Bangladesh J. Bot. **37**(1): 89-92, 2008 (June) **- Short communication**

ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF *EUPATORIUM TRIPLINERVE* VEHL. AGAINST SOME HUMAN PATHOGENIC BACTERIA AND PHYTOPATHOGENIC FUNGI

MD. SHAFIQUR RAHMAN* **AND MOHAMMAD JUNAID**

Department of Microbiology, University of Chittagong, Chittagong-4331, Bangladesh

Key words: Antimicrobial activity, Leaf extract, *Eupatorium triplinerve*

Abstract

Petroleum ether, carbon tetrachloride, chloroform, and ethyl acetate extracts of *Eupatorium triplinerve* Vehl. leaves were investigated for their antimicrobial activities against 11 human pathogenic bacteria and six phytopathogenic fungi. The crude extracts showed good activity against the organisms tested herein. The chloroform extract exhibited the largest zone of inhibition (22 mm in diam with 1000 μg/disc extract) against *Vibrio* and the highest inhibition of fungal radial mycelial growth (73.5% with 100 μg extract/ml medium) against *Colletotrichum corchori*. The chloroform extract exhibited the lowest MIC against *Vibrio* (250 μg/ml) and *C. corchori* (62.5 μg/ml). It appeared that *E. triplinerve* could be a potential natural source of new antimicrobial agent.

Most of the people in rural and urban areas of the world were depended on the medicinal plants for the treatment of infectious diseases. The Ayurvedic and Unani systems of medicines are widely used by the people of Indian subcontinent. Among the different plant derivetives, secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity (Ahmed *et al.* 2002, Rahman *et al*. 1999). *Eupatorium triplinerve* (Compositae) is a slender herb with narrow lanceolate leaves and large number of pedicelled flower-heads at the top of the branch, cultivated in Chittagong and Chittagong Hill Tracts. Extract of the plant is used as antiseptic, and in the treatment of various ulcers and haemorrhages (Ghani 1698). This paper reports the result of antimicrobial activity of *E. triplinerve* on folk usages by traditional practitioners in Tribal areas of Bangladesh.

Fresh leaves of *Eupatorium triplinerve* were collected from Chittagong University campus, Chittagong, Bangladesh. The cleaned leaf samples were cut into small pieces (1-2 cm), air dried and ground to fine powder mechanically. 50 g of the dried powders were kept overnight in petroleum ether, chloroform, ethyl acetate and carbon tetrachloride. The extracts thus obtained were filtered, centrifuged at 5000 rpm for 20 minutes and then concentrated to a gummy material under reduced pressure at 50º C by rotary vacuum evaporator. The gummy material was then transferred to small vials and dried and termed as crude extract.

The crude extract against 11 human pathogenic bacteria *viz.*, *Shigella dysenteriae* AE 14396, *S. sonnei* CRL.(ICDDR,B), *Salmonella typhi* AE 14612, *S. paratyphi* AE 14613, *Bacillus subtilis* BTCC 17, *B. megaterium* BTCC 18, *B. cereus* BTCC 19, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* CRL(ICDDR'B), *Escherichia coli* ATCC 25922 and INABA ET *(Vibrio)* AE 14748 and five phytopathogenic fungi, *viz.*, *Alternaria alternata* (Fr.) Kedissler., *Curvularia lunata* (Wakker) Boedijn, *Colletotrichum corchori* Ikata (Yoshida), *Fusarium equiseti* (Corda) Sacc., *Macrophomina phaseolina* (Maubl) Ashby. and *Botryodiplodia theobromae* Pat were tested.

The *in vitro* antibacterial and antifungal activity of plant material was determined by disc diffusion method (Bauer *et al.* 1966) and poisoned food technique (Miah *et al.* 1990). Mueller-Hinton agar and Potato dextrose agar media were used for culturing of bacteria and fungi, respectively. MICs of the crude extracts were determined by broth macrodilution method (Jones *et al*. 1985).

*Corresponding author.

All the crude extracts (1000 µg/disc) exhibited moderate to good antibacterial activity against the bacterial pathogens tested herein and the largest zone of inhibition (22 mm in diam) was recorded against *Vibrio* (Table 1). Antibacterial antibiotic ampicillin (20 µg/disc) was also found to be active against all the bacteria tested herein.

Table 1. Antibacterial activity of crude leaf extracts from *Eupatorium triplinerve.*

Bacteria	Diameter of zone of inhibition (mm) (Crude extract 1000 µg/disc)				Ampicillin* (20 µg/disc)
	Petroleum ether	Carbon tetrachloride	Chloroform	Ethyl acetate	
Bacillus subtilis Ehr. Cohn	13	17	20	20	19
B. megaterium de Bary	10	16	18	16	16
B. cereus Frank & Frank.	12	19	20	20	18
Staphylococcus aureus Rosen.	13	19	20	21	22
Escherichi. coli	11	17	19	18	10
INABA ET(*Vibrio*)	12	18	22	21	15
Shigella dysenteriae Cast. & Chal.	10	16	18	16	22
S. sonnei Weldin	10	17	18	18	20
Salmonella typhi Warren & Scott.	11	17	19	21	20
S. paratyphi Cast. & Chal	11	16	20	20	17
Pseudomonas aeruginosa Migula	10	16	19	18	10

*Standard antibacterial antibiotic.

The chloroform extract exhibited the lowest MIC value (125 µg/ml) against *Vibrio* (Table 2). Similar antibacterial activity of some other plant extracts has been reported previously (Rahman *et al*. 1998, Sarker *et al*. 1991).

Table 2. MICs of crude leaf extracts from *Eupatorium triplinerve* against test bacteria.

Bacteria	MIC (Crude extract µg/ml medium)			
	Petrolium ether	Carbon tetrachloride	Chloroform	Ethyl acetate
Bacillus subtilis	750	500	250	250
B. megaterium	1000	500	500	750
B. cereus	750	250	250	250
Staphylococcus aureus	750	250	250	250
E. coli	1000	500	250	750
INABA ET (*Vibrio*)	1000	500	125	250
Shigella dysenteriae	1000	750	500	750
S. sonnei	750	500	500	250
Salmonella typhi	1000	500	250	250
S. paratyphi	750	500	250	250
Pseudomonas aeruginosa	1250	750	500	750

The results of the inhibition of fungal radial mycelial growth are summarized in Table 3. It appeared that the crude extracts inhibited the radial mycelial growth of all the test fungi at a concentration of 100 µg/ml medium to varying degrees. The crude extracts exhibited prominent (more than 50%) inhibitions of radial mycelial growth against all the test fungi except *Botryodiplodia theobromae* with carbon tetrachloride, chloroform and ethyl acetate extract, respectively. The highest inhibition (73.5%) of fungal radial mycelial growth was recorded against *Colletotrichum corchori* at a concentration of 100 µg/ml medium using the chloroform. Antifungal

antibiotic nystatin (100 µg/ml medium) exhibited good inhibitions (40.51-75.0%) of radial mycelial growth of all the six fungi tested herein, but it was much less active against *Alternaria alternata, Colletotrichum corchori* and *Fusarium equiseti* compared to that of the carbon tetrachloride, chloroform and ethyl acetate extracts.

Table 3. Antifungal activity of crude leaf extracts from *Eupatorium triplinerve*.

Fungi	Percentage inhibition of fungal mycelial growth (radius in cm) (100 µg/ml medium)				
	Petrolium ether	Carbon tetrachloride	Chloroform	Ethyl acetate	Nystatin*
Alternaria alternata Keissler	30.5	60.0	62.0	62.0	51.55
Curvularia lunata Boedjin	25.5	64.5	65.0	68.0	75.00
Colletotrichum corchori Ikata	30.0	70.0	73.5	66.5	40.51
Fusarium equiseti Sacc.	39.5	60.0	71.5	55.0	44.70
Macrophomina phaseolina Gold.	24.5	70.0	70.0	71.5	71.78
Botryodiplodia theobromae Pat.	31.0	55.5	47.5	40.0	

* Standard antifungal antibiotic.

The lowest MIC (62.5 µg/ml) was recorded against *Colletotrichum corchori* with carbon tetrachloride and chloroform extracts (Table 4). Similar antifungal activities on plant extracts of other plants have also been previously reported (Anwar *et al*. 1994, Miah *et al*. 1990).

Table 4. MICs of crude leaf extracts from *Eupatorium triplinerve* against fungi.

Fungi	MIC (Crude extract µg/ml medium)			
	Petrolium ether	Carbon tetrachloride	Chloroform	Ethyl acetate
Alternaria alternata	750	125	125	125
Curvularia lunata	1000	125	125	125
Colletotrichum corchori	750	62.5	62.5	125
Fusarium equiseti	750	125	125	250
Macrophomina phaseolina	1250	125	125	125
Botryodiplodia theobromae	750	250	500	750

It appears that crude leaf extract of *Eupatorium triplinerve* has antibacterial and antifungal properties and can be used as a novel antimicrobial agent.

Acknowledgements

The authors wish to thank Mr. M.A. Hakim and Dr. M. Nural Anwar, Department of Microbiology, University of Chittagong, Bangladesh for their help and cooperation.

References

Ahmed, A.M.A., M.S. Rahman and M.N. Anwar**.** 2002. Antimicrobial activity of extracts and crude alkaloids isolated from the leaf of *Adhatoda vasica* Nees. Bangladesh J. Life Sci. **15**(**2**) : 125-128.

Anwar, M.N., P.J. Singha, J. Begum and J.U. Chowdhury. 1994. Antifungal activity of some selected plant extracts on phytopathogenic fungi. Bangladesh J. Life Sci. **6**(2): 23-26.

Bauer, A.W., M.M. Kirby, J.C. Sherris, M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disc method. Amer. J. Clin. Path. **45**: 493-496.

Ghani, A. 1998. Medicinal plants of Bangladesh: Chemical constituents and uses. 1st edn. Asiatic Society of Bangladesh. pp. 174.

Jones, N.R., L.A. Barry, L.T. Gavan and J.A. Washington. 1985. Manual of Clinical Microbiology. 4th edn. American Society for Microbiology, Washington D.C. pp. 972- 976.

Miah, M.A.T., H.U. Ahmed, N.R. Sharma, A. Ali. and S.A. Miah. 1990. Antifungal activity of some plant extracts. Bangladesh J. Bot. **19**(1): 5-10.

Raman, M.S., M.N. Anwar and A.Z.M.S. Chowdhury. 1999. Antibacterial activity of secondary metabolites from *Holarrhena antidysenterica* stem bark. Bangladesh J. Microbiol. **16**(2): 101-105.

Rahman, M.S., J. Begum, J.U. Chowdhury and M.N. Anwar. 1998. Antimicrobial activity of *Holarrhena antidysenterica* against *Salmonella typhi*. Chittagong Univ. J. Sci**. 22**(1) : 111-112.

Sarkar, S.D., S. Muniruzzaman and S.I. Khan. 1991. Antimicrobial activity of *Piper chaba* Hunter (Chui). Bangladesh J. Bot**. 20**(2): 179-182.

(Manuscript received on 14 May, 2007; revised on 12 September, 2007)