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NOTE

Antimicrobial Activity of Hexane Extracts of *D. hamiltonii* on Microbial Isolates of Spoiled Vegetables and Pathogenic Microorganisms

Y. VIMALA*, ARUNA CHANDANA KUMARI and K.M. ELIZABETH
Department of Microbiology, College of Science
Gandhi Institute of Technology and Management
Visakhapatnam-530 045, India
Tel: (91)(891)2840473 (O), (91)(891)2519455 (R)

The antimicrobial activity of *Decalepis hamiltonii* was tested by disc diffusion method against microbial isolates from spoiled vegetables and pathogenic microorganisms. The crude aqueous extract, cold hexane extract, hexane distillate and residual extracts of the roots were used for the study. The crude extracts showed largest zones to *E. coli, i.e.*, 15 mm zones. The cold hexane extract showed maximum activity against P_2 (isolate), B (isolate), *Serratia marcescens* and *Rhizopus* species, *i.e.*, 15 mm. The distillate showed 6 mm zones to all the microorganisms tested. The residual extract showed maximum activity against *E. coli* (28 mm), *Aspergillus flavus* (24 mm), P_1 (isolate) (20.0 mm), *Candida albicans* (20.0 mm) indicating that the residual extracts were strongly inhibitory. The results indicate that the residual extracts can be used as broad spectrum antimicrobial agents.

Key Words: *Decalepis hamiltonii*, Hexane extracts, Antimicrobial activity, Microbial isolates, Pathogenic microorganisms, MIC.

Many medicinal plants are screened for their possible antimicrobial activity[1–3]. The methanol, acetone, chloroform and ethyl acetate extracts of *D. hamiltonii* showed antimicrobial activity. In continuation with our work, the hexane extracts of *D. hamiltonii* were tested on microbial isolates of spoiled vegetables and certain pathogenic microorganisms for the antimicrobial activity.

Decalepis hamiltonii (Family Asclepediaceae) is a straggling shrub, roots fasciculated, stem branched, branchlets with winged nodes, flowers yellowish to trichotomous cymes. It is endemic to peninsular India and is restricted to Eastern and Western Ghats. Roots of this plant are used as flavouring agents, appetiser and blood purifier. Root extract was found to be a potent antimicrobial agent and having bioinsecticidal activity on storage pests. Besides these, highly aromatic roots are used as a culinary spice and in preparing a popular cold drink locally known as 'Nannari' which has cooling effect in summer. Roots are used in pickle preparations. The root-specific flavour compound is 2-hydroxy-4-methoxy benzaldehyde.

Decalepis hamiltonii plants were collected from the forests of Kurnool District, Andhra Pradesh. The roots were shade dried and ground to a fine powder, mixed with sterile distilled water to give a concentration of 0.5 gm/5 mL of stock solution which was stored in a refrigerator until further use. The residual hexane extracts were prepared by mixing 100 g of dried and ground root powder with I L of hexane

in an aspirator bottle for 48 h. Later, the solution was collected and subjected to six cycles of distillation until a thick brown coloured paste was obtained. 500 mg of the residual hexane extract was mixed with 5 mL of hexane to give a concentration of 1 μL = 100 mg of root extract.

The microbial strains used in this study were isolated from spoiled vegetables. The vegetables used were *Abelmoschus esculentum, Solanum tuberosum, Momordica cherantia, Luffa acutangula, Citrus lemon.* Biochemical tests were performed to identify the organisms. The isolated organisms were identified as: LF_1 unidentified, LF_2 identified as *Staphylococcus* species, LF_3 as *Candida* species, P_1 as *Pseudomonas* species, P_2 as *Streptococcus* species, B as *Streptococcus pnuemoniae* species, L as *Bacillus* species, AG as the Gram-negative rod. Microorganisms collected from MTCC Chandigarh used for the study were *Pseudomonas aeruginosa* (ATCC 25619), *Salmonella typhi* (ATCC 10749), *Salmonella typhimurium* (ATCC 23564), *Yersinia enterocolitica* (ATCC 9610), *Serratia marcescens.* Fungal strains used were *A. flavus* isolated from peanuts, *Geotrichum* isolated from tomato, *Rhizopus, Fusarium* and *Candida albicans* (ATCC 2091).

All the chemicals, media components and antibiotic impregnated discs used in this study were procured from Hi-media, Mumbai, India. All the bacterial strains isolated from spoiled vegetables were stored on agar slants and fungal strain isolates were stored on Sabouraud's agar slants. Disc diffusion method was performed[5]. Nutrient agar and sabouraud's agar were used to culture bacteria and fungi respectively. Fresh overnight cultures of inoculum (0.1 mL) of each culture containing 10^8 cells were spread on agar plates. Five sterile paper discs (5 mm diameter) were placed in each agar plate and on four discs aqueous extract, cold hexane, distillate and residual extract and on the last disc 20 μL of hexane was placed as control. Ampicillin disc (10 μg) was placed as positive control in all plates inoculated with bacteria and for fungal cultures nystatin (100 units/disc) was placed as positive control. The bacterial cultures were incubated at 37°C for 18–24 h and fungal cultures at 28°C for 48 h; zones of inhibition were measured. The microbes were plated in duplicate and average zone diameter was noted. The minimal inhibitory concentrations (MICs) were determined by tube dilution technique. Different concentrations of residual extracts of *D. hamiltonii* in nutrient broth were serially diluted in duplicate. Control tubes did not receive any extract. Later 10^3 cells of microorganisms collected from MTCC and isolates of spoiled vegetables in 0.02 mL volume were added into each test tube and incubated at 37°C for 18–24 h. The lowest concentration of drug which inhibited the growth was considered as MIC.

The results were presented in Table-1. The residual extract showed maximum zones of inhibition towards *E. coli, A. flavus, Candida* and P_1 which showed 28 mm, 20 mm, 20 mm, 20 mm zones of inhibition respectively and MIC recorded were 200 μg/mL, < 800 μg/mL, < 800 μg/mL, 200 μg/mL respectively. The distillate showed 6 mm zones and hexane control showed 6 mm zones except isolate from P_1.

TABLE-1
ANTIMICROBIAL ACTIVITY OF HEXANE EXTRACTS OF *D. Hamiltonii*

S.No.	Micro-oganisms tested	Zones of Inhibition (mm)						
		Aqueous extract (2 mg/disc)	Cold hexane (20 µL)	Distillate (20 µL)	Residual (2 mg/disc)	Control hexane (20 µL)	Ampicillin (10 units/disc)	MICS µg/mL
1.	LF_1*	8.0	8.0	6.0	10.0	6.0	0.0	< 800
2.	LF_2*	8.0	6.0	6.0	18.0	6.0	6.0	800
3.	LF_3*	8.0	6.0	6.0	8.0	6.0	22.0	800
4.	P_1*	8.0	6.0	6.0	20.0	6.0	0.0	400
5.	P_2*	7.0	15.0	6.0	15.0	25.0	15.0	< 800
6.	AG*	6.0	6.0	6.0	14.0	6.0	17.0	800
7.	B*	6.0	15.0	6.0	13.0	6.0	00.0	800
8.	L*	12.0	6.5	6.0	12.0	6.0	22.0	400
9.	ST	6.0	6.0	6.0	18.0	6.0	0.0	< 800
10.	STM	10.0	6.0	6.0	6.0	6.0	0.0	< 800
11.	YE	8.0	6.0	6.0	8.0	6.0	0.0	< 800
12.	PA	6.0	6.0	6.0	15.0	6.0	0.0	< 800
13.	EC	15.0	6.0	6.0	28.0	6.0	9.0	200
14.	SM	8.0	15.0	6.0	12.0	6.0	0.0	100
15.	AF*	8.0	6.0	6.0	24.0	6.0	30.0†	< 800
16.	RP*	6.0	15.0	6.0	18.0	5.0	30.0†	< 800
17.	GT*	6.0	6.0	6.0	12.5	6.0	22.0†	< 800
18.	CA	8.0	6.0	6.0	20.0	6.0	29.0†	< 800
19.	FS*	7.0	6.0	6.0	23.0	5.0	28.0†	800

*Isolates from spoiled vegetables; †Nystatin, 100 units/disc.
LF_1, LF_2, LF_3: Isolates from *Abelmoschus esculentum.*
P_1, P_2: Isolates from *Solanum tuberosum.*
AG: Isolate from *Luffa acutangula.*
B: Isolate from *Momordica cherantia.*
L: Isolate from *Citrus lemon.*
ST: *Salmonella typhi* (ATCC 10749); STM: *Salmonella typhimurium* (ATCC 23564),
YE: *Yersinia enterocolitica* (ATCC 9610); PA: *Pseudomonas aeruginosa* (ATCC 25619);
EC: *E. coli,* SM: *Serratia marcensens;*
AF: *Aspergillus flavus;* RP: *Rhizopus,*
GT: *Geotrichum;* CA: *Candida albicans* (ATCC 2091);
FS: *Fusarium.*

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