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Antibacterial Activity of Hydroalcoholic Extract of Sea Urchin *Temnopleurus Alexandri* (Bell, 1884)

KEYWORDS: Sea urchin, crude extract , Antibacterial

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ABSTRACT *The present study eludicates and aimed at the antibacterial activity of hydro alcoholic extract of the sea urchin, Temnopleurus alexandri (Bell, 1884). Sea urchins were collected from Chennai coast , Tamil Nadu, India and identified at ZSI, Chennai. Hydro alcoholic extract was prepared by cold percolation method and its antibacterial activity was tested with different concentrations (20, 200, 2000 and 5000 ppm) with 7 bacterial strains i.e., Bacillus subtilis, Enterococcus faecalis, Pseudomonas aureus, Escheria coli, Klebsiella pneumoniae, Proteus vulgaris and Pseudomonas aeroginosa. GC-MS analysis was done to identify and quantify the composition of the crude extract. Minimal inhibitory concentrations were also found.*

Introduction:
Bioactive compounds are extra-nutritional constituents that naturally occur in small quantities in organism (Kris *et al.*, 2002). Over the years many of such compounds have been discovered through bio-prospective endeavors (Yew *et al*, 2013). The exploration of bioactive compounds from the natural product sources still remains important and continues to be stable source of newly discovered compounds.

The sea is a source of novel organic bioactive molecules that have much importance in medicine, physiology, pharmacology and biochemistry. The marine environment may contain over 80% of World's plant and animal species although marine compounds are under estimated in current pharmacopeias, it is anticipated that aquatic environment will become an invaluable source of novel compounds in future. Bioactive chemical compounds can be classified as primary metabolites and secondary metabolites. The secondary metabolites from these organisms have recently gained importance as a potential bioactive compound. Like many other marine organisms, echinoderms have been, and continue to be, examined as a source of biologically active compounds with biomedical applications (Kelly, 2005). A majority of pharmacologically active secondary metabolites have been isolated from echinoderms. But still echinoderms appear to be a rather untapped source in the pursuit of the identification of new and useful products (Petzelt, 2005).

Materials and Methods:
Collection:
Freshly available *T. alexandri* were collected from fish landing centre, Chennai coast. Authentication of the echinoid was done with Zoological Survey of India (ZSI), Chennai (India). The taxonomic classification of the specimen used is as follows

Phylum	:	Echinodermata
Class	:	Echinoidea
Genus	:	*Temnopleurus*
Species	:	*Alexandri*
Authority	:	Bell,1884

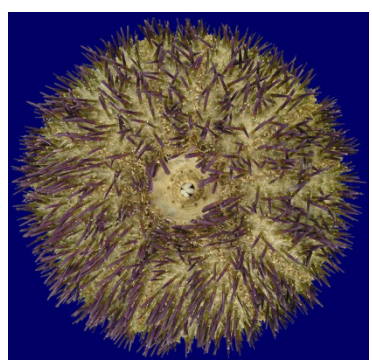

Fig.1: *Temnopleurus alexandri* **(Bell, 1884)**

Extraction:
Extraction was done using cold percolation method. 1 kg of specimen was shade dried at room temperature and ground with a manual mill. The powder (250g) was immersed in 750 ml of alcohol: water (80:20) (1:3 w/v) in an aspirator bottle for a period of 48 hours with occasional shaking. The extract was filtered through a Buchnner funnel with Whatmann No.1 filter paper. The extract was concentrated by removing the ethanol under reduced pressure using rotary evaporator at 40° C and the water was allowed to dry using the water bath. Finally crude extract was obtained. The crude extract was stored at 4° C until further use.

Antimicrobial assay:
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 pneumonia* ATCC 15380, *Proteus vulgaris* MTCC 1771) bacteria were tested. Antimicrobial activity was carried out using well diffusion method (Murray *et al,* 1995 and Olurinola, 1996)Petri plates were prepared with 20 ml of sterile Muller Hinton Agar (MHA) (Himedia). Petri plates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains. Wells were cut and 20ml of the extract was added. Then plates were incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the zone of inhibition formed around the well (NCCLS, 2002). The plates were incubated for 24 hr at 37°C. Zone of inhibition was recorded in millimeters and the experiment was repeated thrice. DMSO was used as

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Negative control. Ampicillin and Streptomycin were used as positive control.

Minimal Inhibitory Concentration (MIC):
MIC was performed according to the standard reference method (Elizabeth *et al.*, 1999). MIC 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 identified and quantified using gas chromatograph (GCMS- Schimadzu) equipped with a DB-5 MS column (mm inner diameter 0.25 mm, length 30.0m, film thickness 0.25µm) mass spectrometer (ion source 200 °C, RI70eV) programmed at 40-650°C with a rate of 4°C/min. Injector temperature was 280°C; carrier gas was Helium (20psi), column flow rate was 1.4ml/min, injection mode – spirit.

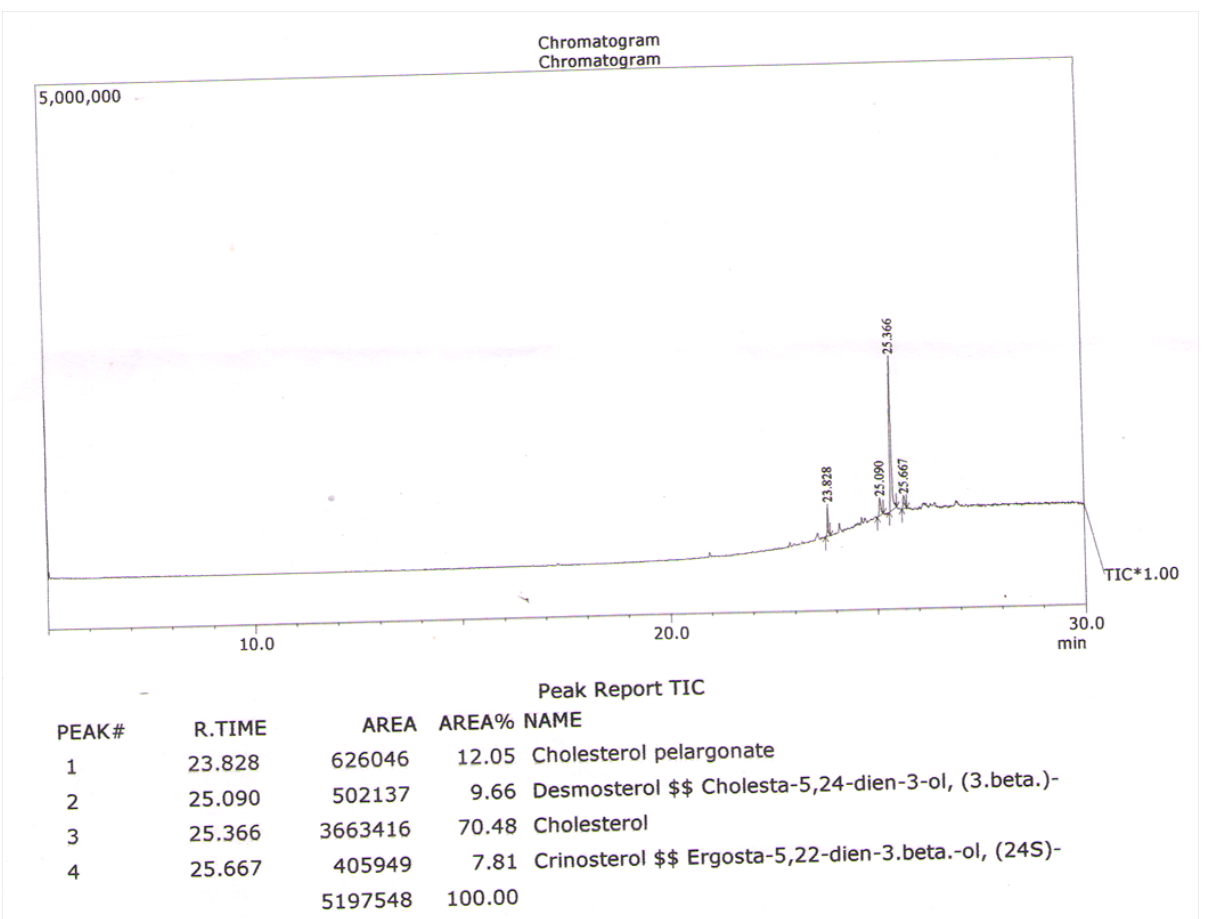
Results:
The yield of extract was 1.05g. The result shows promising antibacterial activity. GC-MS analysis revealed presence of sterols like cholesterol pelargonate, Desmosterol, Cholesterol, Crinosterol and the same has to be confirmed with further studies.

Antimicrobial activity:
Hydro alcoholic extract of *T. alexandri* had very good antibacterial activity for many bacteria tested specifically for *E. coli*, on par with ampicillin except for *P. aeruginosa*, *K. pneumonia* and *B. subtilis* **(Table 1)**. The Zone of inhibition (in mm) were found to be 16mm for *S. aureus* and *E. coli*, 18mm for *E. faecalis*, 8mm for *P. vulgaris* all at the concentration of 5000ppm of extract. Of all the concentrations tested, 5000 ppm was found to have greater antibacterial activity than the other concentrations (20, 200, 2000 ppm) used. The zone of inhibition was found to increase with increased concentration of the extract.

Table 1: Anti bacterial activity of hydro alcoholic extract of *T.alexandri* (Zone of inhibition in mm)

Culture	Streptomycin	Ampicillin	DMSO	20ppm	200ppm	2000ppm	5000ppm
B.subtilis	32	21	-	-	-	-	-
S.aureus	29	16	-	-	-	14	16
K.pneumonia	30	24	-	-	-	-	-
E.coli	30	16	-	-	12	12	16
P.aeroginosa	31	16	-	-	-	-	-
E.faecalis	20	15	-	9	13	15	18
P.vulgaris	31	14	-	-	-	-	8

Fig. 2: GC-MS report of Hydroalcoholic extract



Peak Report TIC

PEAK#	R.TIME	AREA	AREA%	NAME
1	23.828	626046	12.05	Cholesterol pelargonate
2	25.090	502137	9.66	Desmosterol $$ Cholesta-5,24-dien-3-ol, (3.beta.)-
3	25.366	3663416	70.48	Cholesterol
4	25.667	405949	7.81	Crinosterol $$ Ergosta-5,22-dien-3.beta.-ol, (24S)-
		5197548	100.00	

Minimal Inhibitory Concentration (MIC):
MIC **(Table 2)** was found to be as low as 10ppm for *E.faecalis*, 500 ppm for *S.aureus* , 25 ppm for *E.coli.* ; and 2500 ppm for *P.vulgaris*.

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Table 2: Minimal Inhibitory Concentration

Culture	Minimum concentration observed in ZOI (in ppm)	Range of concentration in MIC plates (in ppm)	Minimum inhibitory concentration (in ppm)
S.aureus	2000	2000-31.25	500
E.coli	200	200-3.125	25
E.faecalis	20	20-0.3125	10
P.vulgaris	5000	5000-78.25	2500

Discussion:

Bioactive compounds that possess antibacterial activity are of interest in the field of pharmacology. The Hydro alcoholic extract of *T. alexandri* showed promising antibacterial activity. The major components in the present hydro alcoholic extract could have been responsible for the antibacterial activity.

Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries. Even though pharmaceutical companies have produced a number of new antibacterial drugs in the last years, resistance to these drugs by bacteria has increased and has now become a global concern. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents. Echinoderms have already been reported to contain pharmacologically active compounds with respect to antihistaminic, cytotoxicity, antiangiogenicity, angiogenicity and antibacterial activity (Kelly, 2005). Haug *et al*. (2002) has isolated different extracts from the sea urchin, *Strongylocentrotus droebachiensis*, the sea cucumber *Cucumaria frondosa* and the star fish *Asterias rubens* exhibited antibacterial activity against several strains tested. The crude extract was found to inhibit the growth of several bacteria tested. Abubakar *et al*. (2012) proved that the crude extract of sea urchin has anti-bacterial activity.Since antibacterial agents are of interest in the field of pharmacology, further fractionation, purification and identification of the exact bioactive compound present in the hydroalcoholic extract is of much importance.

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