

Antimicrobial Properties of *Momordica cymbalaria* Hook. F

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Summary

Antimicrobial activity of the fruits of *Momordica Cymbalaria* Hook. F (*Momordica tuberosa*), were tested against different bacteria (including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Shigella sonnei*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus vulgaris*, *Pseudomonas aeruginosa*) and fungi (such as *Candida albicans* and *Aspergillus niger*) by cup plate diffusion method. Minimal Inhibitory Concentration (MIC) values of each active extract were determined. The results obtained showed strong activity of the methanolic extract of the fruits of plant against the bacteria and fungi used as test organisms.

Keywords: *Momordica Cymbalaria*, Antimicrobial activity. MIC

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Introduction

Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide. During the past several years, there has been an increasing incidence of bacterial and fungal infections due to a growth in immunocompromised population such as organ transplant recipients, cancer and HIV/AIDS patients. The changing pattern of clinical evaluation and regulatory requirements for merits and demerits of drugs will be highlighted for future challenges and advances in antimicrobial drug development. Resurgence in the use of herbal medicines worldwide has provided an excellent opportunity to Indian companies to look for therapeutic leads from Indian ancient system of Ayurveda that could be utilized for drug development. (1)

Momordica cymbalaria Hook. F. belongs to the Cucurbitaceae 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 plant is allowed to grow along bunds (boundary of fields), fences and even in the fields for the sake of fruits. However no regular cultivation is practiced. The plant has a tuberous root, which helps to maintain perennial habits, pubescent or sub glabrous. i.e., the plants dry and disappear at the end of the season. The tubers remain in the soil and emerge in the next season. The plant has a monocious stem and is very slender. The leaves are oblicular or reinform with a deeply cordate base. Flowers are unisexual. The male flower peduncle is 5–30 mm long, filiform, puberulous, ebracteate with 2–5 flowers in racemes with a pale yellow corolla and two stamens for each flower. The female flower is solitary on a peduncle of 28 mm length. The fruits are 20–25 mm long, pyriform with 8 sharp ridges, 24× 15mm attenuated at the apex and with the base narrowed into the curved peduncle, which is fleshy, dark green and ribbed. The seeds are 4.6 mm long, ovoid shaped, smooth and shiny. Flowering occurs during October; fruits are harvested from November to January. The yield of each plant is 1.25 to 1.5kg. The tender fruits closely resemble those of a small variety of bitter gourd Athalakkai is used as a vegetable by the rural people of South Tamil Nadu and North Karnataka, India (2). The phytochemicals reported in this plant are tannins, alkaloids, phenols, proteins, amino acids (3), Vitamin C, carbohydrate and β -Carotene (2). The fruits of this plant reported anti diabetic and antihyperlipidemic activities. The tubers were reported as antiovolatory activity. (3, 4)

Furthermore, literature survey of *M. cymbalaria* revealed that traditionally juice of the leaves used for whooping cough, tubers used for abortion, paste of tubers used for applying boils, ulcers, and snake bite (5). No researcher has yet reported antimicrobial activities of fruit this plant. Therefore, it is worth conducting an investigation on the antimicrobial activities of extract of *M. cymbalaria* fruits.

Materials And Methods

Plant material

The fruits of *Momordica cymbalaria* Hook F. was collected in November 2006 from the Bellary, Karnataka, India. The fruit material was taxonomically identified by the Regional Research Institute, Karnataka, India, and the Voucher specimen RRI/BNG/DSRU/F53/2006-07. The fruits were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container.

Preparation of extract

The powder obtained was subjected to successive soxhlet extraction with the solvents with increasing order of polarity i.e. Pet. Ether (60-80°), Chloroform (59.5-61.5°), Methanol (64.5-65.5°) and water. Yield 3.29, 6.19, 11.70, and 15.71% respectively.

Test microorganisms

Strains, including fungi and bacteria were obtained from Microbial Type Culture Collection (MTCC), Chandigarh. *Escherichia coli* MTCC (443), *Staphylococcus aureus* MTCC (96), *Bacillus subtilis* MTCC (121), *Shigella sonnei* MTCC 2957, *Klebsiella pneumoniae* MTCC (109), *Salmonella typhi* MTCC 735, *Proteus vulgaris* MTTC 2813, *Pseudomonas aeruginosa* MTCC (429), *Candida albicans* MTCC (183) and *Aspergillus niger* MTCC (16404) were used as test organisms.

Method of preparation of test organism suspension

Test organism was maintained on slants of medium containing 300 mg of manganese sulphate per liter and transferred to fresh slant once a week. Then the slants were incubated at temperature 32°C for 24 h. Organism was then washed by using 3 ml saline solution from agar slant onto a large agar surface of medium such as Roux bottle containing 250 ml agar. It was incubated for 24 h. Using 50 ml saline solution, the growth from the nutrient surface was washed. Then organism was stored under refrigeration. Inoculum was adjusted at 530 nm, leading to transmission equivalent to 1×10^8 cells/ml.

Antimicrobial assay

Antimicrobial activity of the above mentioned extracts was determined, using a modified cup plate method (Kirby-Bayer method) (6). Muller Hinton agar was used for the growth of bacterial strains and Potato Dextrose agar was used for the growth of fungi. In case of spore producing organisms, sporulated culture was also grown on Potato Dextrose agar. Plant extracts were dissolved in DMSO at a concentration of 2 mg/ml. The standard antibacterial solution containing 20 µg/ml Gentamycin and Amphotericin were prepared. Each plate was inoculated with 20 µl microbial suspension having a concentration of 1×10^8 cells/ml. 0.1 ml extract was added to each cup. The plates containing bacteria were incubated at 37°C for 24h and those containing fungi were incubated at 25°C for 7 days. The positive antimicrobial activity was read based on

growth inhibition zone and compared with the standard drug. All the tests were repeated in triplicates. MIC values were also studied for micro organisms, which were determined as sensitive to the extract in disc diffusion assay. Sterile filter paper discs (6mm in diameter) containing 2.5–1000 µg/disc of plant extracts were placed on the surface of a medium. MIC was defined as the lowest concentration of extract that inhibited visible growth on agar (7)

Results and Discussion

From the results, it could be concluded that the *M .cymbalaria* fruit extracts may be useful as a broad-spectrum antimicrobial agent following extensive investigation. These results may provide a basis for the isolation of compounds of biological interest from *M .cymbalaria* for its potent activity. As shown in Table 1, the methanolic extract of *M .cymbalaria* fruit extract exhibited potent activity against all set of microorganisms used. Petroleum and chloroform extracts showed significant activity against the entire microorganism.

Table 1: Antimicrobial activity of various extracts of fruit of M.Cymbalaria

Organism	Diameter of zone of inhibition (mm)					
	Extracts (2mg/ml)				Standards(20 g/ml)	
	A	B	C	D	E	F
Bacteria						
<i>Escherichia coli</i>	14	12	18	11	18	-
<i>Staphylococcus aureus</i>	13	11	17	10	18	-
<i>Bacillus subtilis</i>	15	11	17	12	19	-
<i>Shigella sonnei</i>	14	10	18	10	18	-
<i>Klebsiella pneumoniae</i>	10	11	16	-	17	-
<i>Salmonella typhi</i>	12	10	15	12	16	-
<i>Proteus vulgaris</i>	11	11	14	-	17	-
<i>Pseudomonas aeruginosa</i>	11	12	15	-	17	-
Fungi						
<i>Candida albicans</i>	12	10	18	12	-	19
<i>Aspergillus niger</i>	13	10	17	11	-	18

A: Petroleum ether extract, B: Chloroform extract, C: Methanol extract, D: Aqueous extract, E: Standard antibacterial agent (Gentamycin 20 µg/ml), F: Standard antifungal agent (Amphotericin 20 µg/ml)

All extracts of the fruit of plant were also tested for their minimum inhibitory concentrations (MIC). The MIC values are shown in Table 2. The presence of activity within the extracts used in the preliminary tests may well depend on the concentration of extracts

Table 2: The MIC values ($\mu\text{g}/\text{disc}$) of *M.cymbalaria* extracts against the microorganisms

Organism	Concentration ($\mu\text{g}/\text{disc}$)			
	Extracts			
	A	B	C	D
Bacteria				
<i>Escherichia coli</i>	10	15	5	100
<i>Staphylococcus aureus</i>	15	15	2.5	100
<i>Bacillus subtilis</i>	15	15	2.5	50
<i>Shigella sonnei</i>	15	15	2.5	100
<i>Klebsiella pneumoniae</i>	50	15	5	-
<i>Salmonella typhi</i>	50	50	10	100
<i>Proteus vulgaris</i>	100	200	10	-
<i>Pseudomonas aeruginosa</i>	100	50	15	-
Fungi				
<i>Candida albicans</i>	50	15	2.5	100
<i>Aspergillus niger</i>	10	50	2.5	100

A: Petroleum ether extract, B: Chloroform extract, C: Methanol extract, D: Aqueous extract.

These results also support the popular use of these plants in tribal/traditional medicine for the treatment of fever, wound infections, and intestinal disorders. Although, the tested plant extracts may contain anti-microbial constituents, further phytochemical and pharmacological studies 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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