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GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS FROM BODY TISSUE METHANOLIC EXTRACT OF MARINE ASCIDIAN *EUDISTOMA VIRIDAE*.

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ABSTRACT

Marine organisms offer an infinite supply of resources for discovering useful therapeutic ents. Apart from foods derived from the marine environment, a wide variety of bioactive bstances are being isolated and characterized several with great promise for the treatment human diseases. Currently, most infectious diseases can be controlled with natural or nthetic drugs. We are still in need of safer, cheaper, and more effective drug products. his study characterized bioactive substances in *Eudistoma viridae* by GC-MS analysis. he GC-MS analysis of the methanol extract confirmed the presence of 12 active mpounds. Several compounds were confirmed in the Ascidian extract, including a ominent steroid peak (26.90%). These compounds may be responsible for different armacological actions.

kEYWORDS: *Eudistoma viridae*, methanol, GC-MS, bioactive compounds.

INTRODUCTION

Natural products have been a major source of new drugs (Uniyal *et al*., 2006)[1] . In the last several decades, research has expanded from land to ocean to seek new drugs, and this diversity has provided a unique source of chemical compounds with potential bio activities that could lead to new drug candidates. The study of marine organisms for their bioactive potential and importance in marine ecosystems has accelerated recently, along with a growing recognition of their importance in human life (Nazar *et al*., 2009)[2]. A number of biologically active compounds with varying degrees of action, such as antifungal, antibacterial, antiviral, antioxidant, anticancer, antileukemic, antiproliferative, cytotoxicity, antibiotic antimalarial activity, inhibitor of protein kinase C and antifouling properties have been isolated from marine sources (Villa and Gernwick,2010)[3]. Some marine ascidians have demonstrated useful activities in the field of biomedicine. The potential of marine ascidians as sources of biologically active products remains largely unexplored in India (Janaki *et al*., 2015)[4].

The chloroform and methanol extracts of Strechopermum marginatum were subjected to GC–MS analysis by Jothibai marget and Kumaresan (2008)[15]. Meenakshi *et al.*, (2012)[13] reported GC mass spectrometry analysis of the simple ascidian *Microcosmus exasperatus*. From tunicates (ascidian) *Trididemnum solidum*, was the first marine compound entered into human cancer clinical trials as a purified natural product (Carte, 1996)[9], but it was unsuccessful in further trials (Davidson, 1993)[11]. Various ascidians, such as Botryllus spp., Didemnum sp were reported for producing anticancer drugs (Azumi *et al*., 1990)[21]. Chandran *et al.,* (2008)[18] highlighted the nature and current status of marine-derived anticancer therapies and the use of marine natural products as anticancer agents. Apart from human medicines, research on marine natural products in the last three decades has also led to the discovery of many chemically and biologically interesting molecules. The technical process of GC -MS study was done at Indian Institute of Crop Processing Technology, Tanjore , Tamilnadu ,India.

MATERIAL AND METHODS

The Ascidian *Eudistoma viridae* was collected from the Tuticorin coast ($8^0$45'N; $78^0$46'E) in the Gulf of Mannar region. The Gulf of Mannar is an established marine national park located on the southeast coast of India between India and Sri Lanka. Samples of *Eudistoma viridae* were collected during low tide from the intertidal area of the Tuticorin coastal area of the Gulf of Mannar and brought to the laboratory for further study. The Ascidians were kept in a glass trough in tap water for 24 hours, for emptying and

cleaning the gut. The entire body tissue was dried at 50°C (constant temperature) for 24 hours in the hot air oven. The dried tissues were powdered, and the methanolic extract of whole body tissue from *Eudistoma viridae* was used for the GC-MS analysis.

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer System comprising an AOC 20i auto sampler and gas chromatography interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions such as Column Elite– 1 fused silica capillary column (30 x 0.25mm ID x 1 EM df, composed of 100 % poly siloxane), operating in electron impact mode at 70 eV: Helium (99.999%) was used as a carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI (split ratio of 10:1) injector temperature 250ºC; and ion- source temperature 280°C. The oven temperature was programed from 110°C (isothermal for 2min), with an increase of 10°C/min to 200°C, then 5ºC/min to 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

The mass spectra were collected using the database of the National Institute of Standard Technology (NIST Ver.21), WILEY, and FAME, which contain more than 62,000 patterns. The unknown components found in the body tissues of *Eudistoma viridae* were matched with the spectrum of the known components stored in NIST, WILEY, and FAME, the MS library, and predicted from Duke's Ethno Botanical Database.

RESULT

GC-MS chromatogram of the methanolic extract of *Eudistoma viridae* shows 12 chemical constituents were identified. Chemical compounds contribute to the pharmacological activity of the Ascidian *Eudistoma viridae*. Chemical compounds were identified based on their retention time (RT), molecular formula, and concentration (peak area %). The GC-MS analysis of the methanol extract confirmed the presence of 12 active compounds, which were identified as follows:

GC-MS analysis of the Ascidian *Eudistoma viridae* revealed the presence of 12 compounds that could be identified as mefloquine, ethyl iso- alcoholate, n-hexadecanoicacid, valeraldehyde, 2-methy-, Quinoline, 3-methyl 2-phenyl-,1-oxide, 2-myristynoyl-pantotheine, octadecane, 3ethyl-5(2-ethyl butyl)-propanedinitrile, 2-[1-[4-(3,5-dimethyl-1-pyrazolyl)phenyl]-2-pyrrolmethylene],cholestanol, stigmasterol, and á sitosterol (Table.1)

Among the 12 identified compounds, cholesterol had the highest percentage of the chemical structures in the purified extracts of *Eudistoma viridae*, as depicted in figures (1-12) respectively. However the

isolation and characterization of compounds may proceed to discover the novel drugs and their pharmacolgical activities .

Fig. 1-12: Chromatogram of the methanol extract of *Eudistoma viridae* by GC- MS

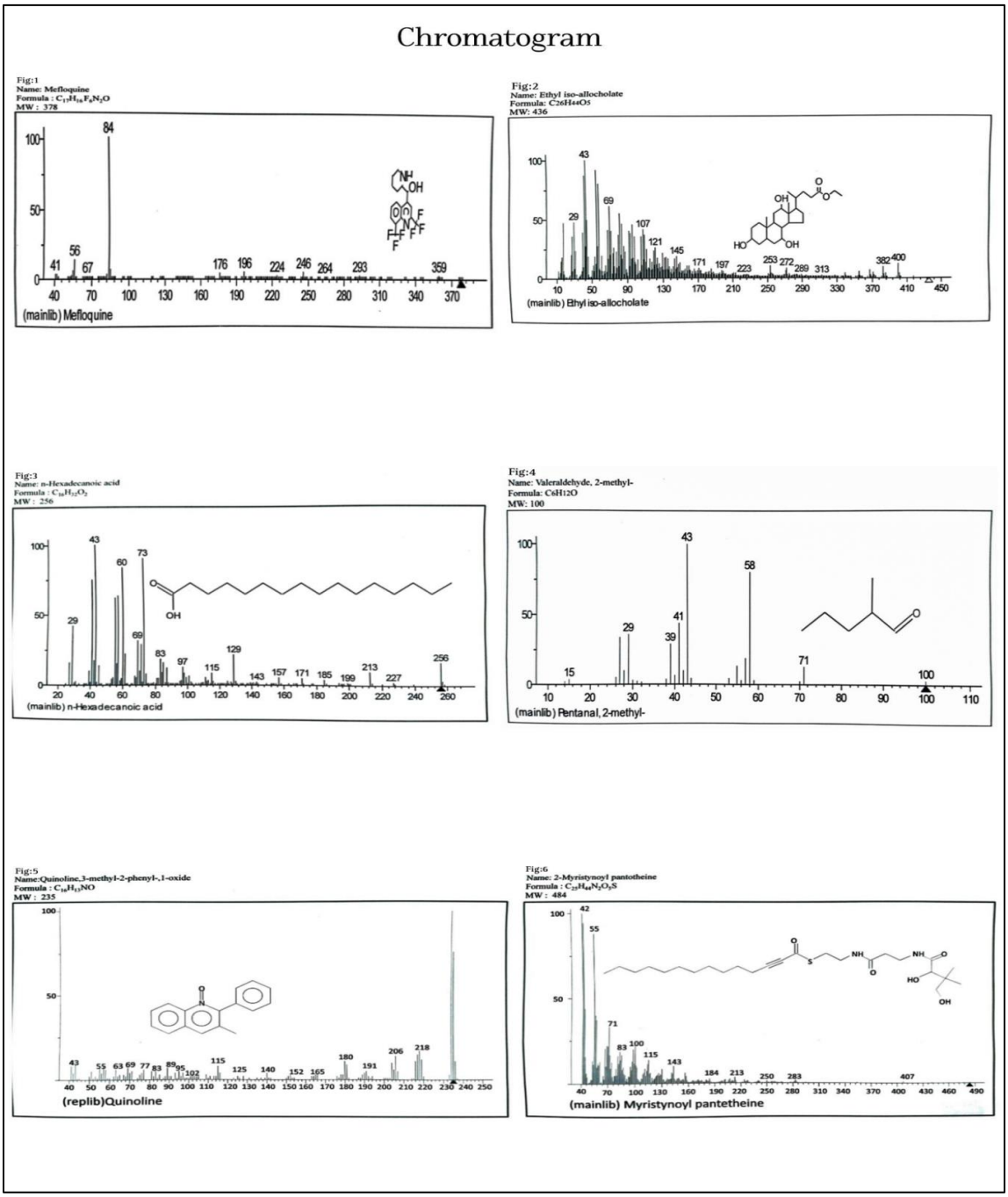

Fig:7
Name: Octadecane,3-ethyl-5-(2-ethyl butyl)-
Formula : $C_{26}H_{54}$
MW : 366

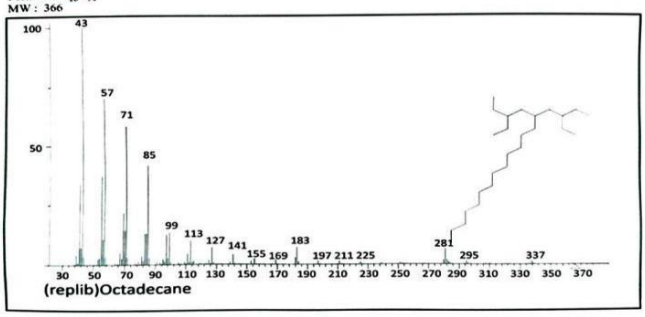

Fig:8
Name: Propanedinitrile,2-[1(-4-(3,5dimethyl-1-pyrazolyl)phenyl]-2-pyrrolyl methylene-
Formula : $C_{19}H_{15}N_5$
MW : 313

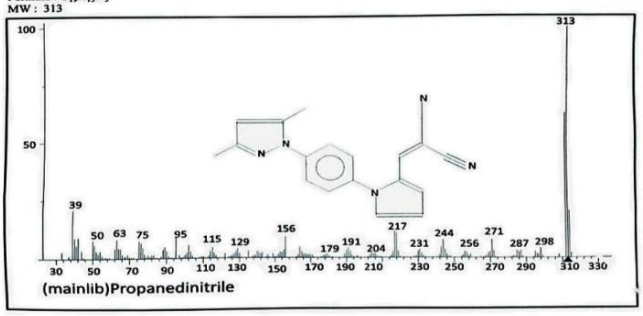

Fig:9
Name: Cholesterol
Formula : $C_{27}H_{46}O$
MW : 386

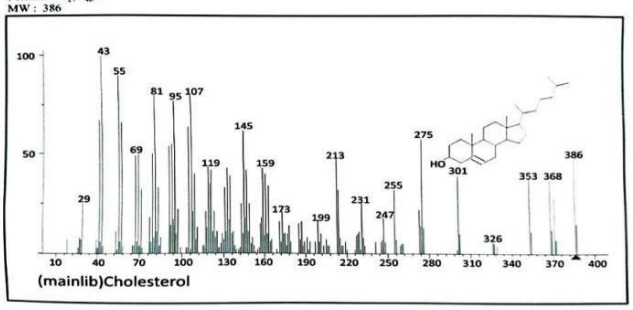

Fig:10
Name: Cholestanol
Formula : $C_{27}H_{48}O$
MW : 388

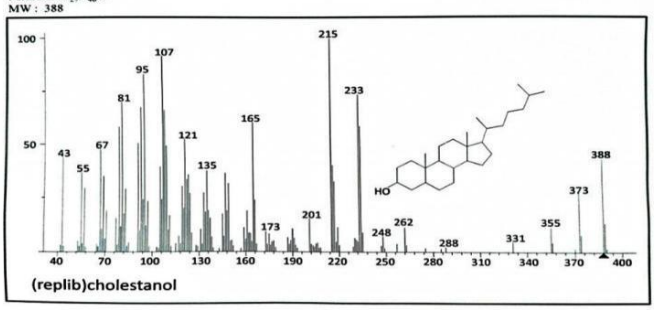

Fig:11
Name: Stigmasterol
Formula: C29H48O
MW: 412

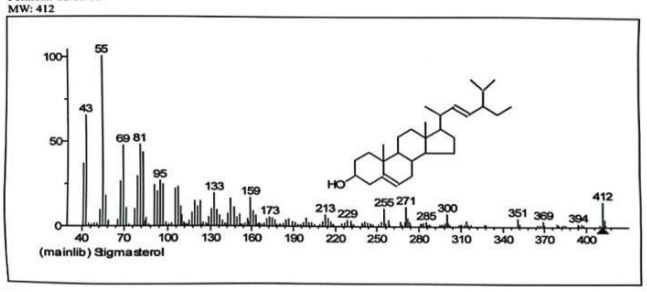

Fig:12
Name: á-Sitosterol
Formula: C29H50O
MW: 414

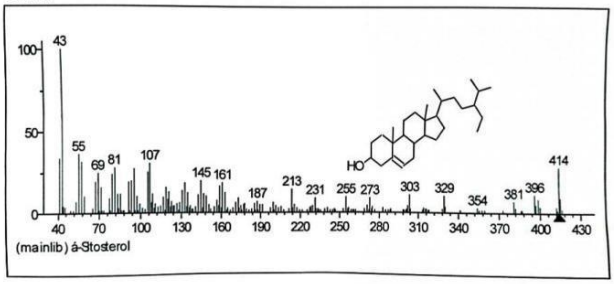

Table 1: Identification of components in the methanol extract of *Eudistoma viridae* by GC-MS

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	Activity
1	3.65	Mefloquine	$C_{17}H_{16}F_6N_2O$	378	2.91	Alkaloid compound	Antimicrobial,Anti-inflammatory, Analgetic
2	10.9	Ethyl isoallocholate	$C_{26}H_{44}O_5$	26	0.50n	Steroid compound	Antimicrobial, Antioxidant, Anti-inflammatory, Antiarthritic, Antiasthma Diuretic
3	13.18	n-Hexadecanoic acid	$C_{16}H_{30}O_2$	256	3.45	Palmitic acid	Antioxidant, Hypercholestrolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hydrolytic 5- Alpha reductase inhibitor
4	17.35	Valerldehyde,2-methyl-	$C_6H_{12}O$	100	1.37	Aldehyde compound	Antimicrobial,Anti-inflammatory, Anti-pyretic
5	19.19	Quinoline,3-methyl-2-phenyl-1-oxide	$C_{16}H_{13}NO$	235	1.75	Alkaloid compound	Antimicrobial, Anti-inflammatory
6	19.82	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	484	1.29	Sulfur compound	Antimicrobial
7	19.93	Octadecane, 3-ethyl-5- (2-ethylbutyl)	$C_{26}H_{54}$	366	0.96	Alkane compound	No activity reported
8	24.02	Propanedinitrile,-[1-4-(3,5-dimethyl-1pyrozlyl) phenyl-2-pyrrolylmethylene]	$C_{19}H_{15}H_5$	313	26.07	Alkaloid compound	Antimicrobial, Anti-inflammatory, Antipyretic
9	29.04	Cholestanol	$C_{27}H_{46}O$	386	26.9	Steroid	Antimicrobial, Antioxidant, Anti-inflammatory, Antiarthritic,Antiasthma Diuretic
10	31.19	Cholestanol	$C_{27}H_{48}O$	388	20.71	Steroid	Antimicrobial, Antioxidant, Anti-inflammatory, Antiarthritic,Antiasthma Diuretic
11	31.19	Stigmasterol	$C_{29}H_{48}O$	412	3.62	Steroid	Antimicrobial, Antioxidant, Anti-inflammatory, Antiarthritic,Antiasthma, Diuretic
12	32.47	a-Sitosterol	$C_{29}H_{52}O$	414	10.48	Steroid	Antimicrobial, Antioxidant, Anti-inflammatory

DISCUSSION

Marine Ascidians, which develop in different environments than terrestrial animals, are the source of a broad range of pharmacological substances. They either express constitutively or the expression is induced by exposure to pathogens (Srikumaran *et al*., 2011)[5]. By virtue of these excellent properties, marine organisms are of interest in terms of pharmaceutical potentials particularly Ascidians (Keivan etal.,2007)[6]. Numerous structurally and pharmacologically important substances with novel antimicrobial, antitumor, and anti-inflammatory properties (Bhadury and Wright, 2004)[7].

Chemical drugs may lead to adverse effects, and researchers have focused on pharmacologically active compounds from natural sources. Extracts of marine mollusks exhibit antibacterial, antifungal, and antiviral activities against pathogenic fish bacteria, and these extracts may also be used in aquaculture (Defer *et al*., 2009)[8]. GC-MS is used to identify the constituents of volatile matter, long- and branched-chain hydrocarbons, alcohols, acids, and esters.

GC-MS analysis of the methanolic extract of *Eudistoma viridae* revealed 12 peaks corresponding to 12 chemical constituents. The results revealed the presence of alkaloids, fluorides, sulfur, chloride, and sterols. In any compound, structural variations were ascertained by determining the concentration of the three major elements viz., carbon, hydrogen, and nitrogen (C,H,N), because the bonding patterns of these three elements are only responsible for structural formation. These probable antimicrobial compounds might be responsible for the antimicrobial activity. The identified compounds have already been established as antimicrobial agents by several authors (Hancock, 2000) [19]. Welling *et al*., 2000 [20]; Selitrennikoff, 2000) [17]

GC-MS analysis of *Eudistoma viridae* revealed the presence of probable antimicrobial compounds such as mefloquine, ethyl -isoallocholate, n-hexadecanoicacid, valeraldehyde, 2-methyl-quinoline, 3-methyl 2phenyl, 1-oxide, 2-myristynoyl pantetheine, octadecane, 3ethyl-5- (2-ethtyl butyl)- propanedinitrile, 2-[1-[4-(3,5-dimethyl-1-pyrazolyl) phenyl] -2-pyrroyl methylene]-, cholesterol, cholestanol, stigmasterol, and ásitosterol, which are responsible for the inhibition of *Escherichia coli*, *Mycobacterium luteus*, and *Pseudomonas florescence*. The present findings are in agreement with GopalaKrishnan., (2011)[12] who reported that hexadecanoic acid, a palmitic acid-derived compound, exhibited antibacterial activity. High concentrations of macrolides extracted from the eggs of the Spanish dancer nudibranch, *Hexabranchus sanguineous*, prevented the growth of pathogenic microorganisms (Pawlik, 1992)[10]. Three antibacterial linear ß - substituted sesterpenes were isolated from Hypselodoris *capensis* (Mc Phail *et al.*, 1998)[16]. These antimicrobial compounds were responsible for the inhibition of bacteria. Unique antitumor

bioactive compounds from tunicates have recently attracted considerable attention in sessile marine ascidians.

GC-MS chromatogram of the methanolic extract of *Eudistoma viridae* showing 12 peaks corresponding to each of the 12 compounds. According to the results, steroid compounds exhibit antiarthritic, hepatoprotective, antiasthma, anti-inflammatory, diuretic, and anticancer activities. On the other hand, alcoholic, fluoro, and aldehyde compounds are responsible for antimicrobial activity. Fatty acids are responsible for nematicide and pesticide activities and can also be used as flavoring agents. Myristic acid is an antioxidant, cancer-preventing, and hyper cholesterolemic nematicide that also acts as a lubricant. In addition to antioxidant and pesticide activities, palmitic compounds act as anti-androgenic, genetic, lubricant and 5 alpha reductase inhibitors. Monounsaturated fatty acids have anti-inflammatory, antiandrogenic, cancer-preventing, insecticide, perfume, 5-alpha reductase inhibitor, and dermatigenic activities. Further GC-MS study of the methanolic extract of *Eudistoma viridae* confirms the presence of the above-mentioned bioactive compounds, which could be responsible for the pharmacological activities, and these compounds are also structurally elucidated, Further purification and clinical trials are needed.

CONCLUSION

Marine environments are major sources of novel drugs. Apart from foods derived from the marine environment, diverse drugs are being isolated and characterized with great promise for the treatment of human diseases. Studies on biomedical screening will provide valuable information on new antibiotic discoveries and provide new insights into the extraction of bioactive compounds from marine organisms.

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