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ANTIBACTERIAL AND PHYTOCHEMICAL SCREENING FROM LEAF AND FRUIT EXTRACTS OF *MOMORDICA CHARANTIA*

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ABTRACT

Momordica charantia is a well known plant in Asia including India which posses wide range of pharmacological activities. These drugs have been used in India as folk remedy in the form of decoctions and infusions to treat bacterial infections and also claimed to be an effective against variety of skin conditions like psoriasis, acne, wounds etc. The present investigation is carried out to study the antibacterial activity and the presence of various phytochemicals of different parts of *Momordica charantia* extracts with different solvents on three microorganisms by disk diffusion method. Methanolic, ethanolic, hexane and aqueous extract of leaves, and fruits of the plant were evaluated for antibacterial activity using the disk diffusion method on three microorganisms (*Bacillus subtilus, Escherichia coli, and Salmonella typhi*). Zone of inhibition was calculated. Results indicate that the different concentrations of various extracts under study exhibit antibacterial activity and among the various extracts, fruit extracts have shown better activity as compared to leaf parts of extracts. Among the various extracts, methanol extracts have shown better antibacterial activity.

KEYWORDS: ***Momordica charantia*, Disc diffusion method, Antibacterial activity, Zone of inhibition.**

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INTRODUCTION

In recent years, several diseases and microbial infections such as respiratory infections, bacterial meningitis, sexually transmitted as well as hospital acquired infections, particularly those caused by the members of the family enterobacteriaceae have shown considerable resistance to a number of antibacterial agents, such as penicillin, ampicillin, and flouroquinolones among many others[1]. *Momordica charantia* or bitter melon, also known as balsam pear or karela, is a tropical vegetable, is a common food in Indian cuisine and has been used extensively in folk medicine as a remedy for diabetes. It is a very common herb having various medicinal properties for the treatment of different kind of disease, viz. antifungal, wound healing and antidiabetic agents[2,3]. Popularity of *Momordica charantia* in various systems of traditional medicine for several ailments (antidiabetic, abortifacient, anthelmintic, contraceptive, eczema, antimalarial, galactagogue, gout, Jaundice, abdominal pain, kidney (stone), laxative, leprosy, leucorrhea, piles, Pneumonia, Psoriasis, Purgative, rheumatism, fever and scabies) focused the investigator's attention on this plant[4]. In developing countries 80% of population continues to use traditional medicine in primary medical problems. *Momordica charantia* is one such plant that has been frequently used as medicine[6-7]. Mo*mordica charantia* Linn. is a well known to possess anti hyperglycemia, anticholesterol, immunosuppressive, antiulcerogenic, anti spermatogenic and androgenic activities anti-HIV, antiulcer, anti-inflammatory, anti-leukemic, antimicrobial, anti-cholesterol, immunosuppressive, and anti-tumor activities[8-10]. Unripe fruits of bitter melon have been found to have the blood sugar lowering capacity, similar to that of insulin and can be used to treat patients with diabetes. The compound that is responsible for this action is non nitrogenous substances charantin, which is a mixture of two compounds, namely, sitosteryl glucoside and stigmasteryl glucoside. Charantin is used to treat diabetes and can potentially replace treatment by injection of insulin, which has not been successful in stimulating the pancreas of the diabetic patients to lower blood sugar to the desire diabetic property such as charantin and others are now being widely accepted as an alternative medicine for diabetes mellitus, and they are free from side effects[11-14]. These chemicals are concentrated in fruits of *Momordica charantia* has shown more pronounced hypoglycemic/antihyperglycemic activity[15]. *Momordica charantia* shown promising effects in prevention as well as delay in progression of diabetic complication such as nephropathy, neuropathy, gastroapresis, cataract and insulin resistance[16]. Despite the existence of potent antibiotic and antifungal agents, resistant or multiresistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs. There is an urgent need to systematically evaluate the plants used in traditional medicine. Such research could lead to new drug discovery or advance the use of indigenous herbal medicines for orthodox treatment. Now a day a renewed interest in traditional medicine is observed and there has been an increasing demand for more and more drugs from plant sources. This revival of interest in plant derived drugs is mainly due to the current widespread belief that green medicine is safer and more dependable than the costly synthetic drugs many of which have adverse side effects[17]. Most recent researches on the plant show that it has ability to inhibit the enzyme guanylate cyclase that is thought to be associated with psoriasis, leukemia and tumor pathogenesis [5]. Therefore, drastic measures should be adopted to control the use of antibacterial agents, to understand the genetic mechanisms of bacterial resistance, and to continue studies to develop new drugs. Ultimately, this may greatly contribute to the provision of more appropriate and efficient antibacterial agents to the patient. In the present study antibacterial study of different parts of plant, extracted with different solvents is compared. The purpose of this work was to perform the chemical prospection of the fruit and leaf extracts and to evaluate the antibacterial activity of *M. charantia* extracts.

MATERIALS AND METHODS

Collection of plant materials

The leaves and fruits of *Momordica charantia* were collected from local market of Tirupati. The leaf and fruit parts were separately shade dried, cut in to small pieces, air-dried and pulverized in to course powder by using a dry grinder and passed through the sieve before being stored in closed vessel for further use.

Microorganisms used: *Bacillus subtilus*, *Salmonella typhi*, and *Escherichia coli*

Extraction

Shade dried leaves, and fruits of *Momordica charantia* were powdered and weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature for 48 hours, using, methanol, ethanol, hexane and water. Alcoholic extract was concentrated under the vacuum in the rotary flash evaporator and successively in hot air oven till solid to semisolid mass. Extracts were stored in an airtight container in refrigerator below 10°C. The different extracts were used in different concentrations.

Culture preparation

A loop full of each tested bacterial strains was aseptically transferred in to 5 ml of maintained media and incubated at 37^0C for 18-24 hours before use. The optical density at 600 nm of each active culture was adjusted using fresh broth to obtain approximately 106 CFU/ml. Bacterial counts were confirmed by plating out on their suitable media and incubated for 48 hours.

Antibacterial Bioassay

Agar well diffusion bioassay

For bioassays, a suspension of approximately $1.5x10^8$ bacterial/ml in sterile normal saline was prepared as described by *Forbes et al*. About 1.5 ml was uniformly spread on nutrient agar media (Hi-media) in glass Petri dishes. Kept aside for 15 mins and excess of suspension was drained and discarded properly. Wells of 6mm in diameter and about 2 cm apart were punched in the agar culture medium using sterile cork borer. Respective concentrations were administered to fullness in each well. Culture plates were incubated at 37^oc for 48 hrs. Bioactivity was determined by measuring Diameter of the Inhibition Zone (DIZ) in nm. The plant extract concentrations were taken from 50, 100, 150 and 200 µg/ml were evaluated for well method. Each experiment was done in triplicates and mean of the DIZ was calculated. Control included the use of solvent without test sample, although no antibacterial activity noted at in the solvent concentration employed for the test. The standard drug ciprofloxacin was used to compare with the soxhlet extracts.

Determination of minimal inhibitory concentration (MIC)

The determination of MIC using the broth dilution method was applied on extract that already proved for their high efficacy against tested microorganisms. The filtrate was evaporated under the vacuum at 45^oC and then freeze-dried to complete dryness. The residue re-dissolved in sterile water to give the stock concentration of 1,000 mg/ml. The stock concentration was serially diluted to nutrient broth in order to observe their activities at lower concentrations. Bacteria inoculums were added into the broth at the concentration of 106 CFU/ml and culture at appropriated temperature. The samples were taken for every 4 hrs microbial count and to evaluate the mode of actions, bacteriostatic and bactericidal action.

Determination of zone of inhibition

Preparation of the extracts

Extract was dissolved in 10 ml of DMSO to get the concentration of 20 mg/ml. Evaluation of the activity was carried out by cup-plate technique using nutrient agar medium and the antimicrobial activity was measured in terms of zone of inhibition.

Procedure

The medium was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121^0 C for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 160^0 C for 1 hours. 30 ml of sterile molten agar medium was seeded by organisms (about 2 ml according to McFarlands standard), in semi hot conditions (40^oC) was

poured aseptically in sterile. Petri plate and allowed to solidify at room temperature. Bores were made on the medium using sterile borer and 0.1 ml of the extract was added to respective bore and 0.1 ml of the standard ciprofloxacin at a concentration of 100 µg /ml was taken as standard. The Petri plates seeded with organisms, containing extract and the standard were kept in a refrigerator at 4^0C for 1 hour to facilitate the diffusion of the extract. After diffusion the Petri plates were incubated at 37^0C for 24 hours in a BOD incubator and zone of inhibition was observed and measured using a scale. The results of the antibacterial activity of *Momordica charantia* are tabulated in tables.

Preliminary phytochemical screening

The condensed extracts were used for preliminary screening of phyto chemicals such as carbohydrates (Molisch's test) cholesterol (Liberman Man Burchard test) protein (Biuret test) aminoacid (ninhydrin test) Alkaloid (Wagner and Dragendroff 'test), flavonoids, tannins, saponins, cardiac glycosides (Keller Killani test) terpenoids (Salkowsi test) and phlobatinins.

PHYTO CHEMICAL ANALYSIS

TEST FOR CARBOHYDRATES

To 2 ml of extract, 2 drops of Molisch's reagent was added and then shaken well. 2 ml of con. H_2SO_4 was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

TEST FOR CHOLESTEROL

To 2 ml of the extract, 2 ml of the chloroform was added in a dry test tube. Then 10 drops of acetic anhydrate and 2 to 3 drops of con. H_2SO_4 was added. A red rose color changed to blue green color.

TEST FOR PROTEINS

To 2 ml of protein solution 1 ml of 40% NaOH solution and 1 to 2 drops of 1% CuSO4 solution was added. A violet color indicated the presence of peptide linkage of the molecule.

TEST FOR AMINOACIDS

To 2 ml of sample added 2 ml of ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

TEST FOR ALKALOIDS

To the extract added 1% HCl and 6 drops of Mayer's reagent and Dragendroff's reagent .An organic precipitate indicated the presence of alkaloids in the sample.

TEST FOR FLAVONOIDS

Five ml of dilute ammonia solution were added to a portion of aqueous filtrate of each plant extract followed by addition of con. H_2SO_4. A yellow coloration is observed which confirms the presence of flavonoids and it disappears on standing.

TEST FOR TERPENOIDS

Five ml of each extract was added to 2 ml of chloroform and 3 ml of con. H_2SO_4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids.

TEST FOR CARDIAC GLYCISIDES

Five ml of each extract was treated with 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was underplayed with 1 ml of con. H_2SO_4. A brown ring of the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas the acetic acid layer, a greenish ring might form just gradually throughout thin layer.

TEST FOR STEROIDS

Two ml of acetic anhydride was added to 0.5 g of ethanolic extract of each sample with 2 ml of H_2SO_4. The color change from violet to blue or green indicated the presence of steroids.

TEST FOR SAPONINS

The extract with 20 ml of distilled water was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins.

TEST FOR TANNINS

Five ml of extract was added to few drops of 1% lead acetate. A yellow precipitate indicated the presence of tannins.

TEST FOR PHLOBATININS

When an aqueous extract of each plant sample were boiled with 1% aqueous HCl, red precipitate was deposited which was taken as evidence for the presence of phlobatinins.

RESULTS

The present study carried out on the plant samples revealed the presence of medicinally active metabolites. The photochemical characters of *Momordica charantia* are summarized in the below table (1). The aqueous extract found to contain carbohydrates, proteins, amino acids, sterols, flavonoids, phlobatanins and terpenoids, cardiac glycosides, and saponins. Ethanolic extract was found to contain carbohydrates, proteins, amino acids, alkaloids, cardiac glycosides, cholesterol, sterol, and phlobatanins. Hexane extract showed the presence of carbohydrates, proteins, amino acids, sterols, alkaloids, cardiac glycosides, saponins, cholesterol. Methanolic extract was found to contain alkaloids, glycosides, cholesterol, saponins, flavonoids proteins and terpenoids.

Table - 1

Phytochemical screening of Momordica charantia extracts

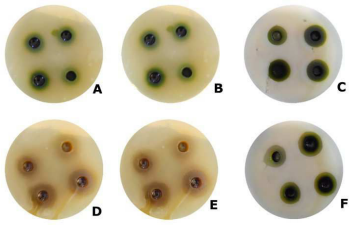
Type of extract	Alkaloids	Glycosides	Carbohydrates	Saponins	Amino acids	Flavonoids	Proteins	Terpenoids	Phlobatanins	Sterioids	Cardiac glycosides
Leaf extract											
Methanol	+	+	+	-	+	-	+	+	+	+	+
Ethanol	+	+	+	-	+	-	+	+	+	+	+
Aqueous	+	-	+	+	+	-	+	+	+	+	+
Hexane	+	-	+	+	+	+	-	+	-	-	-
Fruit extract											
Methanol	+	+	+	+	+	+	+	+	+	+	+
Ethanol	+	+	+	+	+	+	+	+	+	+	+
Aqueous	+	+	+	+	+	-	+	+	+	+	+
Hexane	+	+	+	-	+	-	+	+	-	+	+

Table 2

Determination of zone of inhibition with three different strains of micro organisms

Type of extract	Inhibition zone (µl/mm)											
	Bacillus subtilis				*Escheritia coli*				*Salmonella typhi*			
Leaf	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**
Methanol	5±0.01	6±0.02	7±0.1	9±0.2	4±0.01	6±0.01	7±0.2	8±0.1	4±0.01	5±0.02	8±0.1	8±0.1
Ethanol	4±0.01	5±0.01	7±0.1	7±0.1	4±0.01	5±0.01	6±0.01	8±0.1	2±0.01	0	5±0.02	8±0.1
Hexane	3±0.01	4±0.01	4±0.01	6±0.02	1±0.01	1±0.01	3±0.01	4±0.01	0	0	0	0
Water	0	2±0.01	3±0.01	4±0.01	1±0.01	2±0.01	4±0.01	5±0.01	3±0.01	5±0.01	6±0.02	7±0.02
Fruit	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**
Methanol	4±0.01	5±0.02	6±0.02	7±0.02	4±0.01	5±0.01	6±0.02	7±0.02	1±0.01	2±0.01	3±0.01	4±0.02
Ethanol	3±0.01	3±0.01	4±0.01	5±0.02	3±0.01	4±0.01	4±0.01	5±0.02	0	1±0.01	2±0.01	3±0.01
Hexane	0	1±0.01	2±0.01	3±0.02	0	1±0.01	2±0.01	3±0.01	0	0	0	0
Water	1±0.01	2±0.01	3±0.02	4±0.02	2±0.01	3±0.01	4±0.01	5±0.02	0	3±0.01	4±0.01	5±0.02

Table 3



A) Highest antibacterial activity exhibited by methanol fruit extract against *Bacillus subtilis*
B) Highest antibacterial activity exhibited by methanol fruit extract against *E.coli*
C) Highest antibacterial activity exhibited by methanol leaf extract against *Bacillus subtilis*
D) Highest antibacterial activity exhibited by methanol water fruit extract against *Salmonella typhi*
E) Highest antibacterial activity exhibited by methanol waterleaf extract against *Salmonella typhi*
F) Highest antibacterial activity exhibited by methanol leaf extract against *E.coli*

DISCUSSION

The chemical prospection of *M. charantia* fruit and leaf extracts have indicated the presence of various secondary metabolites (Table 1), that are known to present different therapeutic applications, for example, tannins (antimicrobial, antiviral, moluscicidal and antitumoral), flavonoids (anticarcinogenic, antiviral, antihemorrhagic and antioxidant) [18-21]. The fruit extracts revealed the presence of many metabolites (Table 1), some of them were found in leaf extracts too. Regarding the antibacterial activity, fruit and leaf extracts inhibited the growth of all three tested strains. The methanol extract of fruit and leaf, showed higher antibacterial activity against all three tested strains (Table 2). Hexane extract of fruit and leaf, have not shown the antibacterial effect against Salmonella typhi. In general, the toxic effect to the bacterial membrane and function, because the lipophilic membrane structure, has been used to explain the antimicrobial effect of essential oils and extracts[22-23]. The phyto chemical screening, and qualitative estimation of the plants studied showed that the fruit leaves were rich in carbohydrates, proteins, amino acids, steroids in all the extracts. Some extracts showed presence of tannins, alkaloids and flavonoids too. Steroids were found to be present in all most all the extracts. It should be noted that steroidal compounds are of importance and of interest in pharmacy due to their relationship with such sex hormones. This may be the reason of *Momordica charantia* used as vegetable for expectant mothers or breast feeding mothers to ensure their hormonal balance, since steroidal structure could serve as potent starting material in the synthesis of hormones. The presence of cardiac glycosides has also been reported, by other researchers in *Momordica charantia*, and this plant is widely used in Indian medical system. The plant studied here can be seen as a potential source of useful drugs. Further studies are going on, in this plant to identify further more uses in the field of medicine. The anti oxidant properties of this plant for the use of the diseases as claimed by traditional healers are also being investigated.

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