Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 6, Suppl 1, 2013 ISSN - 0974-2441

Research Article

ANTIMICROBIAL ACTIVITY OF TUBEROUS ROOT EXTRACTS OF *MOMORDICA CYMBALARIA* HOOK.

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Received:17 December 2012, Revised and Accepted:17 January 2013

ABSTRACT

Antimicrobial activity of ethanol and chloroform extracts of the roots of a medicinal plant-*Momordica cymbalaria* used traditionally as potent medicine in healing several ailments such as diarrhea, convulsion, rheumatism, ulcer, skin diseases and used as anti-implantation and anti-ovulatory, anti-diabetic and hepatoprotective agent, was tested against different pathogenic microorganisms by agar well diffusion method. The extents of the growth inhibition of bacteria were measured for each extract and most of the selected bacteria exhibited significant growth inhibition zone. Minimum inhibitory concentration (MIC) and antifungal activity exhibited by root extract against the test organisms by Microtiter plate assay ranged between 1-5 mg/ml. Antimicrobial activities of the crude extracts were comparable to those of the standard antibiotic. Antioxidant evaluation of methanolic root extract of MC was also carried out using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH). This study concluded that *M. cymbalaria* used as a traditional medicinal plant has antimicrobial activity against pathogenic microorganisms.

Keywords: *Momordica cymbalaria*, Antimicrobial activity, MIC, DPPH, medicinal plant.

INTRODUCTION

The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality[1]. Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs [2, 3]. In addition to this, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [4]. Therefore there is a constant need to establish and develop antimicrobial drugs from natural origin that are much safe, reliable and less expensive. Plant based antimicrobials represent a vast untapped source for medicines and they provide enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [5, 6]. Natural antioxidants present in the plants are closely related with their ability to treat various diseases. Antioxidant assays are widely used for assessing medicinal properties of plant material [7].

India is a land of biodiversity in terms of plant species. Various plants have been mentioned in Ayurveda, an ancient Indian Sanskrit literature, for their therapeutic advantages [8].

Momordica cymbalaria Hook (Family: cucurbitaceae) is herbaceous, perennial climber or trailer found very rarely in Maharashtra, and South Indian states of Andhra Pradesh, Karnataka and Tamilnadu. This plant has various medicinal properties. The fruits of this plant resemble with *Momordica charantia* fruits. The fruit extract shows antidiabetic and hypolipidemic effects in alloxan inducing diabetic rats [9]. Fruits also contain higher amounts of Calcium, Potassium, Sodium and Vitamin C than the bitter gourd (*M.charantia*) [10]. The fruit extract has shown antimicrobial activity against bacteria and fungi [11]. The roots are tuberous and are used in Ayurvedic medicines. The roots of MC are used for menstrual irregularities, antifertility, antiovulatory and abortificient activities [12]. The root extracts of *Momordica cymbalaria* has shown antiimplantation activity in rats [13]. The antidiabetic (Type 2) activity was studied with ehtanolic root extract [14]. The root extracts of *Momordica cymbalaria* showed hepatoprotective effect [15].

The present study aimed to investigate the susceptibility of several clinically significant bacterial and fungal strains against crude extracts prepared from the roots of *Momordica cymbalaria*. The minimum inhibitory concentrations (MICs) were also determined. Evidently there are no scientific studies about effect of root extracts against pathogenic microorganisms.

Therefore, the objective of this study was to investigate the antimicrobial activity of *M. cymbalaria* roots.

MATERIALS AND METHODS

Collection of plant material and extraction

The fresh root tubers of *Momordica cymbalaria* Hook were collected from place 'Anala' of Osmanabad District, Maharashtra (India). The tuberous roots were chopped into small pieces and were dried under shade at room temperature for fifteen days. The dried tuber pieces were powdered in grinder and passed through sieve. The dried root powder was stored in air tight container and it was extracted with solvents like ethanol and chloroform in soxhlet apparatus. The extract was filtered, concentrated and dried in vacuum and the residue obtained was stored in a refrigerator at 2-8^{o}C for use in further experiments [16].

Microorganisms used

Fungal and bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Chandigarh. Bacteria like *Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis,* and Fungi like *Penicilium crysogenum, Trichobacterium rubrum, Aspergillus niger, Aspergillus flavus, Rhizopus orizae* were used as test organisms. Bacterial cultures were stored on slants containing Nutrient agar (NA) and fungal cultures were stored on Potato dextrose agar (PDA) and were subcultured on fresh slants once a week. Bacterial suspensions were enriched in NA for 24 hrs and fungi on PDA for 72 hrs and 1-1.5x10^{6} cells/ml fresh inoculums were utilized for antimicrobial activity test.

Antimicrobial screening

The antibacterial tests were carried out using the agar well diffusion method. Petri plates were prepared by pouring 20 ml of nutrient agar for all the bacteria. The inoculum was spread on the top of the solidified media. Once the agar was solidified, they were punched using a sterile cork borer (7-mm diameter). Then wells were filled with 100µl (dissolved in 10% DMSO) of different concentrations of *M. cymbalaria* extracts. 5mg/ml and 10 mg/ml concentrations of both extract were used against all test organisms. Chloramphenicol (1 mg/ml) and DMSO (10%) were used as positive and negative control, respectively. The plates were kept 30 min for diffusion and then incubated at 37 ºC for 24 h. The inhibition zones were compared with that of standard antibiotic chloramphenicol. Each experiment was repeated three times. While antifungal screening was done in 96-well microtiter plates at 595 nm.

Minimal inhibitory concentration (MIC)

MIC values were found out for fungal and bacterial microorganisms. Growth inhibition was measured in 96-well microtiter plates at 595 nm in an ELISA plate reader. The microdilution method was performed in 96-well microtiter plates. 1-5 mg/ml extract concentration was used for the detection of MIC. The sample wells filled with 50 µl of the plant extract and 50 µl test organism suspension. 50 µl test organism suspension with 50 µl Antibiotic chloramphenicol for bacteria and flucanzole for fungi was used as a positive control. The plates were covered in plastic bags. The fungal and bacterial plates were incubated at 28° C for 72 hrs and 37° C for 24 hrs respectively. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each microorganism. The inhibition of cell viability was calculated as follows.

% Inhibition of cell viability = 1- T/C x 100

Antioxidant screening

2,2-Diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging method

DPPH scavenging activity of methanolic extract of MC was determined by the modified method of Koleva *et. al (*2002). Test extracts in methanol were added separately to an equal volume of 100 µM methanolic solution of DPPH and the reaction mixture was kept at room temperature for 15 min. The absorbance of the reaction mixture was recorded at 515 nm using a UV visible spectrophotometer. Ascorbic acid was used as standard. Free radical scavenging activity was calculated using the following formula.

$$\% \text{ of Free radical scavenging activity} = \frac{\{(\text{Control OD}) - (\text{Sample OD})\} \times 100}{(\text{Control OD})}$$

Where control represents reaction mixture containing DPPH and methanol only excluding test extracts, whereas sample represents reaction mixture as described above in method.

RESULTS AND DISCUSSIONS

Table1 shows the effect of ethanol and chloroform extracts of roots of *M. cymbalaria* using agar well diffusion method. There were fine responses of the test organisms to the ethanolic extract as compared with standard antibiotic, while organisms show less response to chloroform extract. *B. subtilis, E.coli, S. typhimurium, S. aureus* and *P. morgani* were susceptible to both concentrations of ethanolic extracts i.e. 5 mg/ml and 10 mg/ml. But in case of chloroform extract was not effective on all test organisms. 5 mg/ml chloroform concentration did not show zone of inhibition. *P. morgani* was only organism to resist the chloroform extracts. Judging by the diameter of the zone of inhibition *S. typhimurium* and *S. aureus* are most susceptible at 5 mg/ml concentration of the ethanolic extract. *E. coli* and *S. aureus* were most susceptible to chloroform extract of *M. cymbalaria.*

Table 1:Antibacterial activity of ethanolic and chloroform root extracts of *momordica cymbalaria*.

Test organism	Zone of inhibition (mm)				
	Ethanol		Chloroform		Ampicilin (mg/ml)
	5 mg/ml	10 mg/ml	5 mg/ml	10 mg/ml	
B. subtillis	5±2.64	10±0.00	**	5±1.00	5±0.00
E. coli	5.3±2.30	9±2.00	**	7±0.00	5.6±1.15
S. typhimurium	6.33±0.57	8.66±0.57	**	3.66±0.57	5.3±0.57
S. aureus	6.33±0.57	8±1.00	**	7±4.35	3±0.00
P. morgani	5±0.00	11±1.00	**	**	11±1.73

Each value is the Mean ± S.D of three independent replicates.
**** - Zone of inhibition was not detected.**

MIC values of extracts of *M. cymbalaria* on test bacteria and fungi.

The MIC values of both the extracts are represented in Table 2 & 3. The MIC values ranged between 1- 5 mg/ml for both root extracts against tested organisms. Therefore, the minimum inhibitory concentration was identified as 2 mg/ml of ethanolic extract for *B. subtilis.* and 5 mg/ml chloroform extract against the selected bacteria.

Table 2:Antibacterial activity

Sr.No.	Microorganisms	Minimum Inhibitory Concentration (MIC) of samples in different solvents (mg/ml)	
		Ethanol	chloroform
1	*B. subtillis*	2	5
2	*E. coli*	5	5
3	*S.typhimurium*	3	5
4	*P. morgani*	5	--
5	*S. aureus*	5	5

Table 3:Antifungal activity

Sr.No.	Microorganisms	Minimum Inhibitory Concentration (MIC) of samples in different solvents (mg/ml)	
		Ethanol	chloroform
1	*P. crysogenum*	5	4
2	*T. rubrum*	5	5
3	*A. niger*	5	4
4	*A. flavus*	4	4
5	*R. orizae*	5	4

The antifungal activity was performed using microtiter plate method. The minimum inhibitory concentration of 4 mg/ml of ethanolic and chloroform extracts were identified as least concentration against *A. flavus* fungus. In antifungal activity, chloroform root extract was more effective than the ethanol extract against all fungal test organisms.

The free radical scavenging activity of extracts was evaluated for their ability to quench the synthetic DPPH radical, measured in terms of percentage (Table 4). DPPH assay has been widely used for the screening of scavenging activity of antioxidant because it is a rapid and sensitive method to detect hydrogen donating ability of plant extracts at low concentrations [17]. Results showed the extract of 200mM and 250 mM posses moderate scavenging activitiy with free radical scavenging percentage of 49.80 and 42.30 % respectively as compared to standard scavenger Ascorbic acid with free radical scavenging percentage of 96%.

Table 4:DPPH radical scavenging activity of the methanolic root extract.

	Methanolic root extract (mM)	Free radical scavenging activity (%)
1	50	25.30%
2	100	36.80%
3	150	20.10%
4	**200**	**49.80%**
5	250	42.30%
6	300	19.50%
7	Ascorbic Acid	96%

Each value in the table was obtained by calculating the average of three experiments.

In general antimicrobial activity increases with increase in concentration of extract as evident by the zone of inhibition and MIC values. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents.

CONCLUSION

From this study we concluded that this medicinal plant has a wide range of antimicrobial activity. Our results show that the antimicrobial effects may be due to its antioxidant and free radical scavenging properties. This study demonstrated that herbal medicine can be as effective as modern medicine to combat pathogenic microorganisms. Using different purification methods, we can purify these antimicrobial compounds which can be used for further pharmaceutical uses.

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