International Journal of Zoology Studies
ISSN: 2455-7269
Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 3; Issue 3; May 2018; Page No. 15-19

FTIR and GCMS studies on the ethanol extract of *Aplidium multiplicatum* from Vizhinjam, Kerala

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Abstract
Ascidians are marine sessile filter feeders that belong to the phylum Chordata and class Ascidiacea. Most recently ascidians have increasingly become the target of natural products research. Ascidians are dominant organisms in marine communities and have a wide geographic distribution. The present investigation is to identify the possible chemical constituents in the extract of ascidian with the aid of FTIR and GC-MS spectral analysis. IR studies indicate the presence of aromatic ring carboxylic acid, phenols, alcohols, phosphates, esters and ethers. GC-MS chromatogram of the ethanolic extract of *A. multiplicatum* showed 17 peaks indicating the presence of 17 chemical constituents The prevailing compounds are 4-Butylbenzoic acid, tridec-2-ynyl ester (41.7%), 5,8,11,14,17-Eicosapentaenoic acid, meth (20.8%), Pyrrolo [1,2-a] pyrazine-1,4-dione, hexahy (13.6%), Phenol, 3,5-dimethoxy-, acetate(10.0%), Vinylbital (8.3%), 4- and Methylimidazole-5-[1,1-dimethylethanol (6.0%) which may be involved in various biological activities like anti-microbial anti-inflammatory, anti-parasitic, anti-carcinogenic etc.. *A. multiplicatum* contains various bioactive compounds in ethanolic extract with various activities like antimicrobial, anti-cancer, diuretic, anti-inflammatory, anti- fungal, antioxidant, pesticide and chemo protective. GC- MS study is the first step towards understanding the nature of active principles.

Keywords: FTIR, GCMS, Ascidian. Chordate, antimicrobial activity, Compound

Introduction
The marine environment is a rich source of both biological and chemical diversity. The ocean cover more than 70% of the earth surface and the marine environment supports rich faunal diversity. Marine organisms would be wonderful sources of biologically active molecules [1]. Because of their extreme environmental conditions like salinity, temperature, pressure etc. and biological factors like competitive and aggressive conditions demands the production of novel and potent active molecules [2]. Marine organisms form a valuable source of novel compounds. Over the past decade, several new therapeutic agents derived from marine sources have entered pre-clinical and clinical trials [3]. Several molecules isolated from various organisms such as algae, fungi, bryozoans, molluscs and ascidians are currently under study at an advanced stage of clinical trials and some of them have already been marketed as drugs [4].

Marine invertebrates, especially ascidians are most prominent sources of new compounds with antimicrobial, anti-viral and cytotoxic potential. Ascidians are marine sessile filter feeders that belong to the phylum Chordata and class Ascidiacea. Most recently ascidians have increasingly become the target of natural products research [5]. The metabolites reported from ascidians are derived from amino acids and it is an important source in drug discovery. Ascidians of the family Didemnidae are recognized as particularly unique sources of modified peptides and alkaloids [6]. Didemnum species are good sources of novel biologically active compounds of various biosynthetic origins [7]. These include well known Tamandarians, Didemnin, Aplidine and related compounds. It displays potent antitumor and immunosuppressive activities as well as G_2 cell cycle check point inhibitors granulatimide and isogranulatimide [8, 9].

Ascidians are renowned for their overwhelming bias towards the production of nitrogenous secondary metabolites. However, with the continued chemical interest in ascidians, an increasing number of non-nitrogen containing metabolites are being isolated. The objective of the present investigation is to identify the possible chemical constituents in the extract of ascidian with the aid of FTIR and GC-MS spectral analysis.

Materials and Methods
Specimen collection and identification
Aplidium multiplicatum was collected from the cement blocks, pilings and pearl oyster cages of Vizhinjam bay (lat 8˚22'35.95"N-76˚59'16.40E") by SCUBA diving at the depth ranging from 4 to 6 m between October and November 2011. The samples were thoroughly washed with sea water, cleaned off sand, mud and overgrowing organisms at the site of collection and transported to laboratory and identified by standard keys of Kott [10, 11, 12] and Meenakshi [13].

Extraction
The freshly collected samples were weighed (20g) and soaked in ethanol for one week and filtering through Whatman No.1 filter paper and the solvents were concentrated by rotary evaporator with reduced pressure and used for further chemical investigations like FTIR and GC-MS analysis. The extraction was done by the methods given by [14].

IR spectral studies
Ethanol extracts were analysed in a liquid cell. This is a small

container made up of NaCl (or other IR-transparent material) which is filled with liquid extract for EPA 418.1 analysis. This forms a longer path length for the sample and it leads to increased sensitivity. Sampling methods include a mull of a powder with hydrocarbon oil (Nujol) or pyrolyzing insoluble polymers and used the distilled pyrolyzate to cast a film. Materials are placed in an Attenuated Total Reflectance (ATR) cell and gases in gas cells. The following conditions were employed; Perkin Elmer Model spectrum RXI; Range 4000nm-400nm; Resolution 4 and Transmittance test mode. The frequencies of different components present in each sample were analysed.

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 × 0.25 mm 1D × 1EMdf, composed of 100% Dimethyl poly siloxane) and it is operating at 70 ev; in an electron impact mode. A scan interval of 2 minutes and fragments from 40 to 550 Da. The percentage of chemical constituents was calculated by comparing the average peak area to the total areas.

Identification of components

Interpretation on mass spectrum of GC-MS was conducted using the data base of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrums of the unknown components were compared with the spectrum of the known components in the NIST library. The common name, molecular weight, molecular formulae and structure of the components of the test materials were ascertained.

Data bases

The details of the chemical compounds present in *A. multiplicatum* were obtained from PubChem (http://pubchem.ncbi.nlm.nih.gov) PubChem is a database of chemical molecules and their activities against biological assays. PubChem contains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bands. The system is maintained by the National Centre for Biotechnology Information (NCBI), a component of National Library of Medicines which is part of the United States National Institutes of Health (NIH). Searching the data base is a broad range of properties including chemical structure, name fragments, chemical formula, molecular weight, XLogp, Hydrogen bond donor and acceptor count.

Results

Interpretation of FTIR

The crude ethanol extracts of *A. multiplicatum* of the FTIR spectrum is intercepted and the results are presented in the tables 1and figures. 2. FTIR studies on the ethanol extracts the spectrum showed the presence of alcohols at frequency level of 3341.38cm^{-1} and 3421.11cm^{-1}. A peak of 2089.24cm^{-1} was observed due to the presence of Isothiocyanate (-NCS). Carbonyl compound was observed at the frequency of 1631.40cm^{-1}. A peak of 1410.57cm^{-1} represents C-C stretching indicates the presence of aromatic amines. O-H bond of phenol or tertiary alcohol was observed at a peak of 1334.01cm^{-1}. The lengthening of the single bonds of the aliphatic amine ring represented by signals in the 1202.04cm^{-1}, 1098.77cm^{-1} region.

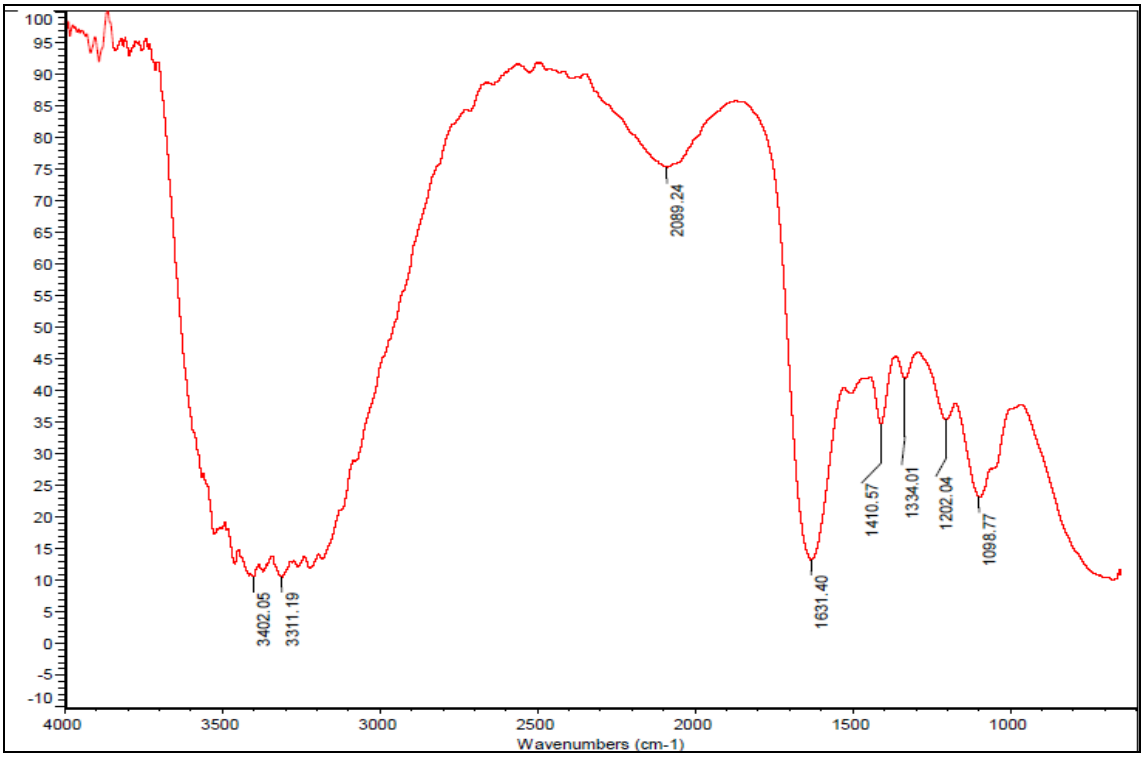

Fig 1: FTIR spectrum of ethanol extract of *Aplidium multiplicatum*

Table 1: IR spectral data of ethanolic extract of *A. multiplicatum*

No	Group Frequency cm-1 of the sample compounds	Functional group assignment and compound	Group frequency cm^{-1}
1	3402.05	O-H stretch, H-bonded-Alcohols, Phenols	3570-3200
2	3311.19	O-H stretch, H-bonded-Alcohols, Phenols	35700-3200
3	2089.24	Isothiocyanate (-NCS)	2150-1990
4	1631.40	C=O stretch, carbonyl compound	1650-1600
5	1410.57	C-C sretch, aromatic amines	1500-1400
6	1334.01	O-H bond, phenol or alcohol	1410-1310
7	1202.04	C-N stretch, aliphatic amines	1250-1020
8	1098.77	C-N strtch-aliphatic amines	1250-1020

Interpretation of Infrared Spectra has been done by the methods suggested by John Coates168

GC-MS analysis of ethanol extract of *A. multiplicatum*

GC-MS chromatogram of the ethanolic extract of *A. multiplicatum* (Fig. 2) showed 17 peaks indicating the presence of 17 chemical Constituents figures 6. 26 to 6. 42. The 20 active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) in the ethanolic extract of *A. multiplicatum* are presented in tables 6. 23 to 6. 36. The prevailing compounds are 4-Butylbenzoic acid, tridec-2-ynyl ester (41.7%), 5,8,11,14,17-Eicosapentaenoic acid, meth (20.8%), Pyrrolo [1,2-a] pyrazine-1,4-dione, hexahy (13.6%), Phenol, 3,5-dimethoxy-, acetate (10.0%), Vinylbital (8.3%), 4- and Methylimidazole-5-[1,1-dimethylethanol(6.0%) which may be involved in various biological activities like anti-microbial anti-inflammatory, anti-parasitic, anti-carcinogenic etc. Of the 17 chemical constituents 16 are first reported from ascidians especially from *A. multiplicatum*.

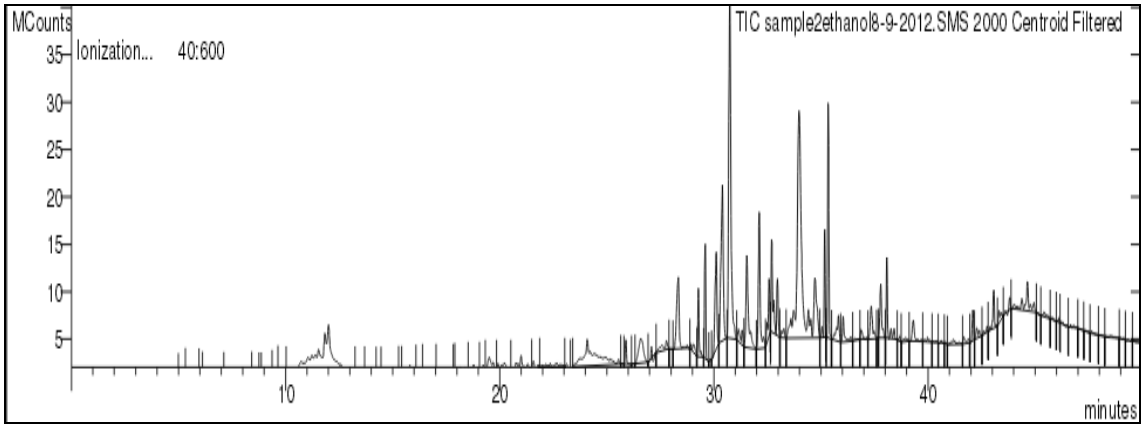

Fig 2: GC-MS spectrum of ethanolic extract of *A. multiplicatum*

Table 2: GC-MS spectrum of ethanolic extract of *A. multiplicatum*

No	RT	Name of the compound	Molecular formula	Molecular Weight	Peak area %
1	25.888	Tetradecanoic acid, ethyl ester	C16H32O2	256.42408	0.3
2	27.088	Pentadecanoic acid, 13-methyl-, methyl e	$C_{17}H_{34}O_2$	270.4507	0.1
3	28.347	Vinylbital	C11H16N2O3	224.25634	8.3
4	29.277	9-Hexadecenoic acid, eicosyl ester, (Z)-	C36H70 O2	534.9398	0.3
5	29.589	Hexadecanoic acid, ethyl ester	C18H36O2	284.5	1.2
6	30.120	Phenol, 3,5-dimethoxy-, acetate	$C_{10}H_{14}O_5$	214.21516	10.0
7	30.412	Pyrrolo [1,2-a] pyrazine-1,4-dione, hexahy	$C_7H_{10}N_2O_2$	154.1665	13.6
8	30.743	4-Butylbenzoic acid, tridec-2-ynyl ester	C24H36O2	356.54144	41.7
9	31.545	4-Methylimidazole-5-[1,1-dimethylethano	C8H14N2O	154.20956	2.6
10	32.114	Dicyclo Octanopyridazine	C16H24N2	244.37516	3.7
11	32.705	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(h	C21H40 O4	356.54	0.5
12	32.968	Heptadecanoic acid, 15-methyl-, ethyl es	C20H40O2	312.5304	0.7
13	33.993	4-Methylimidazole-5-[1,1-dimethylethanol	$C_{14}H_{18}N_2O_2$	246.30492	6.0
14	35.177	Ethyl 5,8,11,14,17-icosapentaenoate	$C_{22}H_{34}O_2$	330.50416	0.9
15	35.339	Fluorometholone	C22H29FO4	376.46166	0.4
16	38.078	5,8,11,14,17-Eicosapentaenoic acid, meth	$C_{22}H_{34}O_2$	330.50416	20.8
17	42.089	1,2,3,4-Tetrahydroisoquinoline, 6,7,8-tr	C12H17NO3	223.26828	2.4

Discussion

Marine natural product chemistry is a new area of research to develop new compounds in the field of biomedical and pharmaceutical industries. The present study provides a good

starting point for the analysis of unique compounds from ascidians for the treatement of human diseases. The presence of strong bands above 3000cm^{-1} indicates the presence of aromatic ring [15]. In a previous study of ethanolic extract of *Phallusia arabica* showed the presence of carbonyl, carboxilic and aromatic ring [16, 17] and [18] have been studied the infrared spectrum of the ethanolic extract of *Ecteinascidia venui* and *Ascidia sydneiensis.* The spectrum of these species revealed the presence of aromaticity, hydroxyl, carboxilic, aliphatic and carbonyl groups. In the present study FTIR spectrum ethanol extract of *A. multiplicatum* showed the presence of phytochemicals such as aliphatic bromo compounds, phenol, tertiary alcohols, carbonyl compounds and carboxylic acids. [18] and [19] and [20] have been discussed about the presence of alcohols, phenols, carboxilic acid, aromatic ring, ethers and aliphatic amines in the methanolic extract of *Phallusia nigra* and *Microcosmus exasperatus.*

The present study was undertaken to identify the bioactive compounds from the solvent extracts of *A. multiplicatum* using GC-MS analysis. Some chemical compounds are reported to have known biomedical value in the pharmacological fields. GC-MS chromatogram of the methanolic extract of *A. multiplicatum* showed 21 peaks indicate the presence of 21 chemical compounds with various biologicl activities like anti-microbial, anti inflamatory, pesticide, chemopreventive, diuretic and anti-oxidant. Similar studies were reported by [21], and they reported that the GC-MS chromatogram of the ethanolic extract of *Didemnum psammathodes* shows the presence of eight biologically active compounds with various activities such as antimicrobial, anti-inflammatory, pesticide, plastizer and anti-inflammatory activity. Again [22] studied the bioactive cmpounds present in the simple ascidian *M. exasperatus* and revealed the presence of 20 chemical compounds having various biological activities. Similar observations on the presence of bioactive compounds have antimicrobial, antifouling, anti-inflammatory, antioxidant and larvicidal activities have been reported from other species of ascidians.

The GC-MS analysis of the crude extract of *A. multiplicatum* revealed that the main phytochemical constituents were hexadecanoic acid -tetra decanoic acid, ethyl ester and octadecanoic acid. These compounds has been already reported from the crude extract of seaweed [26]. Results of the present study matched with a composition study of *Acanthophora spicifera* from India in that palmitic acid, arachinoic acid and eicosapentanoic acid as the dominant fatty acids are present. The capability of hexadecanoic acid that exhibited antimicrobial and antioxidant activities have also been reported by [23].

Fluorometholone (0.4%) is present in the ethanolic extract of *A. multiplicatum* is a glucocorticoid. It is employed usually as eye drops and used in the treatment of allergic and inflammatory conditions of the eye. It has also been used topically in the treatment of various skin disorders [24]. The compound vinylbital is present in the ethanolic extract of *A. multiplicatum*. It is also known as butylvinal, a barbiturate derivative. [25] Reported that vinylbital is a sedative hypotonic drug.

Here, IR studies indicate the presence of aromatic ring carboxylic acid, phenols, alcohols, phosphates, esters and ethers. *A. multiplicatum* contains various bioactive compounds in both methanolic and ethanolic extract with various activities like antimicrobial, anti-cancer, diuretic, anti-inflammatory, anti- fungal, antioxidant, pesticide and chemo protective. GC- MS study is the first step towards understanding the nature of active principles. Further studies are needed to the isolation, purification and structural determination of the chemical compounds responsible for the biological activities which may be lead to the discovery of drug molecules as chemotherapeutic agents in combating various diseases of mankind.

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