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Research Article

A phytochemical and antimicrobial activity of leaf extracts of *momordica cymbalaria hook fenzl.*

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ABSTRACT

Objective: A Phytochemical and antimicrobial activity of leaf extracts of *Momordica cymbalaria Hook Fenzl.*
Method: Distilled water and methanolic crude extracts of leaves of the plant were evaluated for antimicrobial activity using the disk diffusion method and also done MIC, MBC and MIC index on eight reference microorganisms *Escherichia coli,Staphylococcus aureus, Shigellasps, Klebsiellasps, Salmonella, Pseudomonas, Proteus vulgarisand Candida were* used as test organisms.
Result:*Momordica* extracts may be useful as a broad-spectrum antimicrobial agent. Methanolic and Distilled water extract of Momordica exhibited potent activity against all set of micro organisms used. It showed better action against Staphylococcus and least action on Pseudomonas.
Conclusion:Leaf extracts of *M.cymbalaria* demonstrated antimicrobial activity on tested microorganisms. It is having bactericidal property on all used micro organisms.

Keywords: Phytochemical and Antimicrobial activity, *Momordica cymbalaria*, MIC, MBC, MIC index.

INTRODUCTION

About 80,000 species of plants are utilized for treating various diseases in different systems of Indian medicine. Since 1990s there has been a growing shift in interest towards plants as significant sources for new pharmaceuticals. As per the world health organization (WHO) report 80% of the world population, presently use herbal medicine for some aspects of primary health care.[1]Many pharmaceutical companies show interest in plant derived drugs mainly due to the current wide spread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs, which have adverse effects. Since the last decade, the rise in the failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has lead to the screening of several medicinal plants for their potential anti microbial activity.[2,3]Many herbs contains dozens of active constituents that combine to give the plant its therapeutic value.

Momordica cymbalaria Hook. F. belongs to the Cucurbitaceous family. The plant is a perennial herbaceous climber either allowed to trail on the ground or to climb on supports with the aid of tendrils. It is found in the south Indian states of Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu as a weed. The nutritional studies of the fruits of *M.cymbalaria* have reported that they possess a high level of calcium, potassium and vitamin C, in addition to its high crude fiber content.[4] The fruit extracts of *M.cymbalaria* were shown to have antidiabetic, hypolipidemic [5, 6], antidiarrhoeal[7], and antiulcer activity [8]. The roots of the plant are used for menstrual irregularities, anti fertility, antiovulatory, abortificient [9], and hepatoprotective [10] activity.No researcher has yet reported antimicrobial activities of leaves of this plant. Therefore, it is worth conducting an investigation on the antimicrobial activities of extract of *M.cymbalaria* leaves.

MATERIALS AND METHODS

Plant material: The leaves of *Momordica cymbalaria* Hook F. was collected in November 2011from Anantapur district, Andhrapradesh, India. The leaves were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container.

Preparation of extract: A 40g of air-dried leaf powder was soaked in 300ml of organic solvent, viz., methanoland water separately for 24h in around bottom flask at room temperature. Extracts were filtered through the whatman filter paper No.1. The filtrate was allowed to dry at room temperature; methanoland water extracts were weighed and stored in air tight container at 4^0 C till further investigation.

Phytochemical evaluation:Phytochemical examinations were carried out for all the extracts as per the standardmethods. [11]

Detection of alkaloids:Extracts were dissolved individually in dilute Hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

a) Mayer's test: Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide). Formation of a yellow cream precipitate indicates the presence of Alkaloids.

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Organic constituents:

Constituents	Aqueous extracts	Methanolic extracts
Alkaloids	+ve	+ve
Carbohydrates	-ve	-ve
Saponins	+ve	+ve
Triterpinoids	-ve	+ve
Resins	-ve	-ve
Tanins	-ve	-ve
Flavanoids	+ve	-ve
Steroids	-ve	+ve

Sterile tubes are placed in a rack and labeled as fallow.

Tube No	1	2	3	4	5	6	7	8	9
Label	128	64	32	16	8	4	2	Growth control	Sterility control

b) Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown/reddish brown precipitate indicates the presence of alkaloids.

c) Dragendroff's test: Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

d) Hager's test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow colored precipitate indicates the presence of alkaloids.

Detection of carbohydrates:Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml of Conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of Carbohydrates.

b) Benedict's test: Filtrates were treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicates the presence of reducing sugars.

c) Fehling's test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehlings A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of saponins:

a) Froth test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam test: Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phytosterols:

a) Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand.

b) Appearance of golden yellow colour indicates the presence of triterpenes.

c) Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added carefully along the sides of the test tube.

d) Formation of brown ring at the junction indicates the presence of phytosterols.

e) Tshugajeu test: Extracts were treated with chloroform and filtered. Excess of acetyl chloride and a pinch of Zinc Chloride was added, kept aside for some time till the reaction was complete and then warmed on waterbath. Appearance of eosin red colour indicates the presence of triterpenes.

Detection of resins:

a) Acetone-water test: Extracts were treated with acetone. Small amount of water was added and shaken.

Appearance of turbidity indicates the presence of resins.

Detection of tannins:

a) Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids:

a) Alkaline Reagent test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

c) Lead acetate test: Extracts were treated with few drops of lead acetate solution.

d) Formation of yellow colour precipitate indicates the presence of flavonoids.

e) Shinoda test: To the alcoholic solution of extracts, a few fragments of magnesium ribbon and Conc.HCl was added. Appearance of magenta colour after few minutes indicates presence of flavonoids.

f) Zinc hydrochloric acid reduction test: To the alcoholic solution of extracts, a pinch of Zinc dust and Conc.HCl was added. Appearance of magenta colour after few minutes indicates presence of flavonoids.

Anti Microbial activity:The anti microbial activity of the extracts tested individually on gram positive, gram neg*ative bacteriaand fungus. Escherichia coliATCC 25922, StaphylococcusATCC 29213aureus, Shigellasps, Klebsiellasps, Salmonella (clinical isolates only),*

RESULTS

Table-1

Organism	Diameter of zone of inhibition (mm)			
	M.cymbalaria leaf Extracts 2mg/ml		Standard 10µg/ml	
	Methanolic	Distilled water	Gentamicin	Amphotericin
Bacteria				
Escherichia coli	15	10	18	Nil
Staphylococcus aureus	17	15	17	Nil
Shigellasps	14	10	18	Nil
Klebsiellasps pneumoniae	14	10	16	Nil
Salmonella typhi	10	12	18	Nil
Proteus vulgaris	12	10	18	Nil
Pseudomonas aeruginosa	10	Nil	17	Nil
Fungi				
Candida	13	Nil	Nil	18

Table-2

Organism	Minimum Inhibitory Concentration(MIC)			
	M.cymbalaria leaf Extracts (µg/ml)		Standard µg/ml	
	Methanolic	Distilled water	Gentamicin	Amphotericin
Bacteria				
Escherichia coli	64	128	10	Nil
Staphylococcus aureus	64	64	10	Nil
Shigellasps	32	128	10	Nil
Klebsiellasps pneumoniae	32	128	10	Nil
Salmonella typhi	64	64	10	Nil
Proteus vulgaris	64	16	10	Nil
Pseudomonas aeruginosa	128	64	10	Nil
Fungi				
Candida	32	128	Nil	10

*PseudomonasATCC 27853, Proteus vulgarisand Candida (clinical isolates from urine only)*were used as test organisms. Bacteria strains were maintained on nutrient agar at 4^0C and sub-cultured every month in our laboratory.

Agar disc diffusion assay:The anti bacterial activity of the extracts was determined by the disc diffusion method. [12] Briefly, over night bacterial culture were diluted in the Muller-Hinton broth (O.D. 600=0.08) to obtain a bacterial suspension of 10^8 CFU/ml. Petri plates containing 20ml of Muller-Hinton Agar media were inoculated with 200µl of diluted cultures by the spread plate technique and were allowed to dry in a sterile chamber. Five filter paper discs (Whatman NO.1; 6mm diameter) were placed on the inoculated agar surface. A 20µl of the extracts (100mg/ml) were loaded on to the filter paper discs and were allowed to dry completely standard antibiotics gentamicin (10µg), Amphotericin (10µg) and 20µl of DMSO were incubated at 37^0C for 24h. The anti bacteria activity was assessed by measuring the inhibition zone. All the tests were performed triplicate.

Determination of minimum inhibitory concentration (MIC):A minimum inhibitory concentration of an antimicrobial that inhibits growth of a micro organism after 18-24h.The extracts were subjected to the serial broth dilution technique to determine their minimum inhibitory concentration.5 ml of sterile nutrient broth is added to *each* tube. According to the label present on the tube different concentration of plant extracts are added in to them. From the pure cultures of the organism to be tested, prepared a suspension of 5 ml of saline equalent to 0.5 McFarland standards. From above suspension 0.1 ml diluted to 9.9 ml of sterile normal saline. After mixing the contents well, 0.1 ml of this organism suspension is added to the plant extract containing broth tubes through 1 to 7 and also to the growth control tube. Rack is shacked gently to mix the contents and incubated at 37^oC for overnight. Each tube is examined for the presence or

broad-spectrum antimicrobial agent. As shown in Table 1

Table-3

Organism	Minimum Bactericidal Concentration(MBC)			
	M.cymbalaria leaf Extracts (µg/ml)		Standard µg/ml	
	Methanolic	Distilled water	Gentamicin	Amphotericin
Bacteria				
Escherichia coli	64	128	20	Nil
Staphylococcus aureus	128	64	20	Nil
Shigellasps	64	128	20	Nil
Klebsiellasps pneumoniae	64	128	20	Nil
Salmonella typhi	64	64	20	Nil
Proteus vulgaris	128	16	20	Nil
Pseudomonas aeruginosa	Nil	Nil	20	Nil
Fungi				
Candida	32	128	Nil	20

the

Table-4

Organism	**MIC Index (=MBC/MIC)**	
	Methanolic	Distilled water
Escherichia coli	1	1
Staphylococcus aureus	2	1
Shigellasps	2	1
Klebsiellasps pneumoniae	2	1
Salmonella typhi	1	1
Proteus vulgaris	2	1
Pseudomonas aeruginosa	-	-
Candida	1	1
Gentamicin	2	
Amphotericin	2	

absence of turbidity and results are recorded as MIC in µg/ml.

Determination of minimum bactericidal concentration (MBC):A minimum bactericidal concentration is the lowest concentration of an antibiotic required to kill a micro organism. MBC values were also studied for microorganisms, which were tested to the extract by Turbidity MIC**.** All tubes not showing visible growth are sub cultured on to suitable media for the test organism including growth control tube and incubate at 37^{o}C overnight.

MIC index:The MIC index (MBC/MIC) was calculated for each extract and standard control drug to determine whether an extract is bactericidal (MBC/MIC<4) or Bacteriostatic (MBC/MIC>4) on growth of bacterial organisms. [13] Also, the range of MIC index values greater than 4 and less than 32 are considered as Bacteriostatic. [14]

DISCUSSION

From the results which we have obtained, it could beagreed that Momordica extracts may be useful as a methanolic extract of Momordica exhibited potent anti microbial activity against all set of micro organisms used. Distilled water extracts showed activity against the entire microorganism. It showed better action against Staphylococcus and least action on Pseudomonas. We have tested only one fungus that is *Candida* clinical isolates especially from urine on which methanolic extract showed significant action. Although it is an in-vitro study this results may differing in-vivo which has to be done extensively.

CONCLUSION

Results obtained by us support the popular use of these plants for traditionalmedicine forthe treatment of mild fever, Infections (speciallywound infections), and intestinal disorders (like Diarrhoea, Blotting). Even though, the tested plant extracts may contain anti-microbial components, Phytochemical and pharmacological studies are encouraged this will be necessary to isolate the active constituents and evaluate

the anti-bacterial activity against a wide range of microbial populations.

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