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## GC-MS AND FT-IR ANALYSIS OF METHANOLIC EXTRACT OF A MARINE CNIDARIAN *ZOANTHUS SANSIBARICUS* IN SEARCH OF BIOACTIVE COMPOUNDS

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### Keywords:

*Zoanthus sansibaricus*, FT-IR, GC-MS, Bioactive functional groups, Bioactive compounds

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**ABSTRACT:** Present study was aimed to search bioactive functional groups and compounds in the whole animal extract of a marine cnidarians *Zoanthus sansibaricus* by FT-IR and GC-MS. The methanol extract was used to detect bioactive functional groups and compounds. GC-MS of the animal extract was done, and FTIR analysis was thereafter used to identify the functional groups. The bioactive components were identified based on peak values in the region of infrared radiation. Results of GC-MS revealed eight major compounds like Undecane (13.16%), 2,6-Diisopropyl naphthalene (3.47%), Cyclohexasiloxane, dodecamethyl (6.33%), 6, 11-Undecadiene, 1-acetoxy-3, 7-dimethyl (2.84%), n-Hexadecanoic acid (19.91%), Triphenylphosphine oxide (4.34%), beta. - Sitosterol acetate (43.32%) and Dihydroartemisinin, 6-deshydro-5-deshydroxin (6.64%). These are bioactive compounds may act as antimicrobial, antiviral, anti-inflammatory, and antioxidant agents. FT-IR analysis results also indicated the presence of functional groups like N-H (amide group), O-H (carboxylic acid and alcohol), C-H (carboxylic acid and alkanes), and C-F (alkyl halide group) in the compounds.

**INTRODUCTION:** Man has used natural resources for medicinal uses since ancient times, and presently researchers are looking for bioactive compounds from different sources for medicinal use as therapeutic drugs without side effects. The literature survey revealed the presence of various organic molecules from natural sources like in marine animals <sup>1, 2</sup>, algae <sup>3, 4</sup>, terrestrial plants <sup>5, 6</sup>, and terrestrial animals <sup>7</sup>. These organic molecules are termed bioactive compounds, and their activities were tested for their biological and chemical properties.

Many such bioactive compounds from algae, a variety of bacterial species, and from few animals like Porifera, Coelenterate, Molluscs, and Echinoderms are studied and termed as biotoxins having medicinal properties <sup>8-14</sup>. Biological active compounds are experiencing a growing interest in a wide range of applications like geo-medicine, plant science, modern pharmacology, agrochemicals, cosmetics, food industry, nano-bio-science, etc.

Compared to terrestrial, marine organisms have more novel and unique structures owing to the complex living circumstance and diversity of species, and the bioactivities are much stronger <sup>15, 16</sup>. Fewer reports are available on the studies of marine natural products synthesized by the animals and plants were metabolized by associated microorganism's <sup>17-20</sup>. Marine animals and plants are more and more research because of the high content of active compounds, as well as the

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limitation of bioresources supply from the terrestrial sources<sup>21-24</sup>. GC-MS is a technique used in analyzing plant and animal extracts, and it is an interesting tool for testing the amount of bioactive chemicals in the pharmaceutical or food industry<sup>25, 26</sup>. On the other hand, FTIR is an analytical technique based on absorption in the infrared region, which results in changes in the vibrational and rotational states of the molecules<sup>27</sup>. FTIR is used in this study to quantify the several components absorbing in the mid-infrared region (400-5000  $\text{cm}^{-1}$ ), present in the *Zoanthus sansibaricus* extract<sup>28</sup>. Therefore, in the present study, an attempt has been made to detect the presence of possible bioactive functional groups and compounds in the *Zoanthus sansibaricus* extract by using FT-IR and GC-MS analysis.

## MATERIALS AND METHODS:

### Sample Collection and Extract Preparation:

Few live specimens of the Cnidarian species, *Zoanthus sansibaricus* were collected during October - December 2018 from Veraval coast (20°55' N and 70°20' E) off Arabian Sea of Gujarat, Western India. The animal species were identified by WoRMS (<http://www.marinespecies.org/aphia.php?p=taxdetails&id=138757>), and a sample was submitted to the Museum of the Department of Biosciences, Saurashtra University, Rajkot. Immediately upon collection of animals were stored in the 10% polarity methanol. 200 g of whole-body frozen sample was homogenized with 10% methanol, then centrifuged at 5000 rpm for 15 minutes. The supernatant was collected, filtrated, and freeze-dried at 0°C<sup>29</sup>.

### Isolation and Characterization:

**FTIR Analysis:** For identifying the functional group in the extract, FTIR technique was followed. 10 mg of dried powder of *Z. sansibaricus* was encapsulated in the 100 mg of KBr pellet in order to prepare a translucent material, and the resultant sample was loaded in the FTIR spectroscope (Shimadzu, IR Affinity1, Japan). The spectroscopy ranges from 400 to 4000  $\text{cm}^{-1}$  with a resolution of 4 $\text{cm}^{-1}$  was performed.

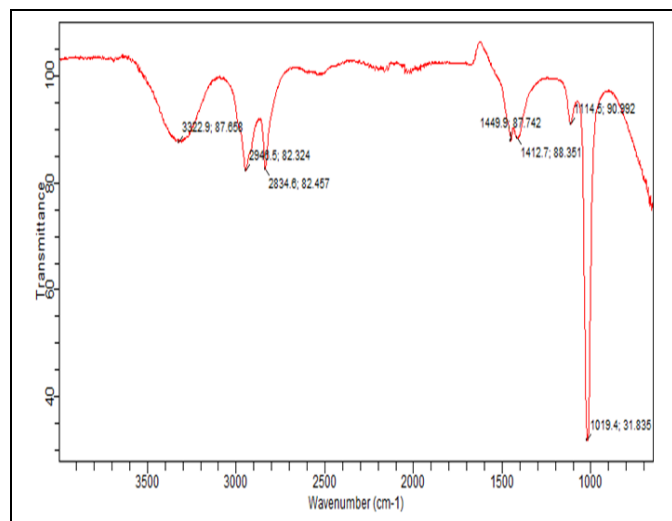
**GC-MS Analysis:** *Z. sansibaricus* extract was prepared in 10% methanol and analyzed with a GC-MS analyzer (GC-MS-QP 2010 plus Shimadzu, Japan). Helium gas was used as a carrier, and its

flow rate was 1 ml/min in split mode (10:1) v/v. Standard GC-MS protocol of Shimadzu equipment was followed for the assay. Mass spectrum of compounds in samples was obtained by electron ionization at 70 eV, and the detector operates in scan mode 50 to 600 Da atomic units. The MS Table was generated through ACQ mode scan within 0.5 seconds of scan interval at speed was 666 and fragments maintained from the 30 to 350 Da. Compound identification was done as per NIST. The component name of the extract materials was identified and calculated the percentage amount by comparing the average peak area to the total area. Unknown components spectrum was compared with the spectrum of the known components stored in the NIST library.

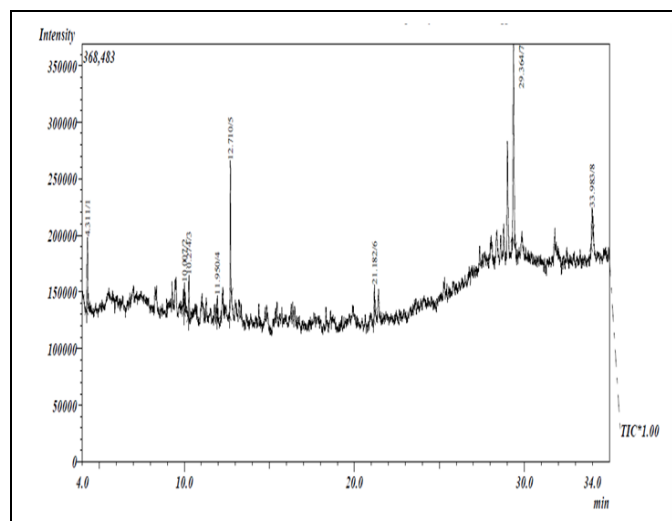
**RESULTS AND DISCUSSION:** Marine invertebrates, which develop in a different environment from terrestrial animals, are the source of a broad range of pharmacological substances. These bioactive compounds either express constitutively, or the expression is induced by exposure to pathogens<sup>30</sup>. Pharmaceutical potentials particularly in invertebrates, are of great interest due to the excellent property of marine organisms to produce bioactive groups and compounds<sup>31</sup>. The present study provides a good starting point for the analysis of unique compounds from Cnidarians, which are highly toxic otherwise, for the treatment of human diseases. GC-MS study is the first step towards understanding the nature of active principles. Further studies are needed like isolation, purification, and understanding the structural determination of the chemical compounds responsible for the biological activities, which may be lead to the discovery of drug molecules.

**FT-IR Analysis:** FT-IR analysis of methanol extract of *Zoanthus sansibaricus* was carried out, and the extract was continuously passing into the FT-IR. The extract was analyzed for identifying the functional groups of the active components based on the peak values in the region of IR radiation. The presence of C-H (alkanes), N-H (amide group), and C-F (alkyl halide group) functional groups was confirmed in the results of FT-IR analysis. The peaks were found at 3322, 2946, 2834, 1449, 1412, 1114, and 1019  $\text{cm}^{-1}$  **Fig. 1**. The peak at 3322  $\text{cm}^{-1}$  showed Hydroxyl group's presence, whereas 2946  $\text{cm}^{-1}$  showed the presence of alkanes and alkyls

group, and  $2834\text{ cm}^{-1}$  peaks showed the presence of carboxylic acid, alkanes and alkyls group. The peaks around  $1449\text{ cm}^{-1}$  showed the presence of the Methyl group,  $1114\text{ cm}^{-1}$  showed the silicon-oxy group, and  $1019\text{ cm}^{-1}$  peak showed the methylene group **Table 1**. Detection of bimolecular composition is the technique of FTIR spectroscopy proved to be a reliable and sensitive method.



**FIG. 1: FT-IR SPECTRUM OF METHANOL EXTRACT OF ANIMAL ZOANTHUS SANSIBARICUS**



**FIG. 2: COMPOUNDS IDENTIFIED FROM METHANOL EXTRACT OF ANIMAL ZOANTHUS SANSIBARICUS BY GC-MS ANALYSIS**

The presence of strong bands above  $3000\text{ cm}^{-1}$  indicates the presence of aromatic ring <sup>32</sup>. The marine animal contains a large amount of primary and secondary metabolites exert a wide range of biological activities on physiological systems <sup>33</sup>. Spectroscopic (FTIR) methods are very rapid and cost-effective than the other conventional methods. Thus, both can be used together or separately to

detect the bioactive constituents <sup>34</sup>. Alkaloids present may possess plasmolytic, anticholinergic, analgesic, stimulants, antimalarial and anesthetic activity and has a record of reducing fever and headache <sup>35</sup>. Alkanes protect animals against water loss and protect against microorganisms, and harmful insects <sup>36</sup>. Alkenes are important in the manufacture of plastics, e.g., as fuel and illuminant, and polythene. They serve as raw materials for the manufacture of alcohols. These groups are used for a general anesthetic, artificial ripening of fruits, making poisonous mustard gas, and ethylene-oxygen flame <sup>37</sup>.

**GC-MS Analysis:** The *Z. sansibaricus* extract was prepared in the methanol, and the compounds were identified by the GC-MS technique **Fig. 1**. Retention time (R) and area (%) showed in **Table 1**. Total eight compounds were present in the methanolic extract of *Z. sansibaricus* by the technique of GC-MS. Components present in the *Z. sansibaricus* were Undercane (13.16%), 2,6-Diisopropyl-naphthalene (3.47%), Cyclohexa-siloxane, dodecamethyl (6.33%), 6, 11-Undecadiene, 1-acetoxy-3,7-dimethyl (2.84%), n-Hexadecanoic acid (19.91%), Triphenylphosphine oxide (4.34%), beta-Sitosterol acetate (43.32%) and Dihydro-artemisinin, 6-deshydro-5-deshydroxin (6.64%) **Table 2**.

**TABLE 1: FT-IR ANALYSIS OF METHANOL EXTRACT OF ANIMAL ZOANTHUS SANSIBARICUS**

Sr. no.	Wave number	Functional group
1	3322	Hydroxyl or alcohol (H-bond OH stretch)
2	2946	Alkanes and alkyls (CH and CH <sub>2</sub> stretching aliphatic)
3	2834	Carboxylic acid, alkanes and alkyls
4	1449	Methyl (Methyl C-H asym./sym. Bend)
6	1114	Silicon-oxy- (SiO <sub>2</sub> )
7	1019	Methylene (Cyclohexane ring vibration)

GC-MS is an important tool for the identification of different chemical constituents from animals, and that would be important for the discovery of new therapeutic agents <sup>38</sup>. Bioactive constituents of the long chain were found to be Undercane, 2, 6-Diisopropyl-naphthalene, Cyclohexasiloxane, dodecamethyl, 6, 11-Undecadiene, 1-acetoxy-3,7-dimethyl, n-Hexadecanoic acid, Triphenyl-phosphine oxide,


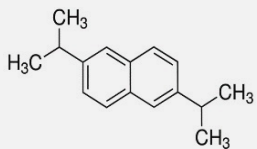
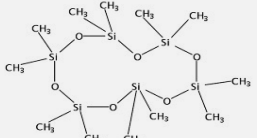
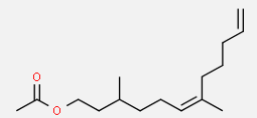

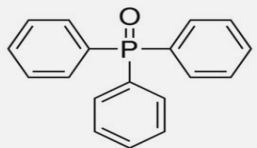
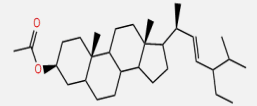
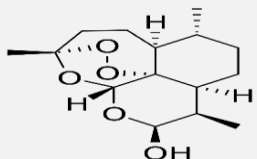
beta-Sitosterol acetate and Dihydroartemisinin, 6-deshydro-5-deshydroxin compounds<sup>39, 40</sup>. FTIR analysis has confirmed the presence of carboxylic acid, alkanes, and alkyls, alkanes, alkyl halide, and alcohol groups.

Hexadecanoic acid (palmitic acid) was the most abundant saturated fatty acid found in the animal and reported in previous studies by Rajeswari et al.,<sup>41</sup>, Anyasor et al.,<sup>42</sup> and Omotosho et al.,<sup>43</sup> to have anti-oxidant, anti-inflammatory, hypocholesterolemic, anti-androgenic, 5- $\alpha$  reductase inhibitor and hemolytic activities. Jiang et al.<sup>44</sup>, Gopalakrishnan et al.

<sup>45</sup>, Mgbeje et al.,<sup>46</sup> have also observed its anticancer and antimicrobial activities respectively. Cyclohex-asiloxane belongs to an aliphatic amine class. This compound is used for the synthesis of organic compounds such as sulphenamide, a base reagent used as accelerators for vulcanization.

Cyclohexamine is also used as a building block for pharmaceuticals, e.g., mucolytic, analgesics, bronchodilators<sup>47</sup>. Hexadecanoic acid, a fatty acid with potential antimicrobial and anti-diarrheal activities, causes growth inhibition and apoptosis induction in human gastric cancer cells<sup>48, 49, 50</sup>.

**TABLE 2: GC-MS ANALYSIS OF METHANOL EXTRACT OF ANIMAL ZOANTHUS SANSIBARICUS**

S. no.	Retenti on time	Name	Molecular Formula	Molecular Weight	Molecular Structure	Peak area %
1	4.311	Undecane	C <sub>11</sub> H <sub>24</sub>	156.31		13.16
2	10.007	2,6-Diisopropyl-naphthalene	C <sub>16</sub> H <sub>20</sub>	212.33		3.47
3	10.274	Cyclohexasiloxane, dodecamethyl	C <sub>12</sub> H <sub>36</sub> O <sub>6</sub> Si <sub>6</sub>	444.924		6.33
4	11.950	6,11-Undecadiene, 1-acetoxy-3,7-dimethyl	C <sub>16</sub> H <sub>28</sub> O <sub>2</sub>	252.392		2.84
5	12.710	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.43		19.91
6	21.182	Triphenylphosphine oxide	C <sub>18</sub> H <sub>15</sub> OP	278.28		4.34
7	29.364	Beta -Sitosterol acetate	C <sub>31</sub> H <sub>52</sub> O <sub>2</sub>	456.74		43.32
8	33.983	Dihydroartemisinin,6-deshydro-5-deshydrox	C <sub>15</sub> H <sub>22</sub> O <sub>3</sub>	250.33		6.64
						100.00

The rich diversity of marine animals has the potential to discover a number of bioactive compounds from them. The biologically active molecules interfere with the prevention of disease at many different points, which is increase the chances of developing selective drugs against a specific disease.

Marine invertebrates have provided many examples of novel secondary metabolites that possess varied chemical status and potent anti-malarial, anti-inflammatory, anti-carcinogenic, anti-bacterial, anti-fungal *etc.*, activities. Therefore, the results of the present study in this direction using *Z. sansibaricus* are promising.

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