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ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF *ALPINIA CONCHIGERA* GRIFF (FAMILY: ZINGIBERACEAE)

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Abstract. The antibacterial activity of the methanol extract of Alpinia conchigera Griff was evaluated by the disc diffusion method against eleven pathogenic bacteria using ciprofloxacin as standards. In the screening, the methanol extract of Alpinia conchigera Griff showed varying degrees of antibacterial activitiy with zone of inhibition ranging from 8.0-24.5 mm, while the highest antibacterial activity was seen against with Vibrio cholerae, Staphylococcus aureus, Salmonella typhi and Salmonella paratyphi.The Minimum Inhibitory Concentrations of the methanol extracts was found to be 31.25-125 µg/mL for bacteria species used in the screening.

Key words. Alpinia Conchigera Griff, Zingiberaceae, MIC, bacterial cultures, methanol extraction.

Introduction. The Zingiberaceae plant, Alpinia conchigera Griff. (Bengali name: Khetranga), is widely cultivated in China, India, Bangladesh (mainly found in hilly areas like in Chittagong and Sylhet) and Southeast Asian country such as Thailand, Indonesia, and Malaysia and this plant consists of aromatic perennial herbs with creeping horizontal or tuberous rhizomes [1]. A. conchigera rhizome oil contained chavicol, chavicol acetate, 1'-acetoxychavicol acetate, eugenol, terpenoids, essential oils, β-bisabolene, 1,8-cineole, β-caryophyllene and cardamomin and used as folk medicine and fungal infection [2, 3] as well as gastrointestinal disorders and preparation of Thai food dishes [4, 5]. The importance of Alpinia conchigera Griff. is in gastric pain, diarrhoea and dysentery in the southeast region of Bangladesh [6]. They are traditionally used as flavoring and medicine such as a tonic, piles, stomachic, febrifuge, cough and mixture for after childbirth. These traditional uses may be explained by the presence of biologically active volatile constituents [7]. The objective of this study is to investigate the antibacterial properties Alpinia conchigera Griff. According to literature review, less research findings of the selected medicinal plant to till date. But further studies are required to explore the medicinal effects of methanol extracts of the above mentioned plant species.

Material and methods. ***Collection of plant materials.***The plants selected for present work *A. conchigera* (Family: Zingiberaceae) were collected from Naramuk, Rajsthali of Rangamati district. After collection, suitable herbarium sheet for each plant with some general information were prepared and send to Bangladesh Council of Scientific and Industrial Research (BCSIR), Baluchara, Chittagong for identification. They provided us the scientific name of the plants. ***Extraction.***The collected plant (leaves and stems) was separated from undesirable materials or plants or plant parts and was shed-dried (35-50°C). The plant was ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until extraction commenced. About 185 g of powdered plant material of *A. conchigera* (Family: Zingiberaceae) was taken in a clean, flat bottomed amber glass container and soaked in1700mL of methanol. The container with its contents was sealed and kept for a period of 10 days accompanied by continuous shaking. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton materials. Then they were filtered by using Whatman filter paper number 1 and the solvent was made to evaporate under the room temperature. The residues were stored in a refrigerator until further studies. ***Bacterial Cultures.*** To investigate the antibacterial activity both the gram (+) and gram (-) species were selected. For the evaluation, the Gm (+) and Gm (-) of clinical isolates: *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysentariae, vibrio cholera, Shigella sonnei and Salmonella paratyphi* were selected respectively. All the clinical species were supplied by the Faculty of Microbiology, Chittagong University, Chittagong, Bangladesh. All the test strains were maintained on nutrient agar slopes and were sub-cultured. These bacteria served as test pathogens for antibacterial activity assay. ***Sterilization.*** Antibacterial screening was done in laminar hood maintaining all precautions required to avoid any contamination derives the test. UV light was kept switch on before half an hour working in laminar hood to avoid any accidental contamination. Petridishes and other glass wares sterilized by autoclaving at a temperature of 121°C and a pressure of 15 Ibs/sq.inch for 15 minutes. Blank discs were first kept in a covered petridish and subjected to dry heat sterilization at 180°C for 1 hour. Then they were kept in a laminar hood under UV light for 30 minutes. ***Culture media preparation.*** The nutrient agar medium (HiMedia Laboratories Pvt. Ltd. India) is used to demonstrate the antibacterial activity and to make subculture of the test organism. The nutrient agar medium contains Beef extract (1.50 g), Peptic digest of animal tissue (5.0 g), Sodium chloride(5.0 g), Yeast extract (1.50 g), agar (15 g), distilled water (q.s to 1000 mL).[*28.0 g is recommended for 1000 mL distilled water].* ***Drugs and Chemicals used for Anti-Bacterial Assay.*** Ciprofloxacin antibiotics (500 µg/disc) and Di-methyl Sulphoxide (DMSO) as solvent. ***Antibacterial Activity.*** Disc diffusion method [8] was used to test the antibacterial activity of the extractives against eleven bacteria. Dried and sterilized filter paper discs (6mm diameter) were then impregnated with known amount of the test substances dissolved in methanol (40µg/mL) using micropipette and the residual solvents were completely evaporated. Discs containing the test material (250µg/disc and 500 µg/disc) were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of Ciprofloxacin (500µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4^0C) for 24 hours to allow maximum diffusion of test samples. The plates were then incubated at 37^0C for 24 hours to allow maximum growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition in millimeter [9]. The experiment was carried out in triplicate and the average zone of inhibition was calculated. ***Minimum Inhibitory Concentration.*** Lowest dose of an antibiotic that dose the inhibition of the growth of a particular no (s) organism (s) upon which it acts is called the 'Minimal Inhibitory Concentration' (MIC). The present study protocol was designed and performed due to the fact that the MEAC are found as a positive antimicrobial agent and the extract acts preciously on a number eleven of gram positive and Gram negative bacteria species are made to test. For MIC test the 'Serial tube dilution technique [10] is used. ***Clinical pathogenic species.*** The above mentioned 11 species of bacteria were screened for minimum inhibitory concentration of the crude methanolic extract of *Alpinia conchigera* Griff. ***Sterilization Technique.*** Test tubes containing Nutrient broth (for bacteria), media and other glass wares were sterilized by autoclaving at a temperature of 121° C and a pressure of 15Ibs/sq.inch for 15 minutes. Then they were kept in a laminar hood under UV light for 30 minutes. ***Culture media preparation.*** The nutrient broth medium (HiMedia Laboratories Pvt. Ltd. India) is used to demonstrate the antibacterial activity and to make subculture of the test organism. The nutrient broth medium contains Beef extract (50 g), Peptic digest of animal tissue (5.0 g), Sodium chloride(1.50 g), Yeast extract (1.50 g), distilled water (q.s to 1000 mL).[*13.0 g is recommended for 1000 mL distilled water].* ***Assay Technique.*** Ten screw cap test tubes were taken and serially marked 1,2,3,4,5,6 and 7 for sample solutions and the rest three T_M for medium, T_{MI} for medium and inoculums and T_{MS} for medium and solvent respectively. 1 mL of nutrient broth medium were taken in all test tubes and sterilized in an autoclave at a temperature of 121°c and a pressure of 15Ibs/sq. inch for 30minutes. After 30 minutes, 1 mL of 1000µg sample was added to the no-1 marked tube and the tube was shaken gently for proper mixing of the content. 1 mL of the content form the 1st tube was added to the no-2 marked tube that action

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Tested bacteria	Determination of Zone of inhibition (mm)			
	MEAC		S	C
	A	B	500µg/disc	-
	250µg/disc	500µg/disc		
Gram Positive Species				
Bacillus subtilis	11	20	24	-
Bacillus megaterium	14	21	25	-
Bacillus cereus	11	23	28	-
Staphylococcus aureus	13	24	27.5	-
Gram Negative Species				
Pseudomonas aeruginosa	12	23	31	-
Escherichia coli	10	22	28	-
Shigella dysentariae	12.5	23.5	32	-
Shigella sonnei	11.5	23	27	-
Salmonella typhi	9	20	23.5	-
Vibrio cholerae	13.5	24.5	24	-
Salmonella paratyphi	8	22	26	-

Table 1. Antibacterial activity of the crude extract of MEAC, standard and blank. MEAC = Methanol extract of Alpinia conchigera Griff. A = 250µg/disc, B = 500µg/disc, S = Standard (ciprofloxacin) and C = Control.

Marked test tubes	Medium added (mL)	Sample solution (µg/mL)	Inoculums added (µl)	Growth of clinical pathogens										
				Gram Positive Species					Gram Negative Species					
				Bacillus subtilis	Bacillus megaterium	Bacillus	Bacillus cereus	Staphylococcus aureus	Pseudomonas aeruginosa	Escherichia coli	Shigella dysentariae	Shigella sonnei	Salmonella typhi	Vibrio cholerae	Salmonella paratyphi
1	1	500	10	-	-	-	-	-	-	-	-	-	-	-	-
2	1	250	10	-	-	-	-	-	-	-	-	-	-	-	-
3	1	125	10	-	-	-	-	-	-	-	-	-	-	-	-
4	1	62.5	10	+	+	+	-	+	+	+	+	-	-	+	
5	1	31.25	10	+	+	+	+	+	+	+	+	+	+	+	
6	1	15.625	10	+	+	+	+	+	+	+	+	+	+	+	
7	1	7.8125	10	+	+	+	+	+	+	+	+	+	+	+	
TMI	1	0	10	+	+	+	+	+	+	+	+	+	+	+	
TMS	1	0	10	-	-	-	-	-	-	-	-	-	-	-	-
TM	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-

Table 2. Minimum inhibitory concentration of the MEAC against the clinical pathogenic bacteria. [MIC = Minimal inhibitory concentration, MEAC = Methanol extract of Alpinia conchigera, T_{MI} = Test tube containing medium and inoculum, T_{MS} = Test tube containing medium and solvent, T_M = Test tube containing medium, (+) = Growth and (-) = No growth]

was performed upto the no.7 marked tubes, after proper mixing 1 mL content from the 7 marked tube was discarded. 10µl of specified bacterial suspension of the clinical pathogen were added to the 1 to 7 and T_{MI} marked tubes by a suitable micropipette (10-100µl). For T_{MS} only 1mL ethanol was added, after shaking 1mL of the mixture was discarded from the tube. T_M contain only 1mL medium. All the test tubes were subjected for incubation at 37°C for 18 hours.

Result and Discussion. ***Determination of Antibacterial Activity.*** The antibacterial activity of the crude extracts were evaluated by the disc diffusion method against 4 gram positive and 7 gram negative pathogenic bacteria using ciprofloxacin as standards. In the screening, the methanol extract of *Alpinia conchigera* Griff. showed 11.14, 11, 13 mm at 250 µg/disc and 20, 21, 23, 24 mm at 500 µg/disc zone of inhibition against four tested Gram (+)ve bacteria like *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium* and *Staphylococcus aureus* respectively and the zone of inhibition was observed against six tested Gram (-)ve bacteria 12, 10, 12.5, 11.5, 9, 13.5, 8 mm at 250 µg/disc and 23, 22, 23.5, 23, 20, 24.5, 22 mm at 500 µg/disc like *Pseudomonas aeruginosa, Escherichia coli, Shigella dysentariae , Shigella sonnei, Salmonella typhi*, *Vibrio cholerae*, *Salmonella paratyphi* respectively. On the other hand, standard antibiotic Ci[rofloxacin (500µg/disc) showed significant antibacterial activity against all tested Gram (+)ve and Gram (-)ve bacteria. ***Determination of Minimum Inhibitory Concentration.*** From the table 1, it was depicted that methanol extract of *Alpinia conchigera* inhibited the growth of *Salmonella typhi, Escherichia coli , Bacillus cereus, Bacillus subtilis.* significantly at the dose of 31.25µg/mL then followed by *Staphylococcus aureus* by

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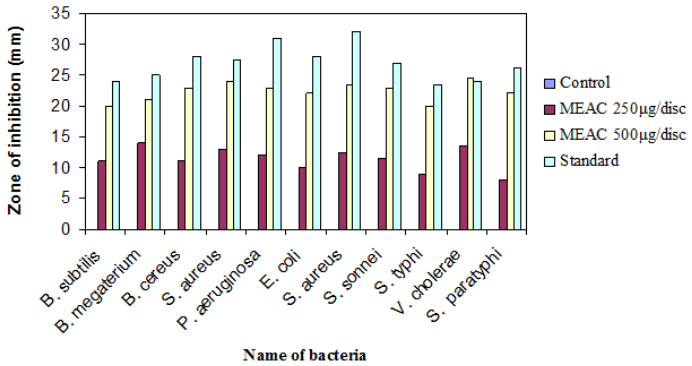

Figure 1. Graphical representation of antibacterial activity of methanol extract of Alpinia conchigera Griff.compared with standard antibiotic and control.

62.50µg/mL, *Bacillus subtilis, Bacillus megaterium, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli, Shigella dysentariae, Shigella sonnei, Salmonella paratyphi* by 125µg/mL.

Conclusions. In the screening, the methanol extract of *Alpinia conchigera* Griff. showed varying degrees of antibacterial activities with zone of inhibition ranging from 8.0-24.5 mm, while the highest antibacterial activity was seen against with *Vibrio cholerae, Staphylococcus aureus, Salmonella typhi and Salmonella paratyphi.* The Minimum Inhibitory Concentrations of the methanol extracts was found to be 31.25-125 µg/mL for bacteria species used in the screening.

References.

1. Kai Larsen, K. 1980. Annotated key to the genera of Zingiberaceae of Thailand. Nat. Hist. Bull.Siam Soc. 28: 151-169.

2. Ghani A. 1998. Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd edition.pp.4-19 Asiatic Society of Bangladesh, Dhaka.

3. E.W.C. Chan, Y.Y.Lim, S.K.Ling, S.P. Tan, K.K. Lim and M.G.H. 2009. Khoo *Caffeoylquinic acids from leaves of Etlingera species (Zingiberaceae).* LWT - Food Science and Technology, Volume 42, Issue 5, Pages 1026-1030.

4. Baby Sabulal, Mathew Dan, Anil John J, Rajani Kurup, Nediyamparambu Sukumaran Pradeep, Renju Krishna Valsamma and Varughese George. 2006. *Caryophyllene-rich rhizome oil of Zingiber nimmonii from South India: Chemical characterization and antimicrobial activity.* Phytochemistry,Volume 67, Issue 22, Pages 2469-2473.

5. K. C. Wong, K. S. Ong, C. L. Lim. 2006. *Compositon of the essential oil of rhizomes of Kaempferia galanga L*. Flavour and Fragrance Journal, Volume 7, Pages 263-266.

6. Yusuf, M.A. Wahab, M.Y., Chowdhury, J.U. and Begum, J. 2007. Some tribal medicinal plants of Chittagong Hill Tracts, Bangladesh. Bangladesh J. Plant Taxon., 14, 117-128.

7. M.A. Sukari, N.W. Mohd Sharif, A.L.C. Yap, S.W. Tang, B.K. Neoh, M. Rahmani, G.C.L. Ee, Y.H. Taufiq-Yap and U.K. Yusof .2008. *Chemical constituents variations of essential oils from rhizomes of four zingiberaceae species*. The Malaysian Journal of Analytical Sciences, Vol 12, No 3: 638 – 644.

8. Bauer A.W., Kibry W. M. M., Sheries J. C., Turek M. 1951. Antibiotic Succeptibility Testing by a Standard Single Disc Method, Am. J. Sci., 1, 103, 195.

9. Roland R. 1982.Antibiotics: An Introduction, F. Hoffmann La Roache and Co. Basle, Switzerland, pp. 70-71.

10. Andrews JM. 2001. Determination of Minimum Inhibitory Concentrations, J. Antimicrobial Chemosphere, 48:5-16.