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Antimicrobial Activity of *Michelia champaca*

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The antimicrobial activity of *Michelia champaca* was assessed against certain human pathogenic microorganisms by disc diffusion method. The crude aqueous extract, cold methanol extract, methanol distillate and residual extracts of flowers were used in the present study. The crude extract showed largest zone of inhibition towards *Rhizopus* species, *i.e.*, 17 mm indicating that this extract was highly effective to this organism. *E. coli* showed 22.5 mm zone of inhibition to cold methanol extract of *M. champaca* flower. *S. aureus* and *M. luteus* were highly sensitive to distilled methanol forming 20 mm zone. The residual extract was strongly inhibitory towards *S. aureus* as it formed 26.5 mm zone and least inhibitory to *S. typhimurium* as it showed 7 mm zone. The MICs of residual extract ranged between 100–800 µg/mL, *S. aureus* with lowest and *B. subtilis* and *Y. enterocolitica* with highest MIC values indicating that *S. aureus* was strongly inhibited by the residual extract and *B. subtilis* and *Y. enterocolitica* were least sensitive to this extract. There was inhibition of amylase and protease production by methanol residual extract of this plant. The number of colony forming units of the air microbial flora was reduced by 88% in 15 min following the spray of residual extract. These results indicate that this floral methanol extract acted as a broad spectrum antimicrobial agent and as it was a very fragrant extract, it can be used as antimicrobial deodorant, antimicrobial perfume and in operation theatres.

Key Words: Antimicrobial activity, *Michelia champaca*, Human microbial pathogens, Air microbial flora.

INTRODUCTION

Michelia champaca (Magnoliaceae) is distributed throughout India. It is a tall evergreen tree obtaining a height of 30 metres and 50–80 cm in diameter. The flowers are yellow to orange red in colour, solitary, fragrant. The roots and root bark is used as purgative, in treatment of abscesses, inflammation and constipation. Stem bark is used as diuretic, for treatment of fever, cough and bronchitis and fruits in psoriasis. Flowers and flower buds are bitter, acrid, astringent, digestive, anthelmintic, refrigerant, expectorant, antipyretic, stomachic, used for treatment of leprosy, malarial fever and ulcers[1]. Magnosprengerine 0.41% and 0.39% is found in bark and root extracts, respectively of Chinese plant. It has been reported that alkaloids from *M. champaca* were used to treat fever and wounds[2]. The antimicrobial activity of several medicinal plants has been reported earlier[3–9]. Recently, the antimicrobial activity of *M. champaca* has been reported where the methanol extract of leaves, seed, stem root bark and heart wood of roots showedbroad spectrum antibacterial and antifungal activity[3]. But the antimicrobial activity of methanol extract of *M. champaca* flower was not tested.

Therefore the aim of the present investigation is to assess the antimicrobial activity of *M. champaca* flower against human pathogenic microorganisms.

EXPERIMENTAL

In the present study, the flowers of *M. champaca* were collected from Visakhapatnam, Andhra Pradesh, India. They were shade dried. Later they were made into a fine powder (1 kg) and mixed in 2.5 L of methanol in 5 L aspirator bottle. Two days later, 20 mL of the surface layer was collected as cold methanol extract. Then the solution was taken in a distillation unit and distillation was performed. The distillate also contained the flower extract instead of having only colourless methanol, which was determined by the yellow colour and designated as distilled methanol extract in the present study which was tested. The residual extract (45 g) was of dark brown colour which was tested against the pathogens. The residual extract was diluted in methanol to give a concentration of 20 μg/μL and used as stock solution which was stored at 4°C for all experiments.

Microorganisms: The following microbial cultures were obtained from MTCC, Chandigarh, India: *Staphylococcus aureus* (ATCC 23564), *Salmonella typhimurium* (ATCC ATCC 23564), *Yersinia enterocolitica* (ATCC 9610), *Escherichia coli* (ATCC 8739), *E. coli* (isolated from drinking water, GITAM) *Pseudomonas aeruginosa* (ATCC 25619), *Rhizopus* species and *Candida albicans* (ATCC 2091). *Bacillus subtilis, Micrococcus* species and *Aspergillus flavus* were isolated from skin infection of human leg. *Geotrichum* species was an isolate of spoiled tomato.

Microbial culture conditions: The bacterial cultures were maintained on Nutrient agar slants or plates (peptone 0.5%, beef extract 0.3%, NaCl 0.5%, agar 2.0%) and fungal cultures on Sabouraud's agar slants or plates (mycological peptone 1%, dextrose 2–4%, agar 2%). Overnight cultures were used in all experiments by inoculating a single colony of each type of culture in respective 5 mL broth and incubating at 37°C for 18–24 h (bacteria) or at room temperature for 48 h (fungi).

Disc diffusion method: Nutrient agar plates or Sabouraud's agar plates were inoculated with 0.1 mL of each fresh culture containing 107 cells by spread plate method. Later five sterile filter paper discs (5 mm) and reference antibiotic discs such as penicillin G (10 unit/disc) and nystatin (100 units/disc) procured from Himedia, Mumbai, were placed in corresponding plates. On one filter paper disc, 4 mg of crude aqueous extract of fruit in 20 μL volume; on the 2nd disc, 20 μL of cold methanol extract; on 3rd disc, 400 μg of residual extract in 20 μL volume; on 4th disc, distilled methanol with flower extract of 20 μL and finally, on 5th disc, methanol control of 20 μL were dropped. Later one reference antibiotic or antifungal disc was placed in each corresponding plate. Then the plates were kept at room temperature for 1 h for the plant extracts to be diffused into the medium. Then the plates were incubated at 37°C for 18–24 h (bacteria) and at room temperature (29°C) for 48 h (fungi).

Determination of minimal inhibitory concentrations (MICs): The minimal inhibitory concentration of residual extract was determined by broth dilution

technique. Duplicates of serial dilutions of respective broth and various concentrations of plant extracts were made in sterile test tubes for 1 mL. The controls did not receive any extract. 50 µL of culture containing 10^3 cells was added to each test tube. The suspension was mixed well by rotating between the palms and poured on to respective agar plates, which were later incubated in the incubator at 37°C for 18–24 h or at room temperature for 48 h in case of fungi. Later, the lowest concentration of the plant extract that inhibited the growth of microorganisms was noted as minimal inhibitory concentration by counting the colony forming units.

The amylase and protease production: The amylase and protease produced by some of these microorganisms were tested by cultivating the microorganisms in the presence (at respective MIC) and absence of this plant extract. These assays were performed according to the method of Sawhney and Singh[10].

Effect of residual extract of *M. champaca* on air microorganisms: To test the antimicrobial potency of this extract on air microflora, it was sprayed at a concentration of 10 mg/mL in a closed chamber and around 10 mL was sprayed. The microbial count was taken at 15 min, 30 min and 1 h after spray. The control plate was exposed to air in the chamber before spraying the extract for 1 h. The percentage reduction in CFU was calculated.

RESULTS AND DISCUSSION

The zone of inhibition produced by crude extract of *M. champaca* (Table-1) ranged from 8–17 mm, *Rhizopus* species with highest zone and *S. aureus* with

TABLE-1
ANTIMICROBIAL ACTIVITY OF *MICHELIA CHAMPACA*

Organism	Zone of inhibition (mm)					
	Crude Extract (4 mg/disc)	Cold extract (20 µL)	Distilled methanol (20 µg)	Residual extract (400 µL)	Pure methanol (control) (20 µL)	Penicillin reference (10 units/disc)
S. aureus	8.0	22.0	20.0	26.5	16.0	25.0
B. subtilis	13.5	16.0	6.0	13.5	12.0	20.0
M. leutius	12.0	18.5	20.0	20.0	14.0	22.0
Y. enterocolitica	15.0	20.0	12.5	12.5	16.0	12.0
P. aeruginosa	12.5	20.5	13.5	19.5	11.5	13.5
E. coli (UTI)	12.0	22.5	14.5	22.0	12.0	12.5
E. coli (W)	15.0	21.0	0.0	20.0	12.0	12.5
S. typhimurium	15.0	10.0	10.0	7.0	12.0	16.0
Rhizopus	17.0	14.0	11.5	15.5	12.0	17.0*
Geotricum species	13.0	15.0	13.0	15.0	11.0	18.0*
A. flavus	12.0	13.0	13.0	12.0	12.0	16.0*
C. albicans	15.0	20.0	17.5	17.0	10.0	20.0*

*Nystatin 100 units/disc

less zone indicating that the crude extract was strongly inhibitory towards *Rhizopus* species and least to *S. aureus*. The cold extract showed zones ranging from 13–22.5 mm, *E. coli* (UTI) with largest and *A. flavus* with lowest zone indicating that the cold extract of this flower was highly effective against *E. coli* and less effective aginst *A. flavus*. The distilled methanol showed zones ranging from 6–20 mm indicating that *S. aureus* and *Micrococcus* species were highly sensitive and *B. subtilis* was less sensitive to this extract. The residual extract of *M. champaca* flower was strongly inhibitory towards *S. aureus* as it formed 26.5 mm zone and least inhibitory to *S. typhimurium* as it showed 7 mm zone. The results of the present study differ from the results of Elizabeth[5], where *C. dygina* was more effective to some of these organisms as they formed larger zones. The MICs of residual extract (Table-2) ranged between 100–800 µg/mL,

TABLE-2
MINIMAL INHIBITORY CONCENTRATIONS OF *M. CHAMPACA* FLORAL METHANOL RESIDUAL EXTRACT

Organism	µg/mL
S. aureus	100
B. subtilis	800
M. leuteus	200
Y. enterocolitica	800
P. aeruginosa	400
E. coli (UTI)	200
E. coli (drinking water isolate)	200
S. typhimurium	ND
Rhizopus	400
Geotricum species	400
A. flavus	400
C. albicans	400

S. aureus with lowest MIC and *B. subtilis* and *Y. enterocolitica* with highest MIC values indicating that *S. aureus* was strongly inhibited and *B. subtilis* and *Y. enterocolitica* least sensitive to this extract. The microorganisms with moderate MIC values were moderately sensitive to this plant extract. There was 100% inhibition of amylase production by residual extract of this plant in *B. subtilis* and *P. aeruginosa* (Table-3) which was 560 µg/mL and 800 µg/mL, respectively before treatment. The protease activity was inhibited by 6-fold following the residual extract treatment in *B. subtilis* and 4-fold in *P. aeruginosa* indicating that this extract was a potential inhibitor of these enzyme activities. There was reduction in the number of colony forming units (Table-4) when this extract was sprayed in a chamber at a concentration of 10 mg/mL methanol. There was 88% reduction in CFU, within 15 min of spray, which slowly increased with increasing time, *i.e.*, after 30 min of spray there was 80% reduction and after one hour there was 70% reduction in CFU indicating that this plant extract has bactericidal effect.

These results indicate that *M. champaca* methanol floral extracts have potent broad spectrum antimicrobial activity. As it was a very fragrant extract, the extracts can be used as antimicrobial deodorants or perfumes and can be used in operation theatres where the abnormal smell and microbes can be removed simultaneously.

TABLE-3
EFFECT OF *M. CHAMPACA* ON AMYLASE AND PROTEASE PRODUCTION

Organism	Amylase without PE (µg/mL)	% inhibition with PE (amylase)	Protease without PE (µg/tyrosine/h)	% inhibition with PE (protease)
B. subtilis	560.0	100	72.5	84
P. aeruginosa	800.0	100	100	80

PE = plant extract.

TABLE-4
EFFECT OF RESIDUAL METHANOL EXTRACT OF *M. CHAMPACA* FLOWER ON AIR MICROBIAL FLORA

Time in min	Number of colony forming units in		% reduction in CFU
	Control	Experimental	
15	25	03	88
30	30	06	80
60	50	15	70

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