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INFRA RED AND GAS CHROMATOGRAM / MASS SPECTRAL STUDIES ON ETHANOLIC EXTRACT OF *DIDEMNUM PSAMMATHODES*

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ABSTRACT

In the present study, the ethanolic extract of *Didemnum psammathodes* have been subjected to Infra red (IR) spectral study and Gas chromatogram/Mass spectral (GC-MS) analysis. Infra red spectral study indicates the presence of aromatic ring, carboxylic acid and moisture or hydroxyl compounds (O-H stretching). The GC-MS analysis revealed the presence of eight compounds. The nature of the compounds identified were steroids, plasticizers, triterpenes and ketones. Major compounds present in this extract are 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- [Synonyms: All-trans-Squalene], Dibutyl phthalate, 1,2-Benzenedicarboxylic acid, diisooctyl ester. The resulting chemical compounds exhibit many biological activities such as anti-microbial, anti-fouling, anti-oxidant, anti-cancer, anti-inflammatory, anti-arthritic, anti-asthmatic, diuretic, pesticide and chemo-preventive.

Key words: Didemnum *psammathodes*, IR spectral studies, GC-MS, Biological activities, Anti-cancer, Anti-oxidant.

INTRODUCTION

Most traditional medicines are developed from nature. Medicinal plants have been used for centuries as remedies for human diseases[1-3]. Fifty percent of prescription drugs are derived from chemicals first identified in plants[4-6], human activities such as drastic transformation of natural landscapes due to urbanisation or deforestation resulted in consequences such as reduction in biodiversity of plants through loss of species[7]. Though, herbal medicines are effective as conventional medicines, they also have the same potential to cause harmful side effects[4,5]. Marine environment is an exceptional reservoir of biologically active products, and it is one of the richest sources for floral wealth and diversity[8]. There is a growing interest worldwide in utilisation of marine extract in pharmacological field because of the existence of marine organism is vast and there is an unexploited source of potential pharmaceutical. Number of natural products isolated from marine organisms is increasing rapidly and now exceeds hundreds as new novel compounds have been extracted from marine invertebrates, especially ascidians and some of them are currently in clinical trials[9]. Marine ascidians have been the focus of intensive chemical investigation in recent years and they are very rich sources for unique and biologically active secondary metabolites[10,11]. These secondary metabolites serve as chemical defence[12,13]. *Didemnum psammathodes* is a colonial ascidian found in the littoral zones attached to the submerged rocks, hull of ships, harbour installations, and materials used for aquaculture operations. Taking into consideration, the pharmacological importance of *Didemnum psammathodes*, the ethanolic extract were subjected using IR spectral study and GC-MS analysis for the first time. This analysis in turn helps to identify the chemical compounds and their biological activities.

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EXPERIMENTAL

Materials and methods

Preparation of the sample

Samples of *Didemnum psammathodes* were collected from Tuticorin coast. The whole animal was cleaned in sea water, dried under shade and homogenized to get a coarse powder. Two grams of dry powder was transferred to a stopper flask and treated with ethanol until the powder was fully immersed. The content of the flask was stirred every hour for the first 6 hours; it was kept aside and again stirred after 24 hours. This process was repeated for 3 days and then the extract was filtered. The filtrate was evaporated to dryness by using a vacuum distillation unit. The final residue thus obtained was then subjected to further studies.

IR Spectral analysis

Infrared spectral study was carried out with the ethanol extract. One mg of the dried extract was mixed with about 100 mg of dried potassium bromide (IR) grade powder. The mixture was then pressed in a special die to yield a transparent disc. The disc was then held in the instrument beam for spectroscopic examination and the resulting IR spectrum was recorded.

GC-MS analysis

GC–MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 mm x 0.25 mm x 0.25 µm df, composed of 5% Diphenyl / 95% Dimethyl poly siloxane), operating in electron impact mode a 70 eV. Helium (99.999%) was used as carrier gas at a constant flow of 1 mL/min and an injection volume of 2 µL was employed (split ratio of 10 : 1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°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 seconds and fragments from 40 to 550 Da.

Identification of components

Interpretation of mass spectrum was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular formula and structure of the components of the test material were ascertained.

RESULTS AND DISCUSSION

Infra red spectrum of ethanolic extract of *Didemnum psammathodes* is given in Fig 1. The absence of strong band above 3000 cm^{-1} (3946.73 cm^{-1}) is due to the presence of aromatic ring, carboxylic acid, which is present in two broad bands namely 3337.64 cm^{-1} and 2352.59 cm^{-1} and it is the characteristic band for O-H stretching vibration which gives evidence for the presence of moisture or hydroxyl compounds[14].

The results pertaining to the GC-MS analysis are given in Fig. 2 and Table 1. Eight compounds were detected in the ethanolic extract of *Didemnum psammathodes*. It revealed the presence of 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione, Dibutyl phthalate, 1,2-diisooctyl ester of Benzenedicarboxylic acid, 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-[Synonyms: All-trans-Squalene], (3á)-Cholesta-4,6-dien-3-ol, (3á)-Cholest-5-en-3-ol-carbonochloridate, 26-Nor-5-cholesten-3á-ol-25-one, and Cholestan-3á-ol. Fig. 1 shows the peak area % of all chemical compounds identified in the extract. A maximum peak area of 45.12% was observed for the compound 2,6,10,14,18,22-Tetracosahexaene,

2,6,10,15,19,23-hexamethyl-, (all-E)-[Synonyms: All-trans-Squalene]. The compounds identified by GC-MS analysis in the ethanolic extract of *Didemnum psammathodes* exhibited multifunctional biological activity. Of the eight compounds identified in *Didemnum psammathodes* extract Dibutyl phthalate, 1,2-diisooctyl ester of Benzenedicarboxylic acid, 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-[Synonyms: All-trans-Squalene], Cholesta-4,6-dien-3-ol, (3á)- and Cholest-5-en-3-ol (3á)-, carbonochloridate have been reported for the first time in ascidians. Compounds such as 9, 9-Dimethoxybicyclo [3.3.1] nona-2, 4-dione, 26-Nor-5-cholesten-3á-ol-25-one has already been reported in ethanolic extract of *Microcosmus exasperatus*[15]. Both *Microcosmus exasperatus* and *Phallusia nigra*[16] revealed the presence of Cholestan-3-ol in their ethanol extract with various biological activities. According to the result, steroid compounds exhibited activities like anti-microbial, anti-cancer, anti-arthritic, anti-asthma, diuretic, and anti-inflammatory. On the other hand, triterpene compound showed in addition to anti-cancer activity other activities like anti-oxidant, pesticide, sun screen, perfumery and chemo-preventive.

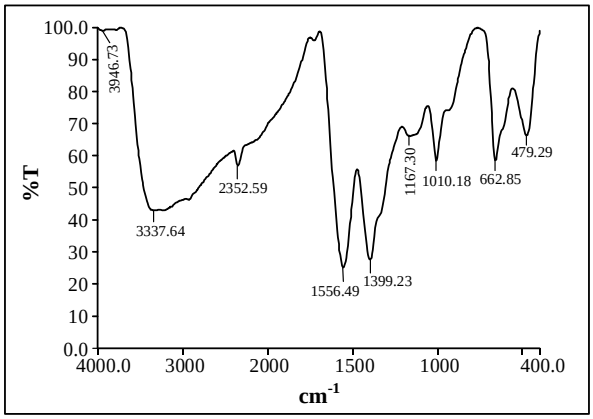

Fig. 1: IR spectrum for the ethanolic extract of *Didemnum psammathodes*

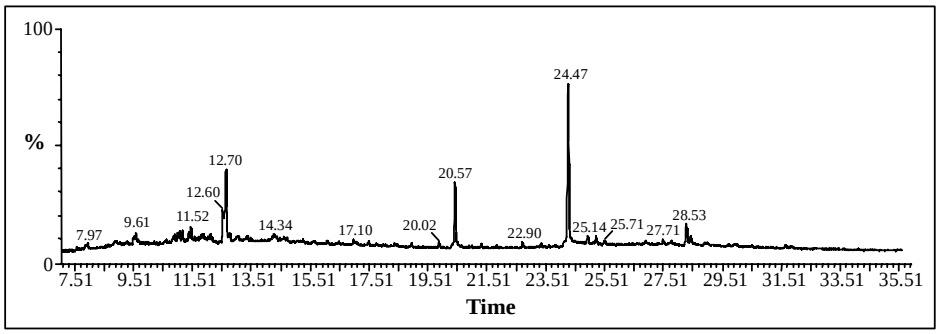

Fig. 2: GC-MS Chromatogram of the ethanolic extract of *Didemnum psammathodes*

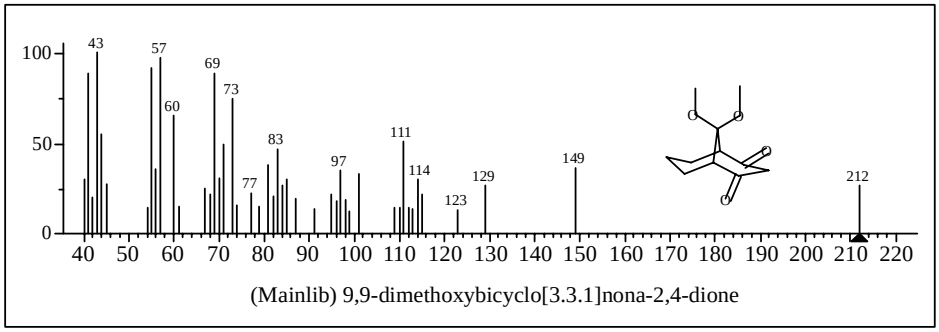

Fig. 3: Mass spectrum of 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione

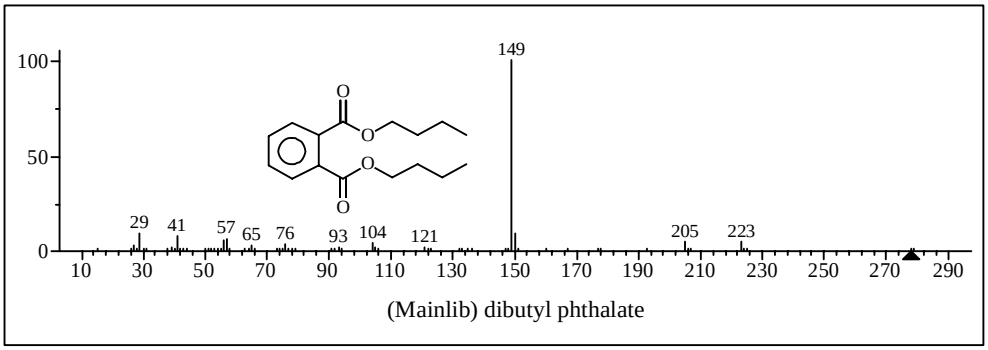

Fig. 4: Mass spectrum of dibutyl phthalate

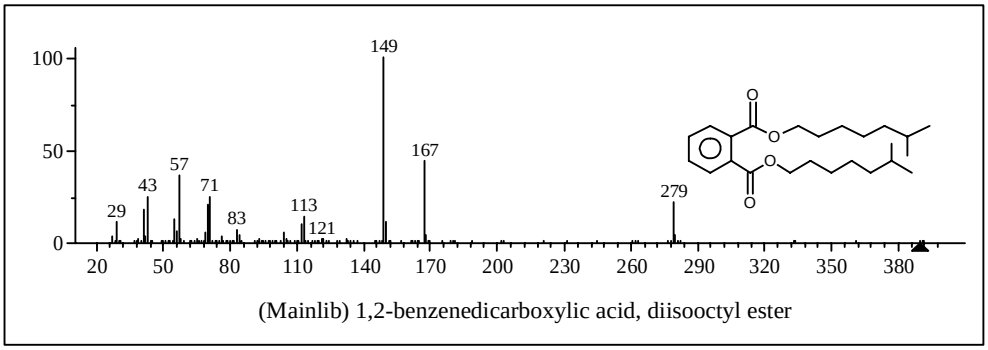

Fig. 5: Mass spectrum of diisooctyl ester of 1, 2-Benzenedicarboxylic acid

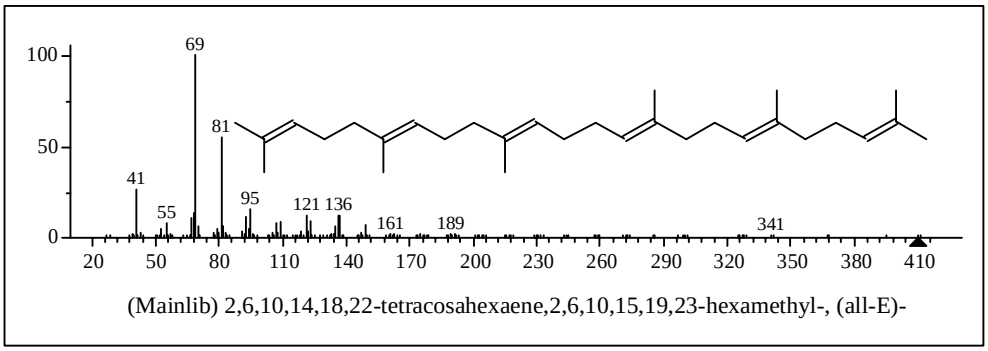

Fig. 6: Mass spectrum of 2,6,10,15,19,23-hexamethyl-, (all-E)-2,6,10,14,18,22-Tetracosahexaene, [Synonyms: All-trans-Squalene]

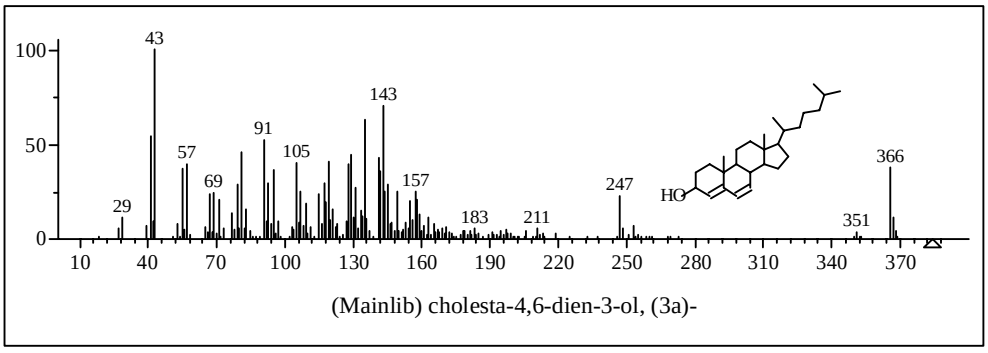

Fig. 7: Mass spectrum of (3á)-Cholesta-4, 6-dien-3-ol

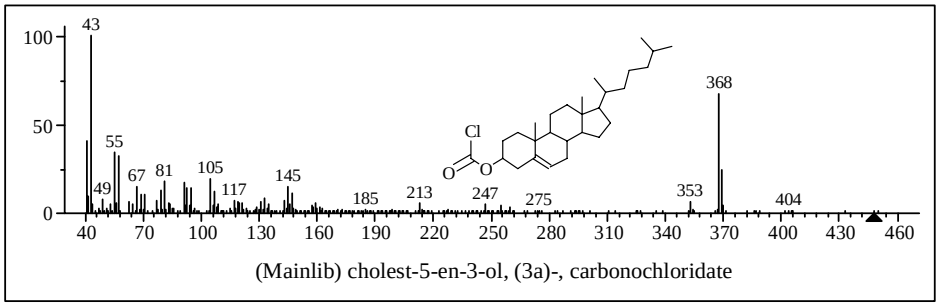

Fig. 8: Mass spectrum of (3á)-Cholest-5-en-3-ol carbonochloridate

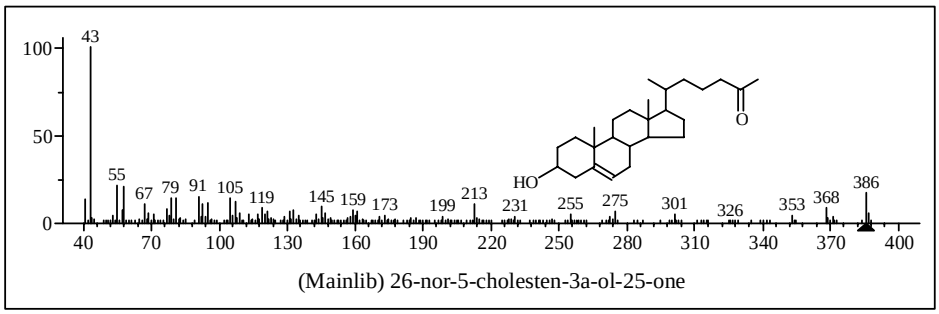

Fig. 9: Mass spectrum of 26-Nor-5-cholesten-3á-ol-25-one

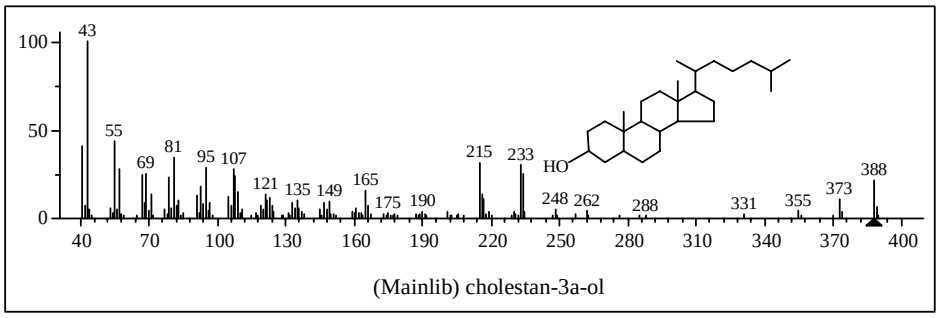

Fig. 10: Mass spectrum of cholestan-3á-ol

Table 1: Activity of components identified in *Didemnum psammathodes*

S. No.	RT	Name of the compound	Molecular formula	Peak area %	Nature of compound	**Activity
1	9.61	9,9-Dimethoxybicyclo[3.3.1] nona-2,4-dione	$C_{11}H_{16}O_4$	4.09	Ketone compound	No activity reported
2	12.70	Dibutyl phthalate	$C_{16}H_{22}O_4$	18.74	Plasticizer compound	Antimicrobial, antifouling
3	20.57	diisooctyl ester of 1,2-Benzenedicarboxylic acid,	$C_{24}H_{38}O_4$	17.50	Plasticizer compound	Antimicrobial, antifouling
4	24.47	2,6,10,15,19,23-hexamethyl-, (all-E)-2,6,10,14,18,22-tetracosahexaene, [Synonyms: All-trans-Squalene]	$C_{30}H_{50}$	45.12	Triterpene compound	Antioxidant, anticancer, pesticide, sunscreen, perfumery, chemo preventive,

Cont…

S. No.	RT	Name of the compound	Molecular formula	Peak area %	Nature of compound	**Activity
5	25.14	(3á)-Cholesta-4,6-dien-3-ol	$C_{27}H_{44}O$	1.69	Steroid	Antimicrobial, anticancer, antiarthritic, antiasthma, diuretic, anti-inflammatory
6	25.42	(3á)-Cholest-5-en-3-ol carbonochloridate	$C_{28}H_{45}ClO_2$	2.13	Steroid	Antimicrobial, anticancer, antiarthritic, antiasthma, diuretic, anti-inflammatory
7	28.53	26-Nor-5-cholesten-3á-ol-25-one	$C_{26}H_{42}O_2$	7.19	Steroid	Antimicrobial, anticancer, antiarthritic, antiasthma, diuretic, anti-inflammatory
8	28.67	Cholestan-3á-ol	$C_{27}H_{48}O$	3.55	Steroid	Antimicrobial, anticancer, antiarthritic, antiasthma, diuretic, anti-inflammatory

**Activity source: Dr. Duke's phytochemical and Ethnobotanical database

CONCLUSION

The GC-MS chromatogram of the ethanolic extract of *Didemnum psammathodes* shows the presence of eight biologically active compounds with various activities like anti-microbial, anti-cancer, anti-arthritic, anti-asthma, diuretic, anti-inflammatory, anti-oxidant, pesticide, sun screen, perfumery and chemo-preventive. GC-MS analysis is the first step towards understanding the nature of active principles. Likewise, IR Studies indicates the presence of aromatic ring, carboxylic acid, O-H stretching vibration and hydroxyl compounds.

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