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Phytochemical Screening and Antibacterial Activity of the Medicinal Plant-Momordica Charantia

KEYWORDS: Momordica charantia, Antifungal, Phytochemical

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ABSTRACT *Medicinal plants are various plants thought by some to have various medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science to have medicinal effects. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. The present investigation is carried out in Momordica charantia leaves. Qualitative phytochemical tests were used to detect the presence of alkaloids, tannins,saponins, flavonoids and phenols. Antimicrobial activity was evaluated for Bacillus subtilis, Streptococcus mutans, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, by using well diffusion methods. Ethanolic plant extract showed a maximum zone of inhibition in Staphylococcus aureus, Escherichia coli by well diffusion method.*

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. *Momordica charantia* belongs to the family *cucurbitaceae.* Found in tropical and sub tropical regions of the world such us India, Asia, South America and widely used as food and medicine. It is commonly known as Bitter gourd. The Latin name of *Momordica charantia* means "to bite" referring to the jagged edges of the leaves, which appear as if they have bitten. The leaves are used for lowering blood sugar level among diabetics. Leaf juice is used for cough, to kill parasites and to heal wounds.

A leaf tea is used to treat diabetes, to expel intestinal gas, promote menstruation and as antiviral agent against measles and hepatitis viruses. Whole plants can be prepared to treat diabetes, skin diseases, sterility in women and chronic ulcers of stomach- plant can be prepare to bring about vomiting. Powder from roots, fruits and seeds can be used as an ingredient in treating hemorrhoids. The fruits and leaves contains alkaloids, saponins, glycosides. An antimicrobial is a compound that kills or inhibits the growth of microbes such as bacteria, fungi, viruses and parasites. The medicinal value of the plant lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. Phytochemicals may protect Human from various diseases. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties.

Bitter melon has been used in traditional Medicine for several other ailments, including dysentery, colic, fevers, burns, painful menstruation, scabies and other skin problems. It has also been used for birth control and also to help childbirth. Bitter gourd leaf extract used for treating some viral infections such as measles, chickenpox etc. the leaf extract contain activity against herpes simplex virus. It is also used for prevention and treatment of malaria.

The fruit is considered as tonic, stomachic, stimulant, emetic, antibilous, laxative and alternative and alternative. The fruit is useful in gout, rheumatism and sub-acute cases of the spleen and liver diseases. It is supposed to purify blood and dispute melancholia and gross humors. It also have anti- diabetic in animal as well as human studies. The fruit juice or a leaf tea is employed for diabetes, malaria, colic, sores and wounds, infections, worms and parasites as an emmenogogue, and for measles, hepatitis and fevers.

Fruit pulp, leaf juice and seeds are antihelmintic. Fruit has also shown the ability to enhance cells up take of glows, to promote insulin release and potentiate the effect of insulin. Fruit and leaves are used in leprosy. It inhibits the enzyme guanylate cyclase, which may benefit people with psoriasis. Bitter melon has been used in various Asian traditional Medicine system for a long time (sathish kumar *et al.,* 2010)

Phytochemicals are naturally occurring, biologically active chemical compounds in plants. The prefix "phyto" is from a Greek word meaning plant. Phytochemicals may protect human from various diseases. In plants, phytochemicals act as a natural defence system for host plants provide colour, aroma and flavor. More than 4000of these compounds have been discovered to date and it is expected that scientists will discover many more. Any one serving of vegetables could provide as many as 100 different phytochemicals. Phytochemicals are protective and disease preventing particularly for some forms of cancer and heart disease (Lakshmi *et al.*, 2011)

MATERIALS AND METHODS

Collection of plant materials *Momordica charantia* leaves were collected from eight to ten years old plants from Coimbatore district and used for the entire course of study.

Sampling of plant material

Fresh leaves of *Momordica charantia* was collected. The leaves were washed thoroughly 2-3 times with running tap water and then air dried under shade. The total dried mass was grounded to a fine powder. The powder obtained after grinding was kept in small plastic bags with proper labeling.

Extraction of plant material

Preparation of ethanolic extracts

10g of sterilized plant leaves were grinded with ethanol. Then they were ground well with the help of mortar and pestle. The plants were subjected to centrifugation for 15 min at 1000 rpm. Again, it was filtered through Whatmann No.1 filter paper. The supernatant were collected with plant extracts of different dilution.

Nutrient Agar medium

Nutrient agar medium is one of the most commonly used medium for several bacteriological strains. After adding all the ingredients into the distilled water , it was boiled to dissolve completely and was sterilized by autoclaving at psi at C

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for minutes. Then it was cooled and poured into petri plates and allowed to solidify.

Agar well diffusion method
The antibacterial activity was tested against aqueous extract of *Momordica charantia*.The inoculum of microorganism was prepared from bacterial culture. About 15-20 ml of Nutrient Agar medium was poured in the sterilized petri dish and allowed to solidify. One drop of bacterial strains was spread over the medium by a sterile cotton swab. Wells of 6mm in diameter and about 2 cm was punctured in the culture medium using sterile cork borer. About (20µl 40µl, 60µl, 80µl, 100µl, 120µl) of plant extracts was added to the wells. Plates were incubated at 37° C for 24 hours. Antibacterial activities were evaluated by measuring the diameters of zone of inhibition.

ANTIFUNGAL ACTIVITY
Collection of plant materials

Momordica charantia leaves were collected from eight to ten years old plants from Coimbatore district and used for the entire course of study.

Sampling of plant material
Fresh leaves of *Momordica charantia* was collected. The leaves were washed thoroughly 2-3 times with running tap water and then air dried under shade. The total dried mass was grounded to a fine powder. The powder obtained after grinding was kept in small plastic bags with proper labelling.

Preparation of ethanolic extracts
10g of sterilized plant leaves were grinded with ethanol. Then they were ground well with the help of mortar and pestle. The plants were subjected to centrifugation for 15 min at 1000 rpm. Again, it was filtered through Whatmann No.1 filter paper. The supernatant were collected with plant extracts of different dilution.

SABOURAUD DEXTROSE AGAR MEDIUM
Sabouraud dextrose agar medium is one of the most commonly used media. After adding all the ingredients into the distilled water it was boiled to dissolve completely and was sterilized by autoclaving at 15 psi pressure at 121°C for 15 minutes. Then it was cooled and poured into petri plates and allowed to solidify.

Agar well diffusion method
The antifungal activity was tested against aqueous extract of *Momordica charantia*. The inoculum of microorganism was prepared from fungal culture. About 15-20 ml of Sabouraud dextrose Agar medium was poured in the sterilized petri dish and allowed to solidify. One drop of fungal strains was spread over the medium by a sterile cotton swab. Wells of 6mm in diameter and about 2 cm was punctured in the culture medium using sterile cork borer. About (20µl, 40µl, 60µl, 80µl, 100µl, 120µl) of plant extracts was added to the wells. Plates were incubated at room temperature for 3 days. Antifungal activities were evaluated by measuring the diameters of zone of inhibition.

PHYTOCHEMICAL ANALYSIS
QUALITATIVE SCREENINIG

Different qualitative chemical tests can be performed for establishing profile of aqueous extract for its chemical composition. The following tests were performed on extracts to detect various phyto constituents present in them.

Detection of Alkaloids (Evans,1997)
Solvents free extract, 50mg is stirred with few ml of dilute hydrochloric acid and filtered. The filtrate is tested carefully with various alkaloidal reagents

Mayer's test (Evans, 997)
To a few ml of filtrate, a drop or two of Mayer's reagents are added by the side of the test tube. A white or creamy precipitate indicates the test as positive.

Detection of Carbohydrates and Glycosides (Rama Krishnan *et al*., 1994)
The extract (100mg) is dissolved in 5 ml of water and filtered. The filtrate is subjected to the following tests.

Miolish's test
To ml of filtrate, two drops of alcoholic solution of α- naphthol are added, the mixture is shaken well and 1 ml concentrated sulphuric acid is added slowly added along the sides of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

Fehling's test
One ml of filtrate is boiled on water bath with 1 ml each of Fehling solution A and B. A red precipitate indicates the presence of sugar

Barfoed's test
To ml of filtrate, ml of Barfoed's reagent is added and heated on a boiling water bath for 2 min. Red precipitate indicates the presence of sugar

Benedict's test
To 0. ml of filtrate, 0. ml Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 min. A coloured precipitate indicates the presence of sugar.

Borntrager's test for Glycosides (Evans, 997)
50 mg of extract is hydrolysed with concentrated Hydrochloric acid for 2 h on a water bath, filtered. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates the presence of glycosides.

Detection of Saponins (Kokate,1999)
The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 min. 2 cm layer of foam indicates the presence of saponins.

Detection of proteins and Amino acids (Fisher,1968;Ruthmann,1970).
The extract (100mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No.1 filter paper and the filtrate is subjected to test for proteins and amino acids.

Ninhydrin test (Yasuma and Ichikawa, 1953)
Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) is added to 2ml of aqueous filtrate. A characteristic purple colour indicates the presence of amino acids.

Detection of Phytosterols (Finar,1986)
Libermann- Burchard's test:

The extract (50 mg) is dissolved in 2 ml acetic anhydride. To this, one or two drops of concentrated sulphuric acid is added slowly along the sides of the test tube. An array of colour change shows the presence of phytosterols.

Detection of Phenolic compounds and Tannins
Ferric chloride test (Mace,1963)

The extract (50 mg) is dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution is added. A dark green colour indicates the presence of phenolic compounds.

Detection of Gum and Mucilages (Whistler and BeMiller, 1993)
The extract (100 mg) is dissolved in ml of distilled water and to this 25 ml of absolute alcohol is added with constant stir-

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ring. White or cloudy precipitate indicates the presence of gums and mucilages.

Detection of Phlobatanins
The extract (0.5g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% Hydrochloric acid solution. Red precipitate shows the presence of phlobatanins.

Detection of steroids
2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H2SO4. The colour changes from violet to blue or green in some samples indicates the presence of steroids.

Detection of Terpenoids (Salkowski test)
0.2g of the extract of the plant sample was mixed with 2 ml of chloroform (CHCl3) and concentrated H2SO4 (3 ml) was carefully added to form a layer. A reddish brown colouration in the interface positive results for the presence of terpenoids.

RESULTS
ANTIBACTERIAL ACTIVITY

The medicinal properties and pharmacological actions of *Momordica charantia* is well used in Indian traditional medicine. Medicinal parts represent a rich source of antimicrobial agents. The plant known to contain various active principles of therapeutic value and to possess biological activity against a number of diseases. Ethanolic extract of *Momordica charantia* inhibited almost all the test organism at concentrations of 20µl, 40µl ,60µl, 80µl, 100µl,120µl, It shows very high activity towards *Staphylococcus aureus, , Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* at the concentration of 120µl and very less activity towards *Streptococcus mutans, Bacillus subtillis*. which is showed in (Table:1)

ANTIFUNGAL ACTIVITY
Momordica charantia has been used in various Asian traditional medicine systems for a long time .The plant known to contain various active principles of therapeutic value and to possess biological activity against a number of diseases. Ethanolic extract of *Momordica charantia* is tested against some fungi such as *Aspergillus niger, Candida albicans, Pencillium chrysogenum, Tricoderma viridae* at the concentration of (20µl, 40µl, 60µl, 80µl,100µl and 120µl). It does not show any antifungal activity against the micro organism used. Although all parts of the plant have demonstrated active antibacterial activity, none have shown activity against fungi. (Jolly *et al.*, 1993)

PHYTOCHEMICAL ANALYSIS
QUALITATIVE SCREENING

The phytochemical analysis of ethanolic extract of *Momordica charantia* was done and the phytochemicals like Carbohydrates, Proteins and aminoacids, Phytosterols, Phenolic compounds and tannins and Steroids are present, which is showed in the(Table:2) Alkaloids, Saponins, Gums and Mucilages, Phlobo-tannins and Terpenoids are absent. This proves that the ethanolic extract of *Momordica charantia* contained bioactive compounds which are responsible for its activity.

TABLE:1 ANTIBACTERIAL ACTIVITY OF *MOMORDICA CHARANTIA*

MICROORGANISMS	ZONE OF INHIBITION (mm) AT DIFFERENT CONCENTRATIONS (µl)						
	Control	20µl	40µl	60µl	80µl	100µl	120µl
Staphylococcus aureus	-	22	21	20	22	21	24
Escherichia coli	-	24	22	20	23	22	25
Pseudomonas aeruginosa	-	20	23	22	21	21	25
Klebsiella pneumonia	-	22	19	21	23	20	25
Streptococcus mutans	-	13	12	14	12	17	15
Bacillus subtillis	-	12	10	11	16	13	16

TABLE:2 PHYTOCHEMICAL ANALYSIS OF MOMORDICA CHARANTIA

S.NO	TESTS	LEAF EXTRACT
1.	Alakaloids	Absent
2.	carbohydrates	Present
3.	saponins	Absent
4.	Proteins and aminoacids	Present
5.	Phytosterols	Present
6.	Phenolic compounds and tannins	Present
7.	Gums and Mucilages	Absent
8.	Phlobotannins	Absent
9.	Steroids	Present
10.	Terpenoids	Absent

DISCUSSION
The leaves of Momordica charantia has been used for thousands of years for thousand of years for its medicinal properties. It is rich in a wide variety of secondary metabolites such as glycosides, alkaloids, proteins, saponins and phytosterols. In the present study on the ethanolic extract was conducted to evaluate the antimicrobial activity of leaves. Leaf of Momordica charantia showed zone of inhibition, therefore it contains effective antimicrobial compounds. Ethanolic extract of leaves have exhibited antibacterial activities against *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia*. It has also been used for gastrointestinal infection and also to lower blood sugar in diabetes. Also the phytochemical screening and qualitative estimation of the plant studied that the leaves were rich in carbohydrates, proteins, amino acids and sterols. Steroids were found to be present in almost all plants. It should be noted that steroidal compounds are of importance and of interest in pharmacy due to their relationship with such sex hormones. Further studies are going on, in these plants to identify furthermore uses in the field of medicine.

CONCLUSION
The hypothesis of obtaining plant based medicine is beneficial to human health based on active profile exposed through in vitro assays. There is a tremendous need for novel antimicrobial agents from different sources. Medicinal plants represent a rich source of antimicrobial and antioxidant agents. Antimicrobial compound is a substance that kill or inhibit the growth of microbes

Momordica charantia L being a very important medicinal plant has the quality of attention with its medicinal values. Conservation and propagation of all medicinal plant is a very important task to be performed in today's world for the conservation of our floral heritage and useful products from them. *Momordica charantia* are used externally for healing

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of wounds and internally for treatment of peptic ulcer. It is used for treatment of jaundice, kidney (stone), leprosy, piles, pneumonia and rheumatism.

In the present study, antimicrobial activity, phytochemical analysis is carried out. The plant was found to possess antibacterial activity against some of the pathogens. Bioactive compounds like steroids, Terpenoids, carbohydrates, proteins and amino acids, phytosterols, phenolic compounds and tannins were identified in the ethanolic extract of the plant.

Further investigation on the isolation of Bioactive components from the plant would help to increase its potential to use the plant as the source of new drugs. This study also encourages cultivation of the highly valuable plant in large scale to increase the economic status of the cultivators in the country.

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