ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF MOMORDICA CYMBALARIA

S. JAMILA JASMIN, (Assistant professor, Department of Microbiology, Sardar Raja Arts & Science College, Vadakkangulam)

DR. JOYS SELVA MARY ALBERT, (Assistant professor, Department of Microbiology, St. Mary's College, (Autonomous) Thoothukudi)

S.GAJENDHINI (Assistant professor, Department of Biochemistry, Pushkaram College of Agricultural Science, Pudukkottai)

Abstract

Antibacterial and Antifungal activity of methanol and chloroform extracts 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 plant extract against the test organisms by Microtiter plate assay ranged between 1-5 mg/ml. Antibacterial activities of the crude extracts were comparable to those of the standard antibiotic. This study concluded that M. cymbalaria used as a traditional medicinal plant has antibacterial and antifungal activity against pathogenic microorganisms.

Keywords: Momordica cymbalaria, Antibacterial activity, Antifungal activity MIC, medicinal plant.

Introduction:

The plants with potential therapeutic values have been used from time immemorial to cure various ailments and infectious diseases. The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality (Williams, 2000). 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 (Pretorius *et al.,* 2003). In addition to this, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions (Ahmad I *et al.,* 1998). 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 (Ozsoy NA, *et al.,* 2008). They are effective in the treatment of

infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Murray M 1995). 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 (Iwu MM, *et al.,* 1999).

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 (Kaushik P 1988).

Momordica cymbalaria (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 (Kameswar Rao B *et al.,* 1999). Fruits also contain higher amounts of Calcium, Potassium, Sodium and Vitamin C than the bitter gourd (M.charantia) (Parvathi S, *et al.,* 2002). The fruit extract has shown antimicrobial activity against bacteria and fungi (Swamy V, *et al.,* 2008). The roots are tuberous and are used in Ayurvedic medicines. The roots of MC are used for menstrual irregularities, antifertility, antiovulatory and abortificient activities (Koneri R, *et al.,* 2006). The root extracts of Momordica cymbalaria has shown antiimplantation activity in rats (Koneri R, *et al.,* 2007). The antidiabetic (Type 2) activity was studied with ehtanolic root extract (Firdous M, *et al.,* 2009). The root extracts of Momordica cymbalaria showed hepatoprotective effect (Koneri R, *et al.,* 2008).

The present study aimed to investigate the susceptibility of several clinically significant bacterial and fungal strains against crude extracts prepared from the plant 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.

Materials and Methods

Collection of plant material

The fresh Plant of *M. cymbalaria* (stem, leaves and fruits) was collected from Tirunelveli district. The fresh plant material was dried under shade. Dried plant material was powdered using mechanical grinder and passed through sieve no.60 to get the powder desired coarseness. Powdered material was preserved in an air tight container.

Extraction procedure

Shade dried plant parts such as stem, leaves and fruits (470 g) were coarsely powdered and subjected to successive solvent extraction using methanol and chloroform by continuous hot extraction (Soxhlet). Each time, the marc (exhausted plant material) was air dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a

rotary flash evaporator.

Microorganisms used

Fungal and bacterial cultures were obtained from Rajas Medical Institutions, Kavalkinaru, Tirunelveli. Bacteria like *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis,* and Fungi like *Penicilium crysogenum, 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 sub cultured on fresh slants once a week. Bacterial suspensions were enriched in NA for 24 hrs and fungi on PDA for 72 hrs.

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 micro titer plates at 595 nm in an ELISA plate reader. The micro dilution method was performed in 96-well micro titer 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^{o} C for 72 hrs and 37^{o} 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.

$$\% \text{ Inhibition of cell viability} = 1 - T/C \times 100$$

Results and Discussions

Table1 shows the effect of methanol and chloroform extracts of M. cymbalaria using agar well diffusion method. There were fine responses of the test organisms to the methanolic extract as compared with standard antibiotic, while organisms show less response to chloroform extract. B. *subtilis, E.coli, S. typhimurium* and *S. aureus* 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. Judging by the diameter of the zone of inhibition of *S. aureus* is most susceptible at 5 mg/ml concentration of the methanolic extract. *E. coli* and *S. aureus* were most susceptible to chloroform extract of M. cymbalaria.

S.No	Test Organism	Zone of Inhibition (mm)				
		Methanol		Chloroform		Ampicillin
		5mg/ml	10mg/ml	5mg/ml	10mg/ml	(mg/ml)
1	*E. coli*	5.7 ± 3.30	9.5 ± 2.20	**	7 ± 0.00	5.6 ± 1.15
2	*P. aeruginosa*	5.5 ± 3.34	8.52 ± 3.02	**	3.66 ± 0.57	5.3 ± 0.57
3	*S. aureus*	6.42 ± 0.22	8.02 ± 1.00	**	7 ± 4.35	3 ± 0.00
4	*B. subtilis*	4 ± 0.00	10.12± 0.00	**	5 ± 1.00	5 ± 1.00

Table 1: Antibacterial activity of methanolic and chloroform extracts of Momordica cymbalaria
**: 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 plant extracts against tested organisms. Therefore, the minimum inhibitory concentration was identified as 2 mg/ml of methanolic extract for *B. subtilis* and 5 mg/ml chloroform extract against the selected bacteria.

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

S.No	Microorganisms	Minimum Inhibitory Concentration (MIC) of samples in different solvents (mg/ml)	
		Methanol	Chloroform
1	*E. coli*	5	5
2	*P. aeruginosa*	3	5
3	*S. aureus*	5	5
4	*B. subtilis*	2	5

Table 2: Antibacterial activity

S.No	Microorganisms	Minimum Inhibitory Concentration (MIC) of samples in different solvents (mg/ml)	
		Methanol	Chloroform
1	*P. crysogenum*	5	4
2	*A. niger*	5	5
3	*A. flavus*	4	4
4	*R. orizae*	5	4

Table 3: Antifungal activity

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 antibacterial and antifungal activity. 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.

References

1. Ahmad I, Mehmood Z, Mohammad F Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol, 1998; 62: 183-193.
2. Firdous M, Koneri R, Sarvaraidu CH, Harish M, Shubhapriya KH NIDDM Antidiabetic activity of Saponins of Momordica Cymbalaria in Streptozotocin-Nicotinamide NIDDM Mice. Journal of Clinical and Diagnostic Research 2009; 3: 1460-1465.
3. Iwu MM, Duncan AR, Okunji CO New antimicrobials of plant origin. In: Janick, J., ed. Perspectives on New Crops and New Uses. ASHS Press, Alexandria, 1999; 457-462.
4. Kameswar Rao B, Kesavulu MM, Apparao C Giri R Antidiabetic and hypolipidemic effect of Momordica cymbalaria Hook fruit powder in alloxan diabetic rats. J Ethanopharmacol 1999; 67: 103-9.
5. Kaushik P, Indigenous Medicinal Plants including Microbes and Fungi. Today and Tomorrow's Printers & Publishers, New Delhi 1988.
6. Koneri R, Balaraman R, Saraswati CD Antiovulatory and abortificient potential of ethanolic extract of roots of Momordica tuberosa Fenzl in rats. Indian J Pharmacol 2006; 38: 111-114.
7. Koneri R, Balaraman R, Saraswati CD Antiimplantation activity of ethanolic root extract of Momordica cymbalaria Fenzl in rats. Indian J Pharmacol 2007; 39(2): 90-96.
8. Koneri R, Balaraman R, Firdous Kumar V Hepatoprotective effects of Momordica cymbalaria Fenzl against carbon tetrachloride induced hepatic injury in rats. Pharmacologyonline 2008; 1: 365-374.
9. Murray M The Healing Power of Herbs. Prima Publishing, CA, 1995; 162-171.
10. Ozsoy NA, Can R, Yanardag, Akev N Antioxidant activity of Silax excelsa L. Leaf extracts. Food chem 2008; 110: 571-583.
11. Parvathi S, Kumar VJF Studies on chemical and utilization of wild edible vegetable athalakkai (Momordica tuberosa). Plant Foods for Human Nutrition 2002; 57: 215-222.
12. Pretorius JC, Magama S, Zietsman PC Growth inhibition of plant pathogenic bacteria and fungi by extracts from selected South African plant species. South African Journal of Botany 2003; 20: 188-192.

13. Swamy V, Jayaveera KN, Bharti Reddy KR Antidiarrhoel acivity of fruit extract of Momordica cymbalaria . The internet J Nutrition and Wellness, 2008; 5(20): 1-9.
14. Williams R Antimicrobial resistance a global threat. Essential Drug Monitor 2000; 1: 28-29.