Bangladesh J Microbiol, Volume 25, Number 1, June 2008, pp 82-84

Short Communication

Antibacterial and Cytotoxic Activity of Longiverbenone Isolated from the Rhizome of *Cyperus scariosu*

M Shafiqur Rahman* and M Nural Anwar

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

[Received 01 April 2008; Accepted 14 June 2008]

Longiverbenone is a naturally occurring sesquiterpene isolated from ethanolic extract of *Cyperus scariosus* rhizome by solvent-solvent portioning and chromatographic technique. The antibacterial activity of longiverbenone was evaluated against eleven potential human pathogenic bacteria using disc diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth macrodilution method. Cytotoxic activity (lethal concentration 50%, LC_{50}) of longiverbenone was determined on new borne brine shrimp (*Artemia salina*). Longiverbenone showed moderate to good antibacterial activity against the test organisms tested herein. It exhibited the lowest MIC (20 µg/ml) and MBC (80 µg/ml) against *Vibrio cholerae*. The LC_{50} of the isolated sesquiterpene was found to be 14.38 µg/ml against new borne brine shrimp.

Keywords: Antibacterial activity, Cytotoxic activity (LC_{50}), *Cyperus scariosu*, Longiverbenone, Minimum inhibitory concentration (MIC)

Plants are still widely used in ethnomedicine around the world. Among the different plant-derived secondary metabolites proved to be the most important group of compounds that showed wide range of antimicrobial activity[1-2]. Microorganisms have developed resistance against many antibiotics and this has created immense clinical problems in the treatment of infectious diseases[3]. This situation forced scientists to search for new antimicrobial substances from various sources, such as medicinal plants. So, there is a continuing need for new antimicrobial agents since none of the available drugs is free from adverse effects and limitation. So, intensive antibacterial and phytochemical investigation is needed in this field.

Cyperus scariosus Br. is a small grass-like herb with angular soft stem and underground rhizomatous tubers and belongs to the family of Cyperaceae. This herb grows plentifully and almost everywhere in Bangladesh as noxious weeds. In tribal areas of Bangladesh, rhizome of *C. scariosus* is widely used as a phytotherapeutic agent against dysentery. The rhizome of this plant contains an amber or light brown viscous essential oil and the extract of the tuber is used as a remedy for fever, diarrhoea, dysentery, cholera and vomiting[4]. The aim of the present work is to evaluate the antimicrobial activity of a pure compound from *C. scariosus* on several pathogenic bacteria, which can cause diseases in man.

Rhizomes of *C. scariosus* were collected from a local garden owned by the Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Chittagong, in December 2004 and it was identified by Dr. M Yousuf of BCSIR, where a voucher specimen (No. 1564a) has been maintained. The collected sample was cleaned, cut into small (1-2 cm) pieces, dried at 50-55°C, pulverized and then 250 g of the dried powder was kept steeped twice overnight in ethanol. The extract thus obtained was filtered, centrifuged at 5,000 rpm for 20 min and then concentrated to a gummy material (12.5 g; 5% w/w) under reduced pressure at 50°C by rotary vacuum evaporator.

Solvent-solvent partitioning of concentrated crude extract was done using the protocol designed by Kupchan and modified by Wagenen[5]. All the fractions were collected separately, dried as usual and tested for any of their antimicrobial efficacy. Of these, only the crude petroleum ether extract fraction showing high antimicrobial activity was subjected to fractionation by column chromatography using silica gel (200-300 mesh) as the adsorbent. From it a total of 24 fractions were collected separately using mixtures containing different proportions of petroleum ether and ethyl acetate with increasing polarity as the eluants. The column-separated fractions thus obtained were examined using thin-layer chromatography (TLC) technique and ultraviolet light (254 and 366 nm) to detect the presence of any fluorescent compound. The R_f-values for each were measured as usual. Fractions showing same R_f-value were mixed together and grouped into eight fractions (C_1-C_8).

All the fractions (C_1-C_8) were tested against a highly sensitive bacterium (*Bacillus megaterium*) and a fungus (*Aspergillus niger*). Of these, the highly antibacterial and antifungal fraction C_1, eluted in petroleum ether, yielded 2.56 g (1.024% w/w) of greenish

***Corresponding author:**
Dr. M Shafiqur Rahman, Associate Professor & Chairman, Department of Microbiology, University of Chittagong, Chittagong 4331, Bangladesh
Tel (Office): (031) 726311-14, Ext 4464; Cell: 01711 959996; Fax: +880 (031) 726310; E-mail: rahmanms68@yahoo.com

brown oil. This oil, on various spectroscopic (*e.g.*, IR, NMR and GCMS) analyses and on comparison of such analytical results with NYST Library data, was identified as longiverbenone, which is a sesquiterpene. NMR and GSMS were performed on respectively a Bruker spectrospin spectrometer and a GC-17A Ver. 3 gas chromatography coupled to a Shimadzu mass spectrometer (Model QP 5050A).

The *in vitro* antibacterial activities of the column separated fractions and of the chemically isolated pure compound longiverbenone of the plant were determined against eleven potential human pathogenic bacteria by disc diffusion method[6]. using Mueller-Hinton (agar and broth) medium. All the results were compared with the standard antibacterial antibiotic ampicillin (20 µg/disc). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by macrodilution broth technique[7]. The LC_{50} test was performed on new born brine shrimp (*Artemia salina*) according to an established method[8]. The median lethal concentration 50% (LC_{50}) of the test compound (1.5625 to 200 µg/ml water) was calculated by trend line fit linear regression analysis of the experimentally obtained data.

All the experimentally observed spectroscopic data of fraction C_1 were found to resemble very closely to the presence of the compound longiverbenone (a sesquiterpene). The compound was obtained as greenish brown oil. Its molecular weight and chemical formula were found to be 218 and $C_{15}H_{22}O$ respectively.

Different concentrations of longiverbenone were screened for their antibacterial activity against the test organisms and the results are summarized in Table 1. It appeared that the compound gave moderate to good antibacterial activity against all the test bacteria except *P. aeruginisa* and *S. aureus*. The largest zone of inhibitions 30, 22, 15 and 8 mm in diameter were recorded against *Vibrio cohlearae* at the concentration of 160, 80, 40 and 20 µg/disc respectively. Standard antibacterial antibiotic ampicillin (20 µg/disc) was also found to be active against all the bacteria tested herein except *P. aeruginosa*.

Table 1. *Antibacterial activity of longiverbenone isolated from rhizome of Cyperus scariosus*

Test organism	Diameter of zone of inhibition (mm)					
	Longiverbenone (µg/disc)					Ampicillin (20 µg/disc)
	160	80	40	20	10	
Bacillus subtilis	25	14	7	0	0	19
Bacillus cereus	26	17	12	7	0	18
Bacillus megaterium	25	14	7	0	0	16
Staphylococcus aureus	0	0	0	0	0	22
Escherichia coli	15	10	0	0	0	10
Vibrio cholerae	30	22	15	8	0	15
Shigella dysenteriae	24	13	8	0	0	22
Shigella sonnei	10	6	0	0	0	20
Salmonella typhi	15	8	0	0	0	20
Salmonella paratyphi	12	10	6	0	0	17
Pseudomonas aeruginosa	0	0	0	0	0	0

The MIC and MBC values of the sesquiterpene of *C. scariosus* rhizome are presented in Table 2. The MIC values of the compound longiverbenone were 20 µg/ml against *V. cholerae*, 40 µg/ml against *Bacillus subtilis*, *B. cereus*, *B. megaterium* and *S. dysenteriae*, 80 µg/ml against *E. coli* and *S. paratyphi*, and 160 µg/ml against *S. sonnei* and *S. typhi*. *S. aureus* was found to resistant to the agent showing growth in the presence of the highest concentration used (320 µg/ml). The MBC values of the agent varied between 80 and 320 µg/ml in case of eight organisms with the lowest (80 µg/ml) against *V. cholerae*. Similar antibacterial activity of plant essential oils and sesquiterpenes has been reported previously[9-10].

Table 2. *Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of longiverbenone isolated from rhizome of Cyperus scariosus against eleven pathogenic bacteria*

Test organism	MIC value (µg/ml)	MBC value (µg/ml)
Bacillus subtilis	40	160
Bacillus cereus	40	160
Bacillus megaterium	40	320
Staphylococcus aureus	NF	NF
Escherichia coli	80	320
Vibrio cholerae	20	80
Shigella dysenteriae	40	160
Shigella sonnei	160	NF
Salmonella typhi	160	320
Salmonella paratyphi	80	320
Pseudomonas aeruginosa	320	NF

NF= Not found.

The cytotoxic activity (LC_{50}) of the compound longiverbenone new born brine shrimp (*Artemia salina*) is presented in Table 3. The LC_{50} of the compound against the brine shrimp was found to be 14.38 µg/ml. The cytotoxic bioassay result of longiverbenone may lead to the exploration of its potential and practical application as a novel less toxic and antimicrobial compound from this plant. Similar cytotoxic activities of plant constituents have been reported previously[11].

Table 3. *Cytotoxic activity (LC_{50}) of longiverbenone on brine shrimp (Artemia salina)*[a]

Longiverbenone (µg/ml)	Log of concentration	Mortality after 24 h (%)		LC_{50} (µg/ml)
		Longiverbenone	Control	
1.5625	0.1938	0		
3.125	0.4949	10		
6.25	0.7959	30		
12.5	1.0969	40	0	14.38
25	1.3979	70		
50	1.6960	90		
100	2.0000	100		
200	2.3010	100		

LC_{50} = Lethal concentration; [a]New born (1-5 h) brine shrimp taken for each case was 10.

Research on natural resources has been encouraged by the World Health Organization (WHO) since last three decades. It is evident from this study that longiverbenone isolated from *C. scariosus* rhizome exhibited moderate to good antibacterial activity against the organisms tested herein. Our results could stimulate further pharmacological studies seeking new antimicrobial agents from the plant resources.

Acknowledgement

The authors wish to thank to Dr. Jaripa Begum, Mr. Jasim Uddin Chowdhury and Dr. M Yousuf, Bangladesh Council for Scientific and Industrial Research (BCSIR), Chittagong, Bangladesh for supplying the samples, spectroscopic recording and analysis of the isolated compound.

References

1. Raman MS, Anwar MN & Chowdhury AZMS. 1999. Antibacterial activity of secondary metabolites from *Holarrhena antidysenterica* stem bark. *Bangladesh J Microbiol*. **16**(2): 101-105.
2. Sarkar SD, Muniruzzaman S & Khan SI. 1991. Antimicrobial activity of *Piper chaba* Hunter (Chui). *Bangladesh J Bot.* **20**(2): 179-182.
3. Davis J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. *Science*. **264**: 375-382.
4. Ghani A. 1998. *Medicinal Plants of Bangladesh: Chemical Constituents and Uses*, 1st edn, pp 195-196. Asiatic Society of Bangladesh, Dhaka.
5. Wagenen BC, Larsen RJS, Cardellina HD, Randazzo ZCI & Justisatienr A. 1933. Ulo santion, a potent insecticide from the sponge *Ulosa ruetleri*. *Organic Chem*. **58**: 335-337.
6. Bauer AW, Kirby MM, Sherris JC & Turck M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Path*. **45**: 493-496.
7. Jones NR, Barry LA, Gavan LT & Washington JA. 1985. *Manual of Clinical Microbiology*, 4th edn, pp 972-976. American Society for Microbiology, Washington DC.
8. Meyer BN, Ferrigni NR, Putman JE, Jacobson LB, Bichols DE & McLaughlin JL. 1982. Brine shrimp: A convenient bioassay for active constituents. *Planta Med*. **45**: 31-34.
9. Aureli P, Costantini A & Zolea S. 1992. Antimicrobial activity of some plant essential oils against *Listeria monocytogenes*. *J Food Product.* **55**: 344-348.
10. Habtemariam S, Gray AI & Waterman PG. 1993. A new antibacterial sesquiterpene from *Premna oligotricha*. *J Natural Product*. **56**: 140-143.
11. Chowdhury AA, Khan OF, Rahman MT, Islam MS. 2003. Cytotoxic and antitumor activity of *Trema orientalis*. *Dhaka Univ J Pharma Sci*. **1**(1-2): 55-58.