Asian Journal of Chemistry Vol. 19, No. 1, (2007), 801–803

NOTE

Antimicrobial activity of Ethyl Acetate Extracts of *D. hamiltonii* on Microbial Isolates of Spoiled Vegetables and Pathogenic Microorganisms

Y. VIMALA*, K. SUMATHI DEVI 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 ethyl acetate extract, ethyl acetate distillate and residual extracts of the roots were used for the study. The crude extracts showed largest zones to LF_1 (isolate), AG (isolate), B (isolate), *Pseudomonas aeruginosa*, *i.e.*, 8 mm zones. The cold ethyl acetate extract showed maximum activity against LF_2 (isolate), *i.e.*, 24 mm. The distillate showed activity against P_2 (isolate) and B (isolate), *i.e.*, 15 mm. The residual extract showed maximum activity against *Aspergillus flavus* (27.5 mm), *Salmonella typhimurium* (27.5 mm), AG (isolate) (25.0 mm), LF_3 (isolate) (25.0 mm) indicating that the residual extracts were strongly inhibitory. The results indicate that the residual extracts can be used as broad spectrum antimicrobial agent.

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

Many medicinal plants are screened for their possible antimicrobial activity[1–3]. The methanol, acetone, chloroform extracts of *D. hamiltonii* showed antimicrobial activity. In continuation with our work[4, 5], the ethyl acetate extracts of *D. hamiltonii* were tested on microbial isolates of spoiled vegetables and certain pathogenic microorganisms for 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, appetizers and blood purifiers. Root extract was found to be a potent antimicrobial agent having bioinsecticidal activity on storage pests. Besides these, highly aromatic roots are used as a culinary spice and in preparing a popular cool 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 g/5 mL of stock solution which was stored in a refrigerator until further use. The residual methanol extracts were prepared by mixing 100 g of dried and ground root powder in 1 L of ethyl acetate 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 methanol extract was mixed with 5 mL of ethyl acetate 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 charantia, Luffa acutangula, Citrus lemon.* Biochemical tests were performed to identify the organisms. The isolated organisms were identified, 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* 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 antibiotics 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 strains isolates were stored on Sabouraud's agar slants. Disc diffusion method was performed[4, 5] by nutrient agar and Sabouraud's agar slants 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 ethyl acetate, distillate and residual and on last disc 20 μL of ethyl acetate 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 30°C for 18–24 h. The lowest concentration of drug which inhibited the growth was considered as MIC.

The results were tabulated in Table-1. The residual extract showed maximum zones of inhibition towards *Aspergillus flavus* and *Salmonella typhimurium* which showed 27.5 mm zone of inhibition and MIC recorded were 800 μg/mL. *Fusarium* showed less MIC, *i.e.*, 25 μg/mL and LF_1, LF_2, SM showed 22.0 mm, 20.0 mm, 23.5 mm zones of inhibition, respectively and MICs as 100 μg/mL.

TABLE-1
ANTIMICROBIAL ACTIVITY OF ETHYL ACETATE EXTRACTS OF
D. Hamiltonii ZONES OF INHIBITION (mm)

S.No.	Micro-oganisms tested	Zones of Inhibition (mm)						
		Aqueous extract (2 mg/disc)	Cold ethyl acetate (20 µL)	Distillate	Residual (2 mg/disc)	Control ethyl acetate (20 µL)	Ampicillin (10 units/disc)	MICS (µg/mL)
1.	LF_1*	8.0	15.0	8.0	22.0	11.0	0.0	100
2.	LF_2*	6.0	24.0	6.0	20.0	13.0	6.0	100
3.	LF_3*	6.0	15.0	6.0	25.0	12.0	22.0	800
4.	P_1*	7.0	7.0	8.0	20.0	13.0	0.0	200
5.	P_2*	7.0	15.0	15.0	14.0	11.0	15.0	100
6.	AG*	8.0	8.0	8.0	25.0	13.0	17.0	800
7.	B*	8.0	14.0	15.0	22.5	13.0	00.0	200
8.	L*	7.0	21.5	8.0	22.5	13.0	22.0	800
9.	ST	7.0	15.0	7.0	25.0	9.0	00.0	800
10.	STM	7.0	7.0	8.0	27.5	11.0	00.0	800
11.	YE	7.0	12.5	10.0	18.0	15.0	00.0	400
12.	PA	8.0	17.5	10.0	25.0	13.0	00.0	> 800
13.	EC	7.0	15.0	8.0	25.0	16.0	9.0	> 800
14.	SM	7.0	25.0	12.0	23.5	11.5	0.0	100
15.	AF*	6.0	13.0	7.0	22.5	9.0	30.0†	800
16.	RP*	6.0	15.0	6.0	22.5	13.0	30.0†	50
17.	GT*	6.0	18.0	6.0	26.5	15.0	22.0†	> 800
18.	CA	6.0	15.0	6.0	6.0	9.0	29.0†	100
19.	FS*	6.0	6.0	6.0	23.0	9.0	28.0†	25

*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.*

REFERENCES

1. R.P. Rastogi and B.N. Mehrotra, Compendium of Indian Medicinal Plants, Vol. 2, Central Drug Research Institute, Lucknow and National Institute of Science Communication, New Delhi (1999).
2. J.M. Sashikumar, M. Remya and K. Janardhan, *Asian J. Microbiol. Biotech. Environ. Sci.*, **5**, 183 (2003).
3. T. Sheela and S. Kannan, *Asian J. Microbiol. Biotech. Environ. Sci.*, **5**, 135 (2003).
4. K.M. Elizabeth, I.P. Duguid, B.P. Marmion and R.H.A. Swain, Microbiologia Medica, Vol. 11, 4th Edn., Fundacao Calouste Gulbenkian Lisbos, pp. 393–403 (1975).
5. K.M. Elizabeth, *Indian J. Microbiol.*, **41**, 321 (2001).

(*Received*: 19 December 2005; *Accepted*: 30 June 2006) AJC-5015