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Antibacterial Effect of Hexane Extract of Sea Urchin, *Temnopleurus alexandri (* Bell,1884)

B.Uma* and R.Parvathavarthini

Department of Zoology, Bharathi women's College, Chennai : 600108, India

Corres.author: umabwc@gmail.com,Phone : 9444905087

Abstract: The present study elucidates that hexane extract of the sea urchin, *Temnopleurus alexandri* has an antibacterial activity. Of the gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* MTCC 441, *Enterococcus faecalis* ATCC 29212) and gram-negative (*Escherichia coli* ATCC 25922,*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 15380,*Proteus vulgaris* MTCC 1771) bacteria tested , hexane extract showed antibacterial activity for all the bacteria tested except *K.pneumoniae*. Various concentrations of hexane extract (5,20,200,2000 and 5000ppm) were tested. Streptomycin and ampicillin were used as positive controls. Lowest (2.5ppm) MIC was noted for *B. subtilis* and *P. aeruginosa* . GC-MS analysis revealed the presence of Pentadecane, Heptadecane, Eicosane, Heneicosane, Docosane as major compounds in the extract. This study shows that hexane extract is a potent antibacterial agent and needs further purification for the specific compound, which is responsible for the said activity.
Key words : Hexane extract – antibacterial activity – GC-MS analysis - *Temnopleurus alexandri.*

Introduction

Marine organisms represent excellent source for bioactive compounds[1] (Bickmeyer *et al*., 2005). Bioactive chemical compounds can be classified as primary metabolites and secondary metabolites, depending on its biosynthetic origin, biochemical role and general occurrence. The secondary metabolites have various functions ,it is likely that some of them may be pharmacologically active on humans and useful as medicines[2] (Briskin, 2000). A majority of pharmacologically active secondary metabolites have been isolated from echinoderms [3](Carballeria *et al*., 1996). Echinoderms seem to have secondary metabolites which are antimicrobial in nature [4,5] . The present study is aimed at assessing the antibacterial activity of the hexane extract from the sea urchin, *T.alexandri.*

Materials and Methods

Collection of animals

Sea urchin, Temnopleurus alexandri (Bell,1884) were collected from fish landing centre, Chennai coast. Authentication of the echinoid was done with Zoological Survey of India (ZSI), Chennai.

Extraction

Shade dried specimens were immersed in Hexane (1:3 w/v) . Hexane extract was obtained by cold percolation and concentrated under reduced pressure using rotary evaporator at 40º C. Finally crude extract was obtained. The crude extract was stored at 4° C until further use.

Microorganisms

Gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* MTCC 441, *Enterococcus faecalis* ATCC 29212) and gram-negative (*Escherichia coli* ATCC 25922,*Pseudomonas aeruginosa* ATCC 27853,*Klebsiella pneumoniae* ATCC 15380,*Proteus vulgaris* MTCC 1771) bacteria were tested .

Antimicrobial assay

Antimicrobial activity was carried out using disc diffusion method [6] . Petri plates were prepared with 20 ml of sterile Muller Hinton Agar (MHA) (Hi-media) for bacteria. The extract was dissolved in 2%

DMSO. Antibacterial sterile (empty) (Sigma) discs were used to load the extract of the required concentration . The test cultures (bacteria 10 8 CFU/ml) were swabbed on top of the solidified media and allowed to dry for 10 min. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using DMSO . Streptomycin and ampicillin (10µg/ disc) were used as positive controls. The plates were incubated for 24 hr at 37º C for bacteria. Zone of inbition was recorded in millimeters and the experiment was repeated twice.

Minimum Inhibitory Concentration(MIC)

MIC was performed according to the standard reference method[7]. MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate. Three replications were maintained.

Gas Chromatography-Mass spectrometry (GC-MS) Analysis

The crude extract was quantified using gas chromatograph (GCMS-Shimadzu) equipped with a DB-5 ms column (mm inner diameter 0.25 mm, length 30.0m, film thickness 0.25µm) mass spectrometer (ion source 200º C, RI70eV) programmed at 40-650 ºC with a rate of 4 ºC/min. Injector temperature was 280 ºC ; carrier gas was He(20 psi), column flow rate was 1.4ml/min, injection mode –split.

Results

Hexane extract of *T.alexandri* had very good antibacterial activity for many bacteria tested almost on par with ampicillin., except *K.pneumoniae* (Table 1). The Zone of inhibition (in mm) were found to be 16mm for *B.subtilis* and 15mm for both *E.faecalis* and *P.aeruginosa*, 14mm for *P.vulgaris* , 12mm for *S.aureus* and 8 mm for *E.coli* , all at the concentration of 5000ppm of hexane extract. Of all the concentrations tested , 5000 ppm was found to have greater antibacterial activity than the other concentrations (5, 20, 200, 2000 ppm) used . The zone of inhibition was found to increase with increased concentration of the extract. MIC (Table 2) was found to be as low as 2.5 ppm for *B.subtilis* and *P.aeruginosa* , 5 ppm for *P.vulgaris* , 50 ppm for *S.aureus* , 200 ppm for *E.faecalis* and 625 ppm for *E.coli*. Of all the bacteria tested , hexane extract of *T.alexandri* was found to have effective antibacterial activity against *B.subtilis* followed by *P.aeruginosa* and *E.faecalis* . Lesser antibacterial activity against *P.vulgaris* , *S.aureus* and finally *E.coli*. But it does not seem to have any antibacterial activity against *K.pneumoniae.*

GC-MS reavealed the presence of 5 major components for the extract. They were Pentadecane, Heptadecane, Eicosane, Heneicosane, Docosane. (Figure 1).

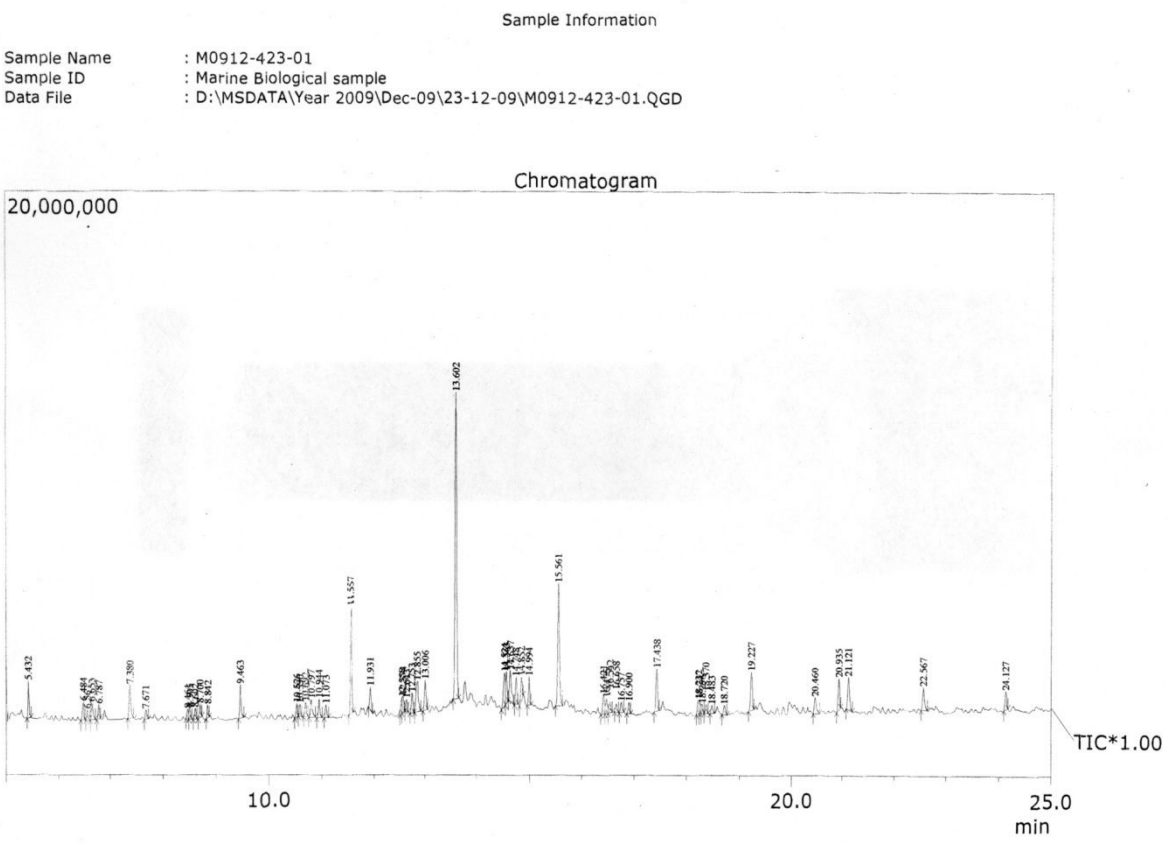
Table 1: Antibacterial activity of hexane extract of *Temnopleurus alexandri*
(zone of inhibition in mm)

Culture	Strep	Amp	DMSO	5ppm	20ppm	200ppm	2000ppm	5000ppm
B.subtilis	32	21	-	-	8	10	12	16
E.faecalis	20	15	-	-	-	10	10	15
E.coli	30	16	-	-	-	-	-	8
K.pneumoniae	30	26	-	-	-	-	-	-
P.vulgaris	31	18	-	-	8	8	14	14
S.aureus	29	16	-	-	-	8	8	12
P.aeruginosa	31	16	-	8	10	12	12	15

Table 2:Minimum Inhibitory concentration (MIC)- of hexane extract of *T.alexandri*

Culture	Minimum concentration observed in ZOI (in ppm)	Range of concentration in MIC plates (in ppm)	Minimum inhibitory concentration (in ppm)
B.Subtilis	20 ppm	20-0.3125	2.5
S.aureus	200ppm	200-3.125	50
E.coli	5000ppm	5000-78.25	625
P.Aeruginosa	5ppm	5-0.0781	2.5
E.faecalis	200ppm	200-3.125	200
P.vulgaris	20ppm	20-3.125	5

Figure 1: GC-MS report of hexane extract of *T.alexandri*



Discussion

The results obtained from the present study revealed antibacerial activity by the hexane extract of *T.alexandri.* Highest activity was observed with the maximum dose of hexane extract and the zone of inhibition was increasing with respect to increasing dose. Echinoderms have already been reported to contain pharmacologically active compounds with respect to antihistaminic , cytotoxicity [8] and antiangiogenicity [9]. The ophuroid *Ophoplocas januarii* from Argentina contained one new antiviral steroidal sulfate [4.] Similarly , Neothyoside is an antifungal triterpene diglycoside from the Gulf of California holothurians Neothyone gibbosa [5].

The major components in the present hexane extract could have been responsible for the antibacterial activity. The biological activities of these major bioactive components in relation to parasitism (Paul *et al*.,2002) , apoptosis (Jae *et al*., 2008) and antimicrobial activity (Liu *et al.*,2010) have already been established , supporting the fact that they might have had the antibacterial activity as well. Since antibacterial agents that possess antibacterial activity are of interest in the field of pharmacology, further fractionation , purification, and identification of the exact bioactive compound present in the present hexane extract is of much importance.

Conclusion

The present study clearly have demonstrated that the hexane extract of *T. alexandri* had antibacterial activity against many bacteria and the major components identified by GC-MS analysis like Pentadecane, Heptadecane, Eicosane, Heneicosane, Docosane could have been responsible for the antibacterial activity of the hexane extract of *T.alexandri.*

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