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Bioactive Potential 6f Tonna Galea (Linne. 1758) From Gulf of Mannar

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Abstract: Crude methanol was prepared from whole *Tonna galea* (Tonnidae) and fractionated by Hexane: Chloroform (F1), Chloroform (F2), Benzene (F3), Benzene: Methanol (F4) and methanol (F5) solvents were analyzed for antimicrobial activity using agar well diffusion technique against several bacterial pathogens. Minimal inhibitory concentrations were then determined for the most potent fraction. To identify the compound responsible for antibacterial activity, the potent fraction was subjected to GC-MS analysis. The broadest antibacterial activity was noted in fraction 1 and fraction 5 The highest activity was exhibited against Vibrio cholerae (0139) in the crude extract.The column purified Hexane: Chloroform fraction showed higher activity against *Vibrio cholerae* 0139 (8mm) and maximum inhibition zone was exhibited against *Aeromonas hydrophila* (10mm). These promising results confirms the presence of Benzenedicarboxylic acid. Mono (2-ethyle xyl) ester, squalene and methyl 3-bromol-1- adamantaneacte by GC-Ms may be related to mollusc defense mechanism and antimicrobial activity against these pathogens.

Key words: Mollusc · Antibacterial · Gulf of Mannar

INTRODUCTION

Most of the pathogens are increasingly resistant to the major classes of the routinely used antibiotic. So there is an urgent need for the discovery of new and novel antimicrobial drugs to effectively combat not only the drugs resistance but also the new disease producers, hence the search for active drugs from alternative sources including marine environment, obviously becomes imperative. The diversified marine organisms assume a great diversity of the discovery of new bioactive substances, the ocean remain as sparingly tapped source for many drugs and contemporary experimental studies indicate that, pharmacologically active substances could be isolated from marine organism [1]. During the last, natural products isolated from marine organisms increases rapidly and hundreds of new compounds being discovered every year [2, 3]. Marine invertebrates offer good source of potential antimicrobial drugs [4-6]. Among the invertebrates, the molluscs are very good source for biomedical important products [7]. Many classes of molluscs exhibit bioactive compounds like antitumour, antileukemic, antibacterial, cytotoxic, anti-inflammatory and antiviral properties [8-10]. Discovered bioactive compounds in molluscs are identified and they are presented specific types of activities [11, 12]. The presence of antimicrobial activity in molluscs has been reported from the mucus of the giant snail *Achatina fulica* [13] and from the egg mass and purple fluid of the seahare *Dobella auricularia* [14]. Proteins and glycoproteins with antibacterial activity have been demonstrated in the digestive organs of various molluscs [15, 16].

A variety of antimicrobial factors, including chlorinated acetylenes, indole derivates [17], glycoproteins [18], proteins [14] have been isolated from molluscs. These reports suggest that molluscs are a rich source for discovering novel lead compounds for the possible development of new types of antibiotics for pharmaceutical use. Keeping the importance of gastropods in terms of bioactive compounds with antibacterial properties, the present study has been undertaken to ascertain the antibacterial activity of extracts from *Tonna galea* (Linne. 1758) (Tonnidae) against some pathogenic bacterial strains.

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MATERIALS AND METHODS

Collection and Preparation of Samples: The molluscs *T. galea* was collected from muddy bottom of deep waters of harbour area of Gulf of Mannar, near by Theraspuram,Tuticorin, situated in the south east coast of India, during April 2010 to December 2010. The collected samples were rinsed with sterile sea water to remove the associated debris and salt. Crude methanolic extracts of mollusca was prepared [19].

Microbial Strains Used: Antimicrobial activity of tissue extracts was determined against 7 different bacterial pathogens, viz., *Bacillus cereus*, *Vibrio cholerae* classical, *Vibrio cholerae* 0139, *Escherichia coli*, *Pseudomonas aerogenosa, Aeromonas hydrophila* and *Salmonella typhi*. These clinical strains were obtained from Department of Basic Biomedical Sciences, Bharathidasan University, Trichy.

Antibacterial Susceptibility Assay: *In vitro* anti bacterial activity was assayed by the disc diffusion method [20]. A known mg of crude gastropod extract was dissolved in 0.6ml of solvent (methanol) and applied to 6mm sterile disc. In the same way for control 0.6 ml of methanol was soaked in sterile disc. Both the discs were allowed to dry at room temperature. Pathogenic bacterial strains were inoculated in sterile broth and incubated at 37°C for 24hr. Pathogens were swabbed on the surface of sterile petri dishes in 20ml of solidified nutrient agar. The control and the experimental discs were placed in the sterile solidified nutrient agar petri plates to asses the effect of solvent and extracts on pathogens. These agar plates were incubated at 37°C for 24hrs and the antibacterial activity was measured accordingly based on the inhibition zone around the disc impregnated with gastropod extract. Antibacterial activity was expressed in diameter zone of inhibition which was measured with the outer side of the disc to inner side of the inhibition zone. Each active extract was tested thrice for confirmation of activity.

Crude extract was fractionated and elusions were made with Hexane: Chloroform (F1), Chloroform (F2), Benzene (F3), Methanol: Benzene (F4) and Methanol (F5). Eluted fractions were assayed for antibacterial activity following the above mentioned disc diffusion method but distilled water was used for control. To estimate the minimum inhibitory concentration of extract three different concentrations such as 1, 10 and 100 mg/ml were prepared and they were tested against the pathogens. After 24hrs of inhibition, the plates were removed and observations were made for inhibition zone against the pathogens. The most potent column chromatography extract of the test animal was subjected to GC-MS study which was carried out on a GC Clarus 500 Perkin Elmer system.

Identification of Compounds: Interpretation on mass spectrum GC-MS was conducted using the data base of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The electrons impact fragmentation patterns of the mass spectra of column fractioned test mollusc unknown components found were matched with the spectrum of the known components stored those in NIST ver.21 National Institute of Standard Technology, the mass spectra library.

RESULTS

Antibacterial activity of extracts from *T. galea* was presented in Figures 1&2a-e and plate 1. The crude methanol extract of *T. galea* the range of activity varied from 2mm (*B.cereus* and *V. cholerae classical*) to

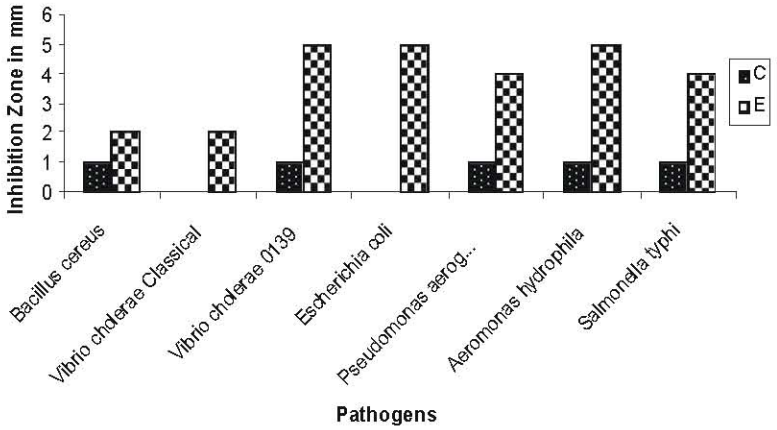

Fig. 1: Antibacterial activity of crude methanol extract of *T. galea* against pathogens

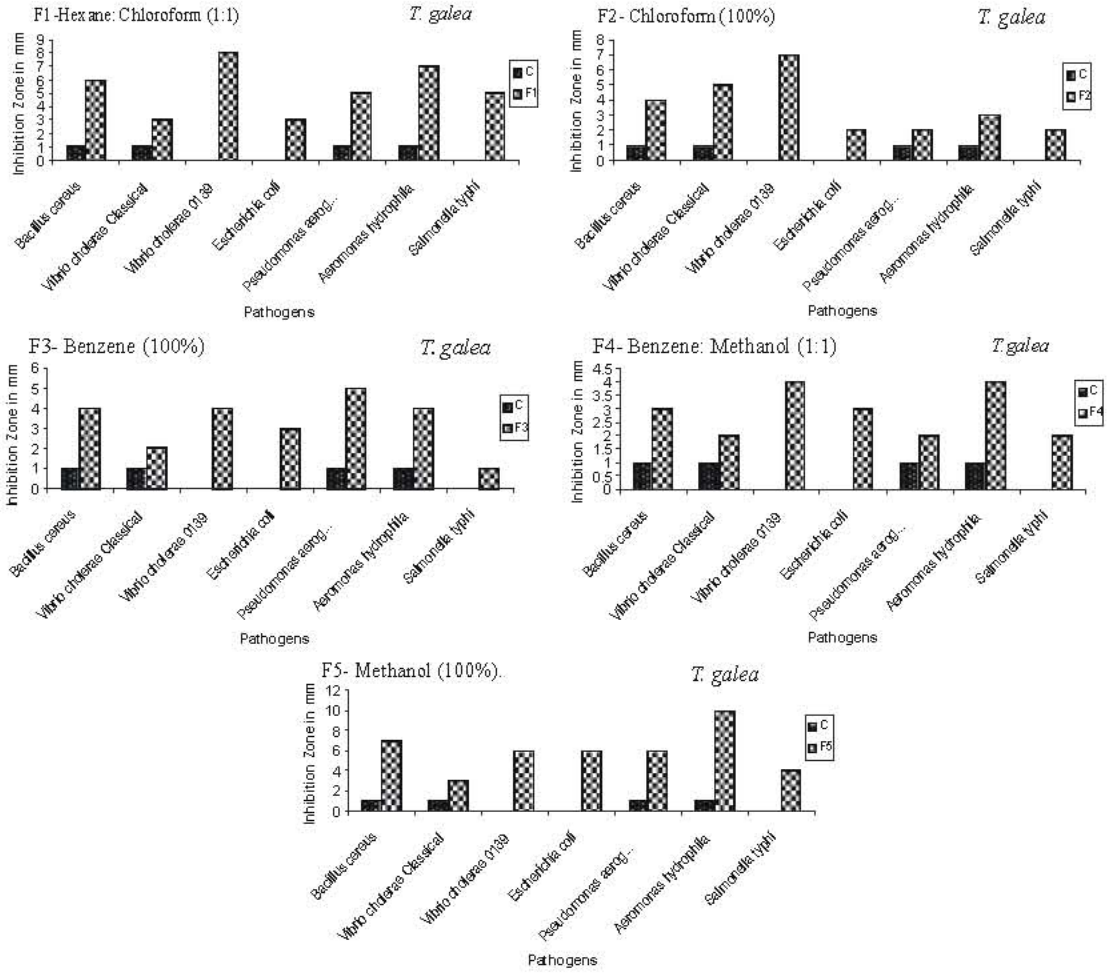

Fig. 2: Antibacterial activity of column fractionated extract of *T. galea* against pathogens

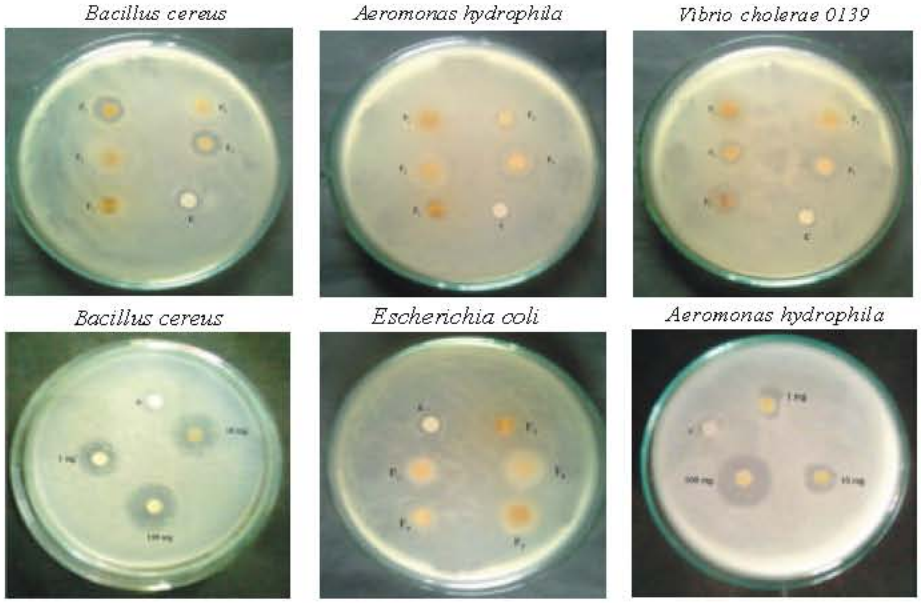

Plate 1: Antibacterial activity of different column fractions and mic of *T.galea*

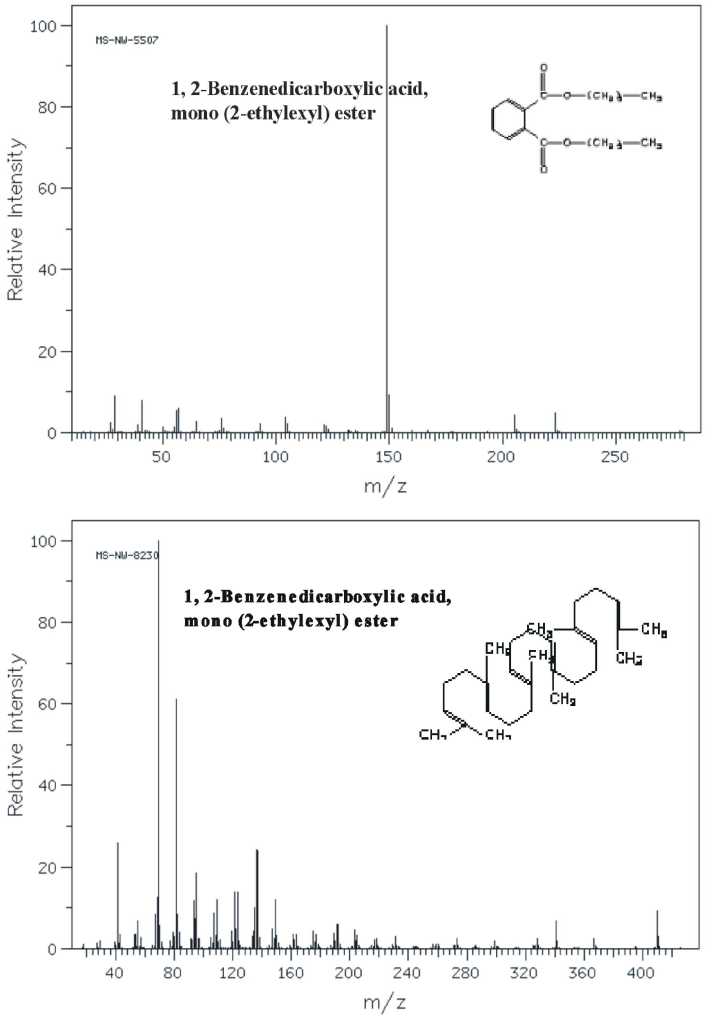

Fig. 3: GC-MS spectra of some compounds present in the methanolic extract of *T. galea*

5mm (*V. cholerae* 0139 and *A. hydrophilic*). Of the five column chromatographic fractions, maximum number of pathogens was inhibited by F1 and F5 fractions (Figs.1&2a-e and plate1). The highest activity of F1 fraction was exhibited against *V. cholerae* 0139 (8mm) and the least against *V.cholerae* classical and *E. coli* (3mm) It also showed inhibitory activity against *B. cereus*, (6mm) and *A. hydrophila* (7mm). F5 fraction showed maximum activity against *A. hydrophila* (10mm) and the lowest against *V.cholerae* classical (3mm). This fraction also arrested the growth of *B. cereus* (7mm), *V. cholerae0139*, *E. coli* and *A. hydrophila* (6mm) respectively. MIC of various fractions revealed that *V. cholerae* 0139 showed maximum inhibitory zone of 7mm at 100mg concentration when treated with F4 fraction and at the same concentration in F5 fraction *A. hydrophila* growth was arrested with the formation of 9mm (Plate 1). The following compounds were identified from mass spectra analysis. Decane, 2, 5, 9-trimethyl-(1.58%), 1, 2-Benzenedicarboxylic acid, mono (2-ethylexyl) ester (20.83%), Squalene (49.94%) and Methyl 3-bromol-1-adamantaneacetate (27.55%), (Fig.3).

DISCUSSION

Several marine molluscan extracts possessed broad spectrum antimicrobial activities affecting the growth of bacteria, fungi and yeasts [9, 21, 22]. Antibacterial activity has previously been described in a wide range of molluscan species [23, 17]. Antibacterial activity of common marine molluscs from Parangipettai coast was studied and reported that the methanol extract of molluscs exhibited significant activity against *Escherichia coli* [8]. This finding corroborates the results of the present study since methanol extract of *T. galea* showed pronounced activity

against *E. coli*. The inhibitory action of the methanol fraction of *Perna viridis* was reported against bacterial and fungal strains [24]. Similar result was also reported in four bivalves against few pathogens and found that methanol extract showed significant activity against *Bacillus subtilis* [25]. Antibacterial activity of opercular extract of *Chicoreus ramosus* and *Pleuroploca trapezium* against six bacterial pathogens was reported [26]. The methanolic extract of *Chicoreus virgineus* and *Chicoreus ramosus* experimentally analyzed and observed the broad spectrum antibacterial activity of body tissue extract [27]. The antibacterial activity *of* a marine mollusc *Babylonia spirata* was screened against *bacterial* pathogens [28]. Similar result was also reported [29] in chloroform extract of *Pterai chinensis* which inhibited eight fish pathogens and the acetone extract in the same animal showed broad spectral activity against all the fish pathogens tested. Acetone column purified fractions of *Trochus tentorium* shown the highest antibacterial activity [30]. The difference in antibacterial activity found in the molluscan extracts may depend on the solvents used for extraction and the compounds extracted [31]. Some of the peptides obtained from oysters inhibited *E.coli* at 330 mg/ml and *Vibrio alginolyticus* at 162 mg/ml [32] and the observed range of *T. galea* due to the methanol extract was well within the range of activity reported earlier.

In the present study the magnitude of inhibition of column fractionated extract of *T. galea* possibly reveal the presence of antimicrobial compounds of the five fractions, t he number of fractions active was F1 and F5, respectively in the column as well as in MIC. *A. hydrophila,* V. *cholerae* 0139, *E.coli and B.cereus* were the most susceptible pathogens in concern with the *T. galea* extract. For the susceptibility of the above mentioned pathogens the following compounds Decane, 2, 5, 9-trimethyl-(1.58%), 1, 2-Benzenedicarboxylic acid, mono (2-ethylexyl) ester (20.83%), Squalene (49.94%), Methyl 3-bromol-1- adamantaneacetate (27.55%) identified from GC-MS analysis might be responsible. Further the squalene is a chemical compound proved to be a preventor of VU rays and also believed to be an antitumour agent. The presence of higher concentration of squalene in a test animal *T. galea* would raise the hope of extraction of antitumour product. Further 2% of squalene in diet given to experimental animals made the animal resistant to gamma radiation [33]. As the molluscan resources are rich and varied in Indian coasts, there exist a great potential for the extraction of bioactive compounds of medical importance at a lower cost.

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