Introduction

The marine biodiversity is largely unexplored and offers great opportunity for the discovery of new bioactive compounds. The chemical constitution of the extracts was recognized using standard methods of chemical and spectral analysis. Chemical examination were carried out to detect the presence of elements like nitrogen, sulphur and halogens that occur in organic functional groups apart from the common ones viz. carbon, hydrogen and oxygen and to ascertain the functional groups present in the compounds.

FT - IR spectroscopy provides valuable information regarding the functional group of a compound as well as the nature of the substituents (Gilbert, 1984). It is also used as a fingerprinting device for establishing the identity of two samples (Kalsi, 2001). The application of Gas chromatography coupled with mass spectrum resulted in the successful elucidation of the compounds from crude extracts (Babu et al., 1997). Mass spectrometry serves as a valuable tool in determining the molecular weight and probable structure even with very small sample size (Silverstein et al., 1998). The Electron Impact Mass spectrometry (EIMS) is useful for volatile compounds.

Thus, an efficient targeted isolation of compounds exhibiting interesting chemical or biological features can be achieved by means of these methods. In the present study, the crude extracts of the flat fishes, *Pseudorhombus malayanus, Pseudorhombus elevatus, Pseudorhombus triocellatus* and *Pseudorhombus natalensis* using the solvents methanol and petroleum ether have been analyzed using GC - MS methods. The chemical compositions of the extracts were ascertained using the data obtained from the elemental, functional group analysis, FT-IR as well as UV spectral studies.
Materials and Methods:

Preparation of questionnaire (LHT, Bangalore, 1996)

The format of questionnaire prepared based on the modified version of Foundation for Revitalization of Local Health Tradations, Bangalore, 1996.

Materials: The members of the family: Paralichthyidae viz.

1. Pseudohombus malayanus
2. Pseudohombus elevatus
3. Pseudohombus triocellatus
4. Pseudohombus natalensis.

are available in the study area (Pondicherry Coastal Waters) almost through out the year. All the four species are taken up for the present study.

Sample collection:

The freshly animals were caught from the bottom travelers and there were kept in icebox on board and transported to laboratory for further analysis. The size of the fishes collected and analyzed varied between 15 cm - 25 cm (Total length).
BIOCHEMICAL INVESTIGATION

EXPERIMENTAL STUDIES:

*Extraction and Fractionation (Shiomi, 1980)*

Extract of all the four fishes in two solvents *viz.* methanol and petroleum ether are taken using soxhlet apparatus for 6 hours following the method of solvents extraction following the method adopted by Shiomi, 1980. The resultant extract after three times of extraction was taken for further experimental studies.

**Chemical analysis:**

The chemical analysis of each of the extract that showed significant pharmacological property, i.e. methanol extract of *Pseudorhombus elevatus* was carried out to ascertain the organic nature of the extract (Fritz Feigl, 1996; Wolfing Vogel: 1997) and their functional groups as well as the elemental analysis (Thomas, 1975: Robert *et al.*, 1985). The characterization was carried out UV, FT-IR and GC - MS, spectrometry at SPIC science foundation, Chennai.

**UV analysis:**

UV spectrum for the test extract was recorded in SHIMADZU-UV-VIS-160 spectrophotometer, Japan, 1mg of sample was dissolved in 3ml of HPLC grade methanol and the $\lambda_{\text{max}}$ was recorded between 200nm and 500nm where the extracts exhibited absorption maxima.

**FT-IR analysis:**

FT - IR spectra were recorded in FT-IR Bruker IFS 85, Germany, A micro drop of crude extract was taken in an IR Golden cell and placed in the cell cavity. FT-IR spectrum was recorded between 4000cm$^{-1}$ and 400cm$^{-1}$ for the entire test extracts under study.
GC-MS analysis:

15 mg of crude extract was dissolved in 1 ml of spectroscopic grade methanol. About 2 ml of each of the dissolved sample was injected into the sample injection port of GC-MS (SHIMADZU QP 5000, Japan). The column (25 mm x 0.25 mm dia) was packed with CBP-1, the temperature increased linearly from 50º C to 320º C and the carrier gas pressure fixed at 79.80 Kpa for the entire test sample studied.

Mass spectra-EIMS (Electron Impact Mass Spectrum) were recorded for each compound separated in succession by GC, the relative intensities corresponding to their R_t of the molecular ion peak and the fragmented ion peaks were normalized with respect to the base peak.
RESULT:

FT-IR vibrational frequencies obtained at 3358 cm\(^{-1}\) inferred the O-H stretching (hydrogen bonded intermolecules), overtone bands at 2490 cm\(^{-1}\), the bands at 2242 and 2074 cm\(^{-1}\) corresponded to N-O bending, C=O stretching at 1604 cm\(^{-1}\), C=O symmetric carboxylate stretching at 1413 cm\(^{-1}\). The C-O and c-\(\text{c}^\text{co}-\text{o}\) stretching appeared at 1208 and 116 cm\(^{-1}\) respectively. O-C=O asymmetric stretching at 1045 cm\(^{-1}\) and a sharp –OH out of plane bending at 971 cm\(^{-1}\). All these characteristic bands of the FT-IR spectrum of the extract indicated the presence of OH, COOH and NO\(_2\) groups. The UV absorption maxima appeared at 294nm with a shoulder at 321 and 379nm indicated the aromatic nature of the compounds.

The Positive EIMS of the compound corresponding to molecular ion peak appeared at m/z 491 (m\(^+\), 10) compatible with the molecular formula C\(_{23}\)H\(_{22}\)N\(_7\)O\(_7\)Scl. The other fragment ions detected at m/z 477(m\(^+\)-CH\(_2\),5), 432(477-COOH,30), 404(432- C=O,5), 356(404-CHCl,20), 342(356-CH\(_2\),15), 282(342-CH\(_2\)CH\(_2\)SH,15), 221(282-CH\(_2\)CH\(_2\)SH,15), 147(221-CH\(_2\)CH\(_2\)NO\(_2\),15), 98(147-CH\(_2\)Cl), 73(98-C=CH,100), 59(CH\(_3\)COOH, 50) suggested the compound to be 5'-ethyne 3'-methoxy 4'-ethanethiol- 2-ethanoate 5-nitroethane –3- chlorodiphenyl 4-ethanoic acid with the structure
The molecular formula and the molecular mass obtained in the EIMS were in good agreement with the above structure.

The molecule ion peak corresponding to the compound 2 at m/z 149 (H⁺, 100) was consistent with the molecular formula C₈H₇O₂N. The fragmentation pattern observed in EIMS with peaks at m/z 104 (m⁺-NCH₃O, 5), 75(104-CH₂-CH₂-CH₃, 20) suggested the compound to be 2,3 dihydroxy indole with the structure.

\[ \text{OH} \quad \text{N-H} \quad \text{OH} \]

The molecular formula and the molecular mass were found to be in good agreement with the structure.
Fig. 2.3 GC-MS of crude methanol extract of *pseudorhombus elevatus*
Fig. 2.2 FT-IR SPECTRUM OF CRUDE METHANOL EXTRACT OF *Pseudorhombus elevatus*
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FIG 2.1 UV spectrum of crude methanol extract of pseudorhombus elevatus
DISCUSSION:

Among the four fishes selected for the present study, the crude methanol extract of *Pseudorhombus elevatus* alone analysed systematically for their chemical constituents since it showed significant bioactivity in the pharmacological and antibacterial studies. The biochemical investigation and meticulous partial characterization revealed the presence of 2 bioactive compounds viz. 5’ ethyne 3’methoxy 4’-ethanethiol, 2-ethanoate, 5-nitroethane, 3-chloro diphenyl, 4-ethanoic acid and 2,3 dihydroxy indole. These two compounds have been systematically characterized based on the available chemical and spectral analysis including EIMS coupled with GC-MS.

Since fishes are unable to synthesis bioactive substances denovo, they depend on a dietary intake and such compound with bioactivity might be the secondary metabolites resulted from the various metabolic processes. Thus the fish *Pseudorhombus elevatus* claim its significance in having two pharmacologically useful compounds with analgesic and anti-inflammatory potential. However further studies on the structural profile of the pure fraction of these bio-active compounds would be of more valuable in future.
Fig. 2.5. EIMS of compound 2 of crude methanol extract of *pseudorhombus elevatus*
Fig. 2.4 EIMS of compound 1 of crude methanol extract of *pseudorhombus elevatus*