with four reagents: Mayer's reagent (potassium mercuric iodide solution), Dragendorff's reagent (potassium bismuth iodide solution), Hager's reagent (saturated solution of picric acid), and Wagner's reagent (iodine and potassium iodide solution).

Test for phytosterols

Lieberman-Burchard test and Salkowski test was performed to identify the presence of phytosterols. The residue was dissolved in few

drops of acetic acid and three drops of acetic anhydride was added followed by few drops of concentrated sulfuric acid. Bluish green colour was formed shows the presence of phytosterol.

Test for fixed oils and fats

A few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats. Spot test was also performed to identify the presence of oil.

Test for gums and mucilages

Test for gums and mucilages was carried on extract by using 90% alcohol and the precipitate was dried in air.

Test of saponins

Foam test was conducted by diluting the extract with 20 ml of distilled and agitated in graduated cylinder **0.1cm layer** of foam was formed and the result was recorded

Test for proteins

Small quantity of extract was dissolved in few ml of water and treated with Biuret, Ninhydrin, Xanthoprotein and Million's reagents,

Test for phenolic compounds

A small quantity of extract was taken in water and FeCl₃ test was performed to identify the presence of phenolic compound.

Thin Layer Chromatography (TLC)

Silica gel coated TLC plates were purchased and was used for the study. A line was drawn on the TLC plate at a distance 2 cm from the base, marks were made on the line for sample application. The sample was spotted on the line with the help of capillary tube and it was allowed to dry. The plate was placed the developing jar with mobile phase. After the solvent reaches $\frac{3}{4}$ th of the TLC plate it is taken out of the jar, the solvent front was drawn. The plates were then kept in iodine jar for few seconds, shaken and taken out. They were examined under the UV/Vis lamp and the spots were circled with pencil. The spots were labelled and the distances from the base lines were measured. The R_f values were calculated by the formula

$$\text{Retention factor (R}_f\text{)} = \frac{\text{Distance travelled by solute from origin}}{\text{Distance travelled by solvent from origin}}$$

Antimicrobial Activity

Microorganisms

The antimicrobial activity of the aqueous and methanolic extracted was tested individually on four different micro organisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis*. All bacterial strains were obtained from department of biochemistry, administrative management college, Bangalore, India. It was investigated by using stokes disc diffusion sensitivity technique and well diffusion methods.

*Stokes disc diffusion method*²²

In stokes disc diffusion method, a loop of bacteria from the agar slant stock was cultured in

nutrient broth over night and spread with a sterile cotton swap into petri plates containing 10 ml of nutrient agar medium. Sterile filter paper discs (9 mm in diameter) impregnated with the plant extract were placed on the cultured plates and incubated at 37°C for 24 hrs. The solvent without extracts served as negative control. Standard antibiotic streptomycin (10µg) was employed as positive control. After 24 hrs of incubation an antibacterial activity was assessed by measuring the inhibition zone. The diameters of the zones of inhibition by the samples were then compared with the diameters of the zones of inhibition produced by the standard antibiotic discs. Each experiment was carried out in triplicate and the mean diameter of the inhibition ones was recorded.

Screening of anti bacterial activity was performed by well diffusion technique. The nutrient agar plates were seeded with 0.1 ml of standardized inoculums of each of the four test organisms. The inoculum was spread evenly over plate with loop or sterile glass spreader. The inoculated plates were incubated at 37°C for 20 minutes. After incubation a standard cork order of 6 mm diameter was used to cut uniform wells on the surface of nutrient agar medium and 10µl of the extracts was introduced in the well and incubated at 37°C for 24 hrs and the one of inhibition was measured in millimeter (mm). Mean zone of inhibition and standard deviations were calculated.

Assessment of In Vitro Antioxidant Activity

Free radical scavenging activity

$$\% \text{ inhibition} = [(\text{Absorbance of control} - \text{Absorbance of test sample}) / \text{Absorbance control}] \times 100$$

A dose response curve was plotted to determine The IC₅₀ values. IC₅₀ is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

ABTS radical scavenging assay²⁵

ABTS radical cations are produced by reacting ABTS (7mM) and potassium persulfate (2.45mM) on incubating the mixture at room

$$\% \text{ inhibition} = [(\text{Absorbance of control} - \text{Absorbance of test sample}) / \text{Absorbance control}] \times 100$$

A dose response curve was plotted to determine the IC₅₀ values. IC₅₀ is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

RESULTS

The results of the phytochemical study are given in table1. TLC fingerprint profiles of both aqueous and methanol extract showed 2 spots. The R_f values are 0.60, 0.34 and 0.56, 0.34 respectively (table2). The extracts showed a broad spectrum of antimicrobial activity by inhibiting the growth of test microorganisms. At

Aqueous and methanolic extract of *Momordica charantia* was used to assess the in vitro antioxidant activity. Antioxidant scavenging activity was studied using 1, 1-diphenyl, 2-picrylhydrazyl free radical (DPPH) (24). Various concentration of test solution in 0.1ml was added to 0.9 ml of 0.1 mM solution of DPPH in methanol. Methanol only (0.1ml) was used as experimental control. After 30 minute of incubation at room temperature, the reduction in the number of free radical was measured, reading the absorbance at 517nm. Ascorbic acid was used as reference standard. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. Ascorbic acid was used as reference standard.

The percent inhibition was calculated from the following equation:

temperature in dark for 16 hours. The solution thus obtained was further diluted with PBS to give an absorbance of 1.000. Different concentrations of the test sample in 50µl were added to 950µl of ABTS working solution to give a final volume of 1ml. The absorbance was recorded immediately at 745nm. Gallic acid was used as reference standard. The percent inhibition was calculated from the following equation:

the concentration of 100 mg/ml methanolic extract showed significant rate of inhibition in *E.coli* showing 6 mm inhibition zone by disc diffusion method. But in well diffusion method same concentration showed maximum zone of inhibition (8 mm) against *Bacillus subtilis* and *Klebsiella pneumonia* (table3). In vitro antioxidant results showed that the methanolic extracts of leaves were more potent than aqueous extracts in both DPPH and ABTS methods (table4).

DISCUSSION

The leaves of *Momordica charantia* has been used for thousands of years for its medicinal properties. It is rich in a wide variety of secondary metabolites such as glycosides, alkaloids, phytosterols, proteins, saponins and phytosterols which has been found invitro to have antimicrobial properties.²⁶ In this connection the present study on the methanolic and aqueous extracts was conducted to evaluate the antimicrobial activity of leaves. Aqueous extract of leaves showed milder antimicrobial activity compared to methanolic extract, which certainly indicates that methanolic extract contain higher concentration of active antimicrobial agents such as alkaloids, glycosides, volatile oils, which are all found in more abundant amount in *Momordica charantia*.^{27,28} Preliminary results of the activity of antimicrobial agents such as plant active components, antibiotics are usually expressed invitro as zones of inhibition around the chemical this is in comparable to the work of Gislene *et.al.*,(2000) on the antibacterial activity of the plant extract and phytochemicals on antibiotic resistance bacteria in rail according to them any chemical that demonstrates activity with zones of inhibition of 7 mm and above is acceptable as being active, the leaf extract of *Momordica charantia* showed 8 mm inhibition zone, therefore it contains effective antimicrobial compounds. Leaf extracts of *Momordica charantia* showed road spectrum antimicrobial activity since various water, ethanol, and methanol extracts of leaves have exhibited antibacterial activities against *E.coli*,

Pseudomonas. Besides, extract of the entire plant has shown antiprotozoal activity against *Entamoeba histolytica* and its fruit extract has demonstrated antibacterial properties against *Helicobacter pylori*, the bacteria causing cancer. In addition to these properties, it has also been used as appetite stimulant, treatment for gastrointestinal infection and to lower blood glucose sugar in diabetes. Its use for treatment of certain types of cancer and viral infections has also been reported. Recently, researchers have found that *Momordica charantia* contains several proteins that inhibit HIV in vitro, these proteins, known collectively as ribosome inactivating proteins (RIPs) are alpha-momorcharin beta- momorcharin and MAP-30 (Momordica anti-HIV protein).²⁹ As research is still in progress, it is unclear which active ingredients are having clinical usefulness.

CONCLUSION

From our study and with previous literature survey we can come to conclusion that the *Momordica charantia* leaves are rich in phytochemicals which has free radicals scavenging activity and further studies can be made to isolate and identified the chemical nature of the antioxidant present in the plant.

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Table 1: Preliminary phytochemical screening of *Momordica charantia* leaves

S.No.	Phytochemicals	Test	Aqueous	Methanol
1	Glycosides	a) Molisch's test	++	+++
		b)Fehling's test	++	+++
		c)Benedict's test	++	+++
		d)Barfoed's test	++	+++
2	Phytosterols	a)Libermann burchard test	+++	+++
		b)Salkowski test	+++	+++
3	Proteins	a)Biuret test	++	++
		b)Ninhydrin test	++	++
		c)Xanthoprotein test	++	++
		d)Millon's test	++	++
4	Alkaloids	a)Mayer's test	++	++
		b)Drangandroff's test	++	++
		c)Hager's test	++	++
		d)Wagner's test	++	++
5	Flavonoids	a)With aqueous NaOH solution	++	++
6	Phenolic compounds	a)FeCl ₃ test	++	++
7	Tannins	a) FeCl ₃ test	-	+
8	Saponins	a)Foam test	+	+
9	Fats & Fixed Oils	a)spot test	+	++
		b)Saponification test	+	++
10	Gums& Mucilages	a)With 90% alcohol	-	-

Key: +++ =High, ++ =Moderate, + =Normal concentration, - = Absent.

Table 2: TLC profile of *Momordica charantia* leaves

Extract	No. of spots visible by UV	R _f values
Aqueous extract	2	0.60 0.34
Methanol extract	2	0.56 0.34

Table 3: Antimicrobial activity of *Momordica charantia* by disc diffusion and well diffusion method

Methods	Extracts (100 mg/ml)	Microorganism/ zone of inhibition in mm*			
		EC	PA	BS	KP
Disc Diffusion	Aqueous	1.5±0.5	1.1±0.3	1.1±0.3	1.1±0.2
	Methanol	5.98±0.3	5.08±0.3	4.98±0.2	2.92±0.2
Well Diffusion	Aqueous	1.8±0.5	1.5±0.3	2.4±0.4	2.3±0.3
	Methanol	6.9±0.3	6.0±0.3	8.1±0.4	8.1±0.3
Gentamicin (10mg/ml)		20.0	18.0	22.0	.0

Key: EC= Escherichia coli; PA= Pseudomonas aeruginosa; BS= Bacillus subtilis; KP= Klebsiella pneumonia
*Each value was expressed as the mean±SD. (N=3)

Table 3: Antioxidant activity of *Momordica charantia* extracts

Methods	Extracts (100mg/ml)	Concentration(µg) / % inhibition				IC 50	Standard
		25	50	75	100		
DPPH Ascorbic acid (4 µg/ml)	Aqueous	11± 0.24	24± 0.37	59± 0.91	83± 0.34	66.25	50±0.21
	Methanol	18±0.12	33±0.23	50±0.22	90±0.14	73.30	
ABTS Gallic acid (4 µg/ml)	Aqueous	16±0.23	28± 0.18	38±0.32	59±0.30	53.75	57±0.41
	Methanol	18±0.32	28±0.13	42±0.18	63±0.26	56.25	

Each value represents the mean ± SD. N=3,

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