ANTIBACTERIAL ACTIVITY OF CLOVE EXTRACTS AGAINST PHAGOGENIC STRAINS INCLUDING CLINICALLY RESISTANT ISOLATES OF *SHIGELLA* AND *VIBRIO CHOLERAE*

SK. NAZRUL ISLAM* ABU J. FERDOUS,**
MONIRA AHSAN AND A.B.M. FAROQUE****
**Institute of Nutrition and Food Science,*
University of Dhaka, Dhaka 1000, Bangladesh.
***Department of Pharmacy, University of Dhaka,*
Dhaka 1000, Bangladesh.

ABSTRACT

Clove extracts (petroleum ether, chloroform and ethanol extracts) were tested *in vitro* for their antibacterial activity against forty isolates of pathogenic bacteria including clinically *resistant* (resistant to amipicillin and nalidixic acid) strains of twenty five *Shigella* and four. *Vibrio.* All of the isolates except *Pseudomonas aeruginosa* showed promising sensitivity to the extracts.

Introduction

Clove is the dried (flower bud of *Eugenia caryophyllus,* family Mytraceae (Tyler*, et al.;* 1988). It has a wide range of medicinal properties; even it is now commonly used in Western medicine (Tyler *et. al.* 1988, Chopra *et. al.,* 1982). Recently antibacterial property of clove has been reported (Briozzo *et. al,* 1989, Watanabe *et. al,* 1985, Ueda *et. al,* 1982). In this paper the antibacterial activity of clove extracts, especially against clinically resistant isolates of *Shigella dysenteriae* type I*, Shigetlla flexneri* and *Vibrio cholerae* has been discussed.

Materials and Methods

Extraction: Powdered clove (25 gm) was extracted separately with petroleum ether(60-80 b.p) chloroform and ethanol with occasional shaking for 48 hours. The extracts were then filtered and concetrated to dryness under reduced pressure at 45°C. Extractions with petroleum ether yielded 1.75 gm oily mass and that with chloroform and ethanol yielded 5.0) gm and 3.5 gm dried mass respectively.

Microorganisms: Identified pure isolates of *Shigella* and *Vibrio cholerae* were collected from the patholgical laboratory of International Centre for Diarrhoeal Disease Research, Banglades, Dhaka and the rest of the strains were obtained form the

*Corresponding Author

microbiology laboratory of the Institute of Nutrition and Food Sciences, University of Dhaka.

Test Materials and Antibiotic Discs: Solutions of petroleum ether, chloroform and ethanol extracts at a concentration of 100 mg per ml were prepared respectively in petroleum ether, chloroform and ethanol. Twenty microlitres of each of the solutions was adsorbed on sterile Matricel filter paper discs and kept for few hours under vacuum for removal of the solvents. Standard ampicillin (10ug/ disc), tetracycline (30ug/ disc), nalidixic acid (30 mg/disc) and gentamicin (10pgldisc), were used in the experiment.

Sensitivity testing procedure: The sensitivity of the bacterial isolates to the clove extracts was done by disc diffusion method (Bauer et al, 1966). Tryptic soy agar (DIFCO) plates were coated with standard suspension (10 cells/ml) of the bacterial isolates using sterile cotton swabs. The test materials and antibiotic discs were than placed on the inoculated plates with sterile needle. The plates were then kept in the incubator at 37^{o}C for 24 hours.

Results and Discussion

Infectious diseases afflict around three billion people of less developed countries (Walsh and Warren, 1979). The currently used antibiotics (Such as ampicillin, chloramphenicol, co-trimoxazole) are becoming insensitive to these infections (Ahsan, 1989) which has resulted in the treatment of the infectious diseases quite difficult. Hence it is of utmost importance to look for newer effective antimicrobial agent. Attempt has been initiated to study the antibacterial activity of clove extracts.

Forty bacterial isolates which includes eleven common pathogenic strains and twenty nine multiple drug resistant isolates of *Shigella dysenteriae* type, 1, *Shigella flexneri* and *Vibrio cholerae* strains, were tested *in* vitro for their sensitivity to the petroleum ether, chloroform and ethanol extracts of clove (Table 1 & 2). The activity of the extracts was compared with that of ampicillin, tetracycline, nalidixic acide and gentamicin. Almost all of the isolates tested except *Pseudomonas aemginosa,* showed promising sensitivity to each of the extracts (2 mg/disc). Against the strains of *Shigella* and *Vibrio cholerae,* petroleum ether extract gave 12-28 mm zone of inhibition followed by chloroform extract, 10-22 mm and ethanol extract, 9-21 mm (Table 2); but against the common pathogens relatively smaller zone of inhibition was observed (Table 1). On the contrary, most of the isolates of *Shigella dysenteriae* type 1 were found resistant to ampicillin. Six isolates of *Shigella dysentenae* type 1 were resistant to nalidixic acid, the drug of choice in *Shigella* infections. *Escherichia coli* (ETEC 144.2) showed resistance to tetracycline. Among the antibiotics, only gentamicin was effective against all isolates of *Shigella* and *Vibrio cholerae,* but this antibiotic cannot be used freely owing to its toxicity, especially for ototoxicity and nephrotoxicity (Sande and Mandell, 1985).

Recently activities of essential oil of clove on various strains of bacteria and fungus

have been reported (Briozzso *et al.,* 1989). Ethanol and water extract of clove have also anti- bacterial properties (Watanabe *et al,* 1985). Ueda *et al*, (1982) reported antimicrobial effect of ethanol extract of clove.

Table-1

Antibacterial activitiy of clove extracts against pathogenic bacteria. The values in the table indicate zone of inhibition in mm.

Bacteria	Strain No.	P-ext.	C-ext.	E-ext.	TC
		2 μg/disc		30 μg/disc	
		Zone of inhibition in mm			
Bacillus subtilius	QL40	20	16	17	19
Sarcine lutea	QL166	18	17	21	32
Staphylococcus aureus	QL102	10	9	13	20
Streptococcus feaecalis	ATCC	21	18	17	22
Streptococcus B-hemolyticus	CRL	14	11	14	12
Aeromonas hydrophilia	5328	19	15	17	25
Escherichia coli (ETEC)	144.2	15	12	17	--
Klebsiella sp.	BTCC13	14	13	15	22
Proteus vulgaris	QL144	15	14	17	23
Pseudomonas aeruginosa	QL147	--	--	9	10
Salmonella typhi	CRL	20	18	21	16

P-ext. = Petroleum ether extract
C-ext. = Chloroform extract
E-ext. = Ethanol extract
- = No inhibition
TC = Tetracyline

Table-2
Anti-Shigella and anti-Vibrio activity of clove extracts.
The values indicate zone of inhibition in mm.

Bacteria	Strain No.	P-ext.	C-ext.	E-ext.	AM 10	NA 30	GM 10
			2 μg/disc			μg/disc	
			Zone of inhibition in mm				
	115250	25	15	17	--	9	13
	14731	24	14	18	--	26	13
	114835	28	21	15	--	24	16
	115166	22	15	14	--	25	14
	115173	20	11	14	--	--	13
	5563	19	20	21	--	--	14
	5372	22	18	21	--	--	12
Shigella	5202	23	20	21	--	--	14
dysenteriae	5544	19	18	18	--	9	11
type 1	TL-2236	15	14	13	--	16	23
	TL-2248	20	13	12	--	22	28
	TL-2176	12	11	11	--	17	25
	TL-2156	15	14	12	--	15	25
	TL-2155	13	14	12	--	20	24
	TL-2149	12	10	9	--	13	32
	TL-2124	19	18	15	--	--	33
	114830	21	15	14	23	25	14
	115253	21	12	12	21	17	11
	115160	--	--	13	24	25	15
	2701	22	15	12	--	22	12
Shigella	5175	22	17	18	--	22	14
flexneri	5126	18	16	17	22	19	11
	5337	21	22	--	--	26	15
	A = 18R	20	12	14	--	22	12
	SH-4	19	16	17	24	16	12
	5256	23	27	21	--	18	14
	5219	27	25	24	--	20	15
Vibrio	5245	25	24	23	--	18	14
cholerae	5095	23	24	23	--	19	15

P-ext. = petroleum ether extract A
C-ext. = chloroform extract
E-ext. = Ethanol extract

M = Ampicillin
NA = Nalidixic acid
GM = Gentamicin

The present work is an elaborate one, involving antibacterial activity of petroleum ether, chloroform and ethanol extract of clove against various bacterial strains, especially those against multiple drug resistant strains of *Shigella* and *V. cholerae* This study revealed that clove would be a good antibacterial drug in the treatment of infectious diseases, provided if it is found effective and nontoxic in *in viva* study. It is important to isolate the active antibacterial constituent(s) of clove. This is the first report on the clove for its antibacterial activity against the strains of *Shigella* and *Vibrio cholerae,* especially against multiple drug resistant isolates.

Acknowledgement

We are grateful to Dr. Zia Uddin Ahmed of International Centre for Diarrhoeal Disease Research, Bangladesh, for providing the isolates of *Shigella* and *Vibrio cholerae* strains.

References

Ahsan, M. (1989), M. Phil. Thesis, Department of Pharmacy, University of Dhaka, P.4.

Bauer, A.W., Kirby, W.M., Sherris, J.C., and Turek, M. (1966), *Amer. J. Clin. Path.* **45**: 493.

Briozzo, J., Nunez, L., Chirife, J, Herszage, L. and Dequino, M. (1989), *J. Appl. Bacteriol*. **66**(1): 69.

Chopra, R.N., Chopra, I.C., Handa, K.L. and Kapur, L.D. (1982), Indigenous Drugs of India, Academic Publishers, New Delhi, p. 172.

Sande, M.A. and Mandell, G.L. (1985). The Pharmacological Basis of Therapeutics, Ed. Goodman, L.S. and Gilman, A., The Macmillan Company. New York, p.1161.

Tyler, V.E., Brady, L.R. and Robbers, J.E. (1988). Pharmacognosy, Lea and Febiger, Philadelphia, p.131.

Ueda, S., Yamashita, H., Nakajima, Y. and Kuwabara, Y. (1982). Nippon Shokuhin Kogyo Gakkaishi, **29**(2): 111.

Walsh, J.A. and Warren, K.S. (1979). *N. Engl. J. Med*. **301**: 967.

Watanabe, H., Bagiawati, S. and Tamura, N. (1985). *Shokuhin Shosha*, **20**(1-2): 27.