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Analysis of Pharmacologically Active Compounds in *Stomopneustes variolaris* Exoskeleton Using Hplc and Gc-Ms Techniques

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Abstract
In recent years worldwide extensive efforts have been made for the identification of bioactive compounds from marine natural resources. Many pharmacologically active compounds have been extracted, characterized and purified from various marine animals with wide range of pharmacological properties. More novel compounds await discovery from the organisms that inhabit the world's oceans. In the present study the ethanolic extract of *S. variolaris* (Common slate pencil sea urchin) exoskeleton has been subjected to chromatographic purification. Of the three major peaks obtained the high peak was subjected to GC-MS analysis. The studies on the active constituents in the high peak exoskeleton fraction clearly showed the presence of seven compounds. The compound Dimethyl sulfoxide is the major constituent in the exoskeleton fraction.

Keywords: Echinodermata, *Stomopneustes variolaris*, Slate pencil sea urchin, Pharmacologically active compounds, GC-MS, HPLC

Introduction
The ocean realm is a massively complex environment and it contains 97% of the Earth's water. The oceanographers have stated that only 5% of the world ocean has been explored. The ocean fringe is the highly competitive and most protective regions on the planet. The animals that thrive in this stressful environment are using vast array of bioactive compounds synthesized by them to overcome the stress [1]. These compounds play an important role in defense mechanism, catching prey and highly repellent material to predator.

The chemistry and biological activities of marine natural products have been studied by many scientists. From 1969 to 1999 about 300 patents on natural products were issued. Moreover, approximately 2,500 new pharmacologically active metabolites were reported from a variety of marine organisms during the decade from 1977 to 1987 [2]. So far, over 20,000 natural products have been isolated and identified from various marine organisms [3]. Interestingly, the majority of marine natural products involved in preclinical and clinical trials are produced by invertebrates. Among them, sponges (37%), coelenterates (21%) and microorganisms (18%) are the major source of biomedical compounds followed by algae (9%), echinoderms (6%), tunicates (6%), molluscs (2%) and bryozoans (1%) [4].

The invertebrate phylum Echinodermata is the largest group of marine deuterostome. The echinoids (sea urchins) are belongs to this phylum, a word meaning 'spiny skins'. They have spines that protect them from predators. Because of their longer evolutionary history, echinoids likely possess a valuable source of novel compounds. Several publications have suggested that sea urchins are a rich source for bioactive compounds. The chemical diversity of secondary metabolites isolated from echinoderms includes terpenes, alkaloids, triterpene glycosides, steroidal glycosides, fatty acids and related compounds [5, 6, 7, 8].

The sea urchin shells are known to contain various polyhydroxylated naphtoquinone pigments such as spinochromes [9] and echinochrome A [10]. Similar structural compounds are also found in the gonads of sea urchins [11]. These compounds have antioxidant and bactericidal effects. In India, commercially processed (dried) sea urchin shells are used as craft. The sea urchin *Stomopneustes variolaris* is a commonly found sea urchin in India, distributed along the coast of Vizagapatnam to Kanyakumari. However, there is no information regarding the active constituents in this species. To address this lacuna, the present study was carried out to identify the pharmacologically active compounds from exoskeleton ethanolic extract of common slate pencil sea urchin, *Stomopneustes variolaris*.

2. Materials and methods

2.1. Sample collection and extraction

Live specimens of sea urchin *S. variolaris* were handpicked by divers during low tide time in the coast of Kanyakumari (Lat. N08°06′ 49′′; Long. E077°73′353′′), India. The sea urchin was taxonomically identified and authenticated by scientists of Central Marine Fisheries Research Institute (CMFRI), Vizhingam, Kerala. All individuals were cleaned with tap water and the exoskeleton was dissected and shade dried (Figure 1 and 2). After complete drying the exoskeleton (Shell with spines and pedicellariae) was coarsely powdered using mortar and pestle. The extraction procedure was followed by modified method of Chellaram *et al*., [12]. 100gms of dried samples were soaked in ethanol for 5 days at normal room temperature, then the extract was filtered by Whatman filter paper No.1 and the solvent was concentrated by rotary vacuum evaporator (Superfit Rotavap- at 45°C) under reduced pressure and temperature, the resultant residue was stored at 4ºC for further analysis.

Fig 1: Gross view of *S. variolaris*

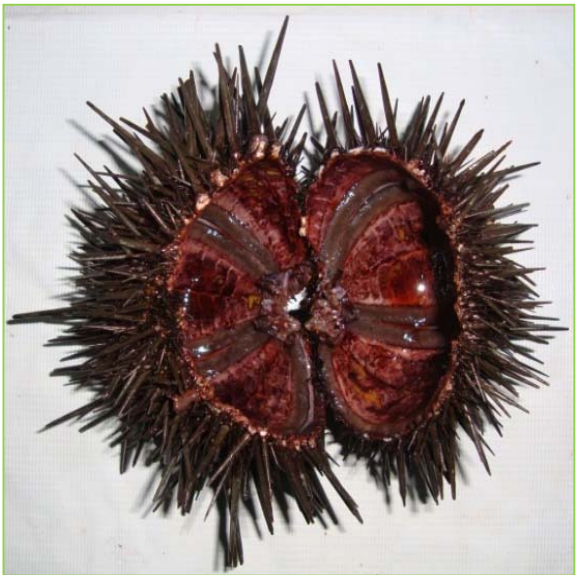


Fig 2: Exoskeleton of *S. variolaris*

2.2. Purification of pharmacologically active compounds by using HPLC technique

The HPLC analysis of crude ethanolic extract of *S. variolaris* exoskeleton was carried out with chromatographic system consist of autosampler with 100 µl fixed loop and an UV-Visible detector. The separation was performed on a SGE ProtecolPC18GP120 (250mm × 4.6 mm, 5µm) column at ambient temperature. The mobile phase consists of methanol to water (70:30 v/v) and the separations were performed by using isocratic mode, elution performed at a flow rate of 1 ml/min. The samples were run for 15 min and detection was done at 280 nm by UV detector. All chromatographic data were recorded and processed using autochro-3000 software. Fractions representing each peaks were eluted separately and used for GC-MS analysis

2.3. Identification of pharmacologically active compounds by using GC-MS technique

GC-MS analysis was made by following the method of Joachim and Hubschmann [13]. The high peak fraction thus obtained from HPLC purification of ethanolic extract of exoskeleton was further used for the identification of pharmacologically active constituents by using GC-MS analysis. 2 µl of the fraction was then subjected to GC-MS analysis.

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: columnElite-1 fused silica capillary column (30 × 0.25 mm ID × 1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature is 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2min), with an increase of 10°C/min, to200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550Da.

Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for pharmacologically active compounds by using the standard mass spectral database of WILEY and NIST [14, 15].

3. Results

3.1. HPLC analysis

In the present study, the crude ethanolic extract of *S. variolaris* exoskeleton was purified by HPLC. Of the three major peaks obtained the first peak recorded at the retention time of 1.880 min occupies 41.65% of the total area. The second peak was noticed at 2.073 mins representing 51.50% of the total area. Of the two major peaks, the first peak with maximum height was subjected to GC-MS analysis. The third peak at the retention time of 3.367 min was a minor peak representing about 6.85% of the total area (Figure 3 and Table 1).

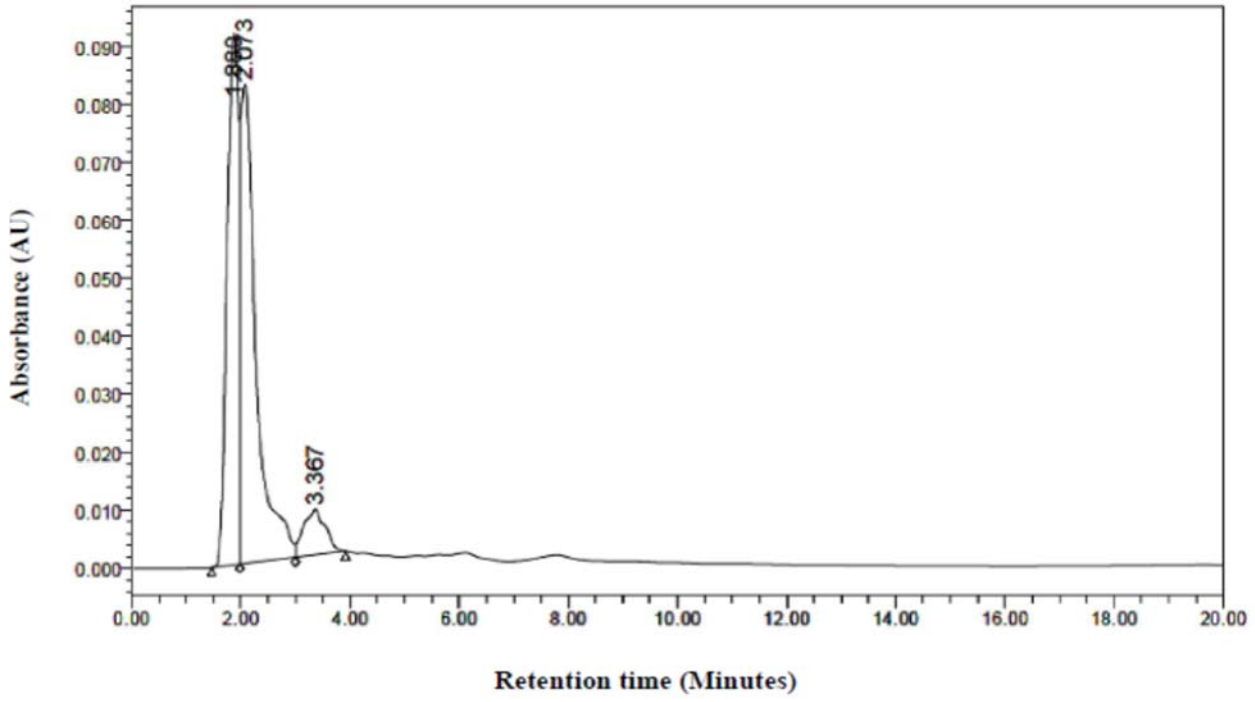


Fig 3: HPLC chromatogram of *S. variolaris* exoskeleton

Table 1: HPLC analytical report of *S. variolaris* exoskeleton

S. No	RT	Area	%Area	Height
1	1.880	1334882	41.65	91598
2	2.073	1650411	51.50	82662
3	3.367	219551	6.85	7840

3.2. GC-MS analysis

The results pertaining to the GC-MS analysis of *S. variolaris* exoskeleton fraction were given in figure 4 and table 2. Seven compounds were detected in the *S. variolaris* exoskeleton purified fraction. The GC-MS analysis of the exoskeleton extract showed Dimethyl sulfoxide (RT- 2.019 min), Hexadecanoic acid, ethyl ester (RT- 30.030 min), 9-Octadecenoic acid, 12 hydroxy-, methyl ester, [R- (z)]- (RT- 35.911 min), Tetradecanoic acid, 12- methyl-, methyl ester (RT- 25.048 min), 2, 3- Dihydroxypropyl elaidate (RT- 33.526 min) were the major constituents and 17- (1, 5- Dimethylhexyl)- 3 hydroxy- 10, 13- dimethyl- 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 15, 16, 17- Tetradecahydro- 1H- cyclopenta [A] phenanthren- 15- one (RT- 50.574 min) and Propanephosphonic acid, bis (trimethylsilyl) ester (RT- 23.590 min) were the minor constituents.

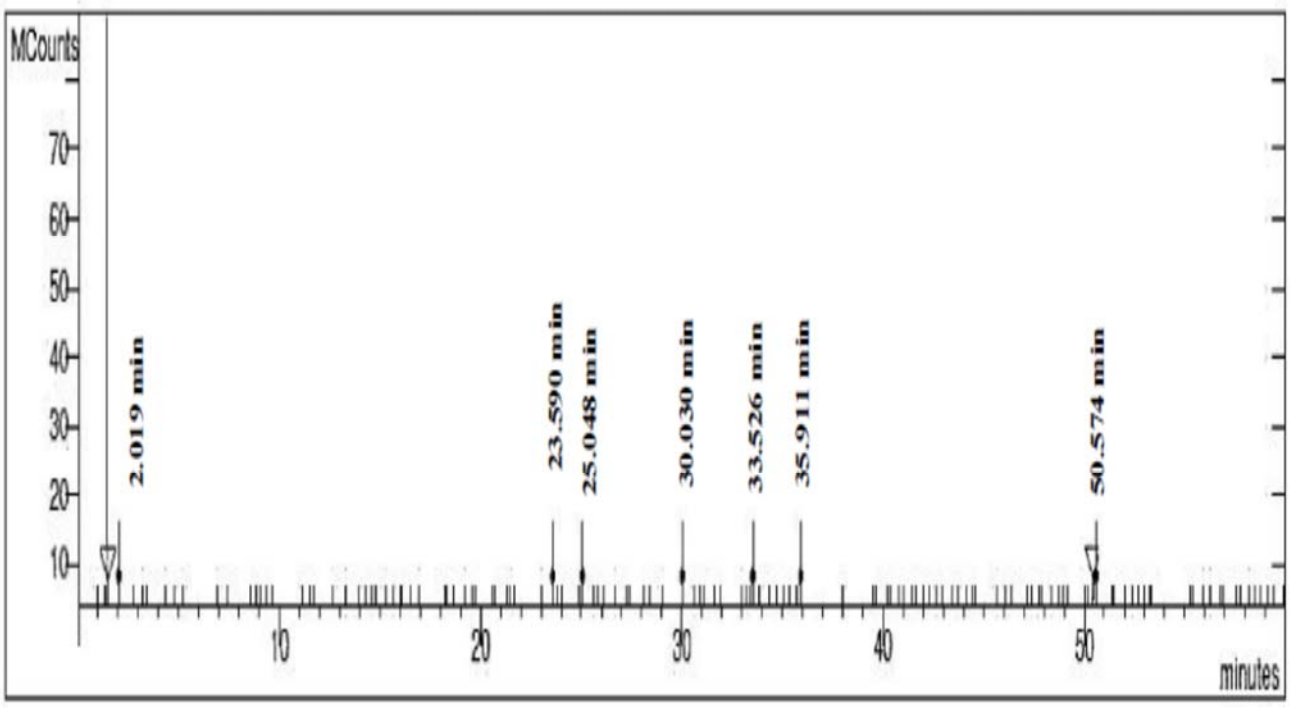
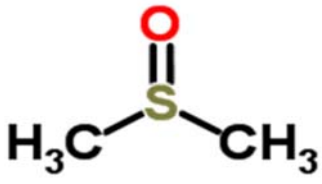
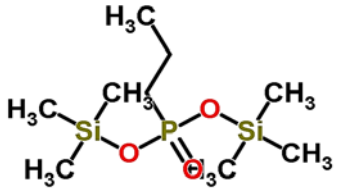
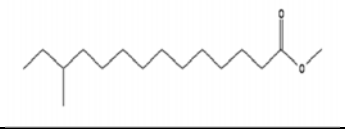
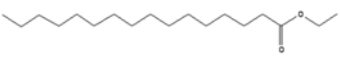
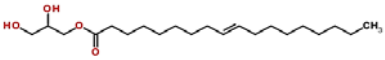
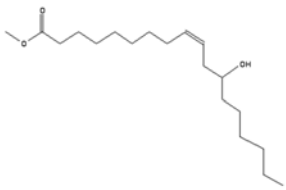
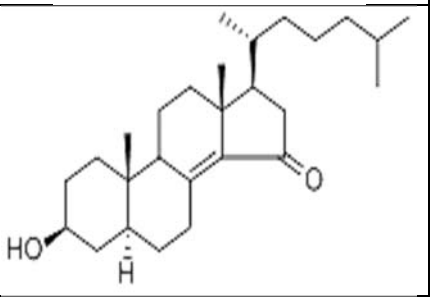


Fig 4: GC-MS spectrum of *S. variolaris* exoskeleton

Table 2: GC-MS analytical report *S. variolaris* exoskeleton fraction

S. No	Name of the compound	Analytical report		Chemical Structure
1	Dimethyl sulfoxide	**RT (min)**	2.019	
		Peak area	149334	
		MF/MW	$C_2H_6O_3$/ 78.13344	
		Synonyms	DMSO; Methyl sulphoxide; Methylsulfinylmethane	
2	Propanephosphonic acid, bis (trimethylsilyl) ester	**RT (min)**	23.590	
		Peak area	12610	
		MF/MW	$C_9H_{25}O_3PSI_2$/ 268. 437805	
		Synonyms	Phosphonic acid, propyl-, bis (trimethylsilyl) ester	
3	Tetradecanoic acid, 12-methyl-, methyl ester	**RT (min)**	25.048	
		Peak area	55519	
		MF/MW	$C_{16}H_{32}O_2$/ 256.4241	
		Synonyms	Methyl 12- methyltetradecanoate; Methyl tetradecanoate, 12- methyl	
4	Hexadecanoic acid, ethyl ester	**RT (min)**	30.030	
		Peak area	145241	
		MF/MW	$C_{18}H_{36}O_2$/ 284.4772	
		Synonyms	Palmitic acid, ethyl ester; Ethyl hexadecanoate; Ethyl palmitate	
5	2, 3- Dihydroxy propyl elaidate	**RT (min)**	33.526	
		Peak area	40888	
		MF/MW	$C_{21}H_{40}O_4$/ 356.539886	
		Synonyms	2, 3- Dihydroxypropyl (9E)- 9- octadecenoate	
6	9- Octadecenoic acid, 12 hydroxy-, methyl ester, [R- (Z)]-	**RT (min)**	35.911	
		Peak area	55950	
		MF/MW	$C_{19}H_{36}O_3$/ 312. 4873	
		Synonyms	Methyl ricinoleate; Ricinoleic acid methyl ester	
7	17- (1, 5- Dimethylhexyl)- 3 hydroxy- 10, 13- dimethyl- 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 15, 16, 17- Tetradecahydro- 1H- cyclopenta [A] phenanthren- 15- one	**RT (min)**	50.574	
		Peak area	13641	
		MF/MW	$C_{27}H_{44}O_2$/400.64	
		Synonyms	Cholestolone; 15 Ketosterol	

4. Discussion

The echinoderms not only contain pharmaceutically useful compounds but also produce toxic substances responsible for seafood poisoning and allergy. The exoskeleton of the sea urchin is associated with test to which the spines and pedicellariae are attached. The spines protect the urchin from predators. They inflict a painful wound when they penetrate human skin, but are not dangerous. But the pedicellariae are venomous [16]. The studies on the active constituents in the exoskeleton fraction clearly showed the presence of seven compounds. Some of the chemical constituents are reported to have a known biomedical and pharmacological value.

The compound Dimethyl sulfoxide (RT- 2.019 min) is the major constituent in the exoskeleton fraction. It is commonly known as DMSO. In medicine, the Dimethyl sulfoxide is predominantly used as an anti- inflammatory, antioxidant and analgesic agent. Jacob and De-la-Torre [17] summarized that the DMSO alone or in combination with synergistic molecules, may help to neutralize pathological products harmful to the heart and brain in medical disorders involving head and spinal cord injury, stroke, memory dysfunction and ischemic heart disease. Even at very low dose, dimethyl sulfoxide has a powerful protective effect against paracetamol induced liver injury in mice [18].

The compound Hexadecanoic acid, ethyl ester (RT- 30.030 min) is a fatty acid ester. It has antiandrogenic, antioxidant, hypocholesterlemic, nematicidal and pesticidal properties [19]. Sarumathy *et al.* [20] evaluated the nephroprotective and antioxidant activities of hexadecanoic acid, ethyl ester. The compound 9- Octadecenoic acid, 12 hydroxy-, methyl ester, [R- (Z)] - has antioxidant properties [21]. Charles *et al.* [22]

reported the presence of tetradecanoic acid, 12- methyl-, methyl ester in the leaf extract of *Alseodaphne semecarpifolia.* 17- (1, 5- Dimethylhexyl)- 3 hydroxy- 10, 13- dimethyl- 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 15, 16, 17- Tetradecahydro- 1H-cyclopenta [A] phenanthren- 15- one (RT- 50.574 min) is a 15- ketosterol and it has antiviral and cytotoxic properties. In the previous studies, Wang *et al*. [23, 24, 25] reported the cytotoxic 15- ketosterol from the star fish *C. semirgularis*. The bioactive compound Propanephosphonic acid, bis (trimethylsilyl) ester (RT- 23.590 min) has hypocholesterolemic and antioxidant properties. Hypocholesterolemic and antioxidant property could therefore be highly effective in the treatment of atherosclerosis. Furthermore, the lipophilicity of this compound predicts that it will become incorporated in the LDL and protect against the damages caused by oxidative stress. Hexadecanoic acid, ethyl ester and 9- Octadecenoic acid, 12 hydroxy-, methyl ester, [R-(Z)]- found in the exoskeleton fraction of *S. variolaris* are derivatives of saturated fatty acids. Saturated fatty acids and their derivatives from plant sources known to exhibit antimicrobial activity.

5. Conclusion

In conclusion, the present work revealed that *S. variolaris* exoskeleton are good sources of pharmacologically active compounds. This study also suggesting that the promising compounds from these untapped sources to be evaluated for curing many diseases.

6. Acknowledgement

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