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BIOACTIVE POTENTIAL OF FUSINUS NICOBARICUS FROM GULF OF MANNAR

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Abstract: The whole body crude extract of *Fusinous nicobaricus* with Methanol,Chloroform, Ethylacetate, Methanol and Chloroform (1:1) and Ethyl acetate and Chloroform (1:1) solvent were assayed against six human bacterial pathogens viz Vibrio *cholerae Ogawa, Salmonella typhi, Shigella flexneri Escherichia coli, Mycobacterium tuberculosis and Pseudomonas fluorescens* using disc diffusion technique. Among the crude extracts of methanol, ethylacetate, chloroform and methanol and ethylacetate (1:1) and ethylacetate and chloroform (1:1) extracts of *F.nicobaricus* maximum inhibition zone was obtained in ethylacetate followed by ethylacetate and chloroform (1:1) extract and the most sensitive pathogens inhibited were *P.fluorescens, S.flexneri, S. typhi* respectively. To identify the compound responsible for antibacterial activity the most potent ethyl acetate extract was subjected to GC-MS analysis. 14 compounds were identified and characterized. Of the fourteen compounds Cyclohezane, isothiocynato, Phenol, 2,4-bis (1,1-dimethylethyl), 9,12-Octadecadienoic acid(Z,Z), 1,4-Benzenediamine,N-(1,3-dimethylbutyl)-N'-ph enyl, 1-Hexadecanol,2-methyl,1,2-Benzenedicarboxylic acid, diisocytyl ester, 1-Docosanol, Methoxyacetic acid, 2-pentadecyl ester, Cholesterol and Ergost-5-en-3-ol,(3a), might be responsible for antibacterial activity.

Keywords: Antibacterial activity, inhibitory zone, GC-MS analysis, test pathogens.

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INTRODUCTION

Marine organisms comprise approximately a half of the total biodiversity, thus offering infinite source to discover useful therapeutics. The marine environment comprises of complex ecosystem an extremely diverse reservoir of life, with a plethora of organisms and many of these organisms are known to possess bioactive compounds as a common means of defense. Apart from the food that is derived from the marine environment, a wide variety of bioactive substances is being isolated and characterized several with great promise for the treatment of human disease. Infectious diseases caused by bacteria, fungi and viruses are still a major threat to public health, despite the tremendous progress in human medicine. As a result of the continuous evolution of microbial pathogens towards antibiotic-resistance, there have been demands for the development of new and effective antimicrobial compounds.

One solution to the global crisis of antibiotic resistance is the discovery of novel antimicrobial compounds for clinical application. In the last several decades, research has expanded from land to ocean in order to find new drug and this diversity has provided a unique source of chemical compounds with potential bioactivities that could lead to potential new drugs candidates.

A number of biologically active compounds with varying degrees of action, such as antimicrobial, antitumour, anticancer, antileukemic, antibacterial, antiviral, antiproliferative, cytotoxic and photoprotective, as well as antibiotic and antifouling properties, have so far been isolated from marine sources (Benkendorff *et al*., 2001; Villa.2010; Mayer *et al*., 2011; Blunt., 2011; Rajaganapathi *et al*., 2002; Prem and Patterson, 2002;; Haug *et al*., 2003).

The phylum Mollusca, which includes soft-bodied invertebrates, the second largest phylum in the animal kingdom makes up a major part of the world's marine invertebrate fauna. The potential of marine molluscs as a source of biologically active products is largely unexplored in India. Hence a broad based screening of marine molluscs for bioactive compounds is necessary. Considering all the above facts the present study has been undertaken to test the molluscan extract against human pathogens and to find out the functional groups of the most potent fractions.

MATERIALS AND METHODS

PREPARATION OF EXTRACTS

The molluscs ***Fusinus nicobaricus*** *is* commonly known as Nicobar spindle is a species of sea snail, a marine gastropod mollusc was collected from muddy bottom of deep waters of Gulf of Mannar, near by Theraspuram, Tuticorin, situated in the South east coast of India, in the month of December 2014. The collected samples were rinsed with sterile sea water to remove the

associated debris and salt. Test animals were first carefully removed from their shells. The flesh was cut into small pieces and air-dried. The dried animal sample was ground well into a fine powder in a mixer grinder. Each 150 gram of this fine powdered samples were soaked in AR grade of methanol, chloroform and ethyl acetate and methanol (1:1) and chloroform and ethylacetate (1:1) and chloroform for 15 days and these extract were filtered through WhatMann No.1 filter paper and evaporated to dryness in rotary evaporator and the dried extract was stored at 0°C for further use..

MICROORGANISMS USED

The test organisms were supplied by the Basic Biomedical Science, Bharathidasan University, Trichy. Six bacterial stains viz Vibrio *cholerae Ogawa, Salmonella typhi, Shigella flexneri Escherichia coli, Mycobacterium tuberculosis and Pseudomonas fluorescens* were used in the study. The organisms were sub cultured on Muller Hinton Agar medium, incubated at 37°C for 24hrs and stored at 4°C in the refrigerator to maintain stock culture.

PREPARATION OF MEDIA

The medium was prepared by dissolving nutrient agar (Hi Media Laboratories Pvt. Ltd.,) in distilled water and autoclaving at 121°C for 15 minutes. It is used for preliminary antibacterial study.

PREPARATION OF INOCULUM

Stock cultures of *V. cholera Ogawa, S. typhi, S. flexneri, E. coli, M. tuberculosis and P. fluorescens* were maintained at 4°C in slopes of nutrient agar. Active cultures for experiment were prepared by transferring a loopful of microorganisms from stock cultures to test tubes of nutrient broth and incubated for 24 hours at 37°C.

ANTIBACTERIAL SUSCEPTIBILITY ASSAY

In vitro anti bacterial activity was assayed by the disc diffusion method (Bauer *et al.*, 1996). A known mg of crude extract was dissolved in 0.6 ml of solvent (methanol) and applied to 6mm sterile disc. In the same way for control 0.6 ml of respective solvent 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 24 hrs. Pathogens were swapped on the surface of sterile petridishes in 20ml of solidified nutrient agar. The control and the experimental discs were placed in the sterile solidified nutrient agar petriplates to assess the effect of solvent and extracts on pathogens. These agar plates were incubated at 37°C for 24 hrs. After 24hrs the plates were removed and observations were made for inhibition zone against the pathogens. The most potent fractions of test mollusc was subjected to GC-MS to

characterize the possible compounds responsible for antimicrobial activity. 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.

RESULT AND DISCUSSION

The result of antibacterial activity of crude methanal, chloroform, ethylacetate, methanal and chloroform (1:1) and ethylacetate and chloroform (1:1) extracts of *F. nicobaricus* were given in Figures 1-5.

Antibacterial activity of *F. nicobaricus* the result revealed that the crude methanol extract showed the highest activity against (3mm) *S.flexeneri* and *E.coli* (3mm) (Table-1) and the lowest against *S.typhi* (1mm) (Fig-1). Similar observation was made by (Pasiyappazham Ramasamy *et al.*, 2012) in crude extract of methanolic extract of gastropod *Hemifusus pugilinus* with highest activity against *E.coli* . The present finding agrees well with the result of broad spectral activity for the methanolic whole body extract of Phalium glaucum against six human pathogenic bacteria (Thilaga *et al.*, 2014). Rajaganapathi (2002) also reported that methonalic extracts exhibited inhibition against *Streptococcus pyogenes.*

Crude extract of Chloroform was exhibited the highest activity (4mm) against *S.flexneri* (Fig-2). The crude extract of ethylacetate showed the maximum activity against *P.fluorescens* (8mm) (Fig-3) and very meager activity was exhibited against *M. tuberculosis* (1mm). *S.flexneri* exhibited the uppermost activity (5mm) in crude extract of Methanol and Chloroform (1:1) (Fig-4). Earlier work performed by Chellaram *et al.,* (2004) on the antibacterial activity of the winged mollusc *Pteria chinensis* reported that out of the six solvents used the extract obtained from the acetone and chloroform exhibited higher antibacterial activity against human pathogens which stands by the present work (). In ethylacetate and chloroform (1:1) extract the highest activity was obtained against (9mm) *P.flurescens* (Fig-5) and very trace activity against *M.tuberculosis* (2mm) (Fig-5). In the similar way Periyasamy *et al* .,(2012) reported that *Babylonia spirata* exhibited the antibacterial activity of Ethanol, acetone , methanol, chloroform, ethylacetate, extracts the maximum inhibition zone 12 mm was observed against *Psudomonas aeruginosa* in the crude ethanol extract of *Babylonia spirata* and the minimum inhibition zone (2 mm) was noticed against *Staphylococcus aureus.*

Among the crude extracts of methanol, ethylacetate, chloroform and methanol and ethylacetate(1:1) and ethylacetate and chloroform(1:1) extracts of *F.nicobaricus* maximum inhibition zone was obtained in ethylacetate followed by ethylacetate and chloroform(1:1) extract and the most sensitive pathogens inhibited were *P.fluorescens, S.flexneri,S. typhi* respectively. Anbuselvi *et al.*, (2009) reported that the maximum inhibition zone was observed against Eschericha *coli*, Shigella dysendriae, *Klebsiella pneumoniae and Vibrio cholerae* in the

ethyl acetate extract of *Trocus tentorium*. Since the ethylacetate extract exhibited maximum activity among the tested pathogens the crude extract was subjected to GC-MS analysis to find out the responsible compound for the inhibitory activity of the tested human pathogens. The following compounds were identified from mass spectra analysis (Table-1 & Figure -6) Cyclohezane, isothiocynato, Phenol, 2,4-bis (1,1-dimethylethyl), Benzene, 1,3,5-tri tert-butyl, Tetradecanoic acid, n-Hexadecanoic acid, 9,12-Octadecadienoic acid(Z,Z), 1,4-Benzenediamine,N-(1,3-dimethylbutyl)-N'-ph enyl, 1-Hexadecanol,2-methyl,1,2-Benzenedicarboxylic acid,diisocytyl ester, Tetradecane, 2,6,10-trimethyl, Heptacosane, Octacosane, 1-Docosanol, Methoxyacetic acid, 2-pentadecyl ester, Cholesterol, Hentriacontane, Ergost-5-en-3-ol,(3a),Octadecane,3-ethyl-5-(2-ethylbutyl). The present findings are in agreement with (Emiliano Manzo *et al.*, 2007) who reported that two novel triterpenoids, aplysoils A and B, ßEtzionin a tyrosin derived compound exhibited antibacterial activity against *Bacillus subtilis.* An antimicrobial peptide from the seminal plasma of the mud crab *Scylla serrata* was isolated by Wang *et al.*, (2007). A polyproline type AMP isolated from the Chilean scallop *Argopecten purpuratus* showed antifungal activity against *Fusarium oxisporum* and *Saprolegnia parasitica* (Arenas *et al.*, 2009).

CONCLUSION

The present study indicates that the whole body extracts of *F. nicobaricus* would be a good source of anti bacterial agent and would replace the existing inadequate and cost effective antibiotics. It is worthy to note that the product from natural source is good for health and devoid of side effects. However further investigations involving characterization and purification of the active extracts as drugs and application of the extracts as drug for human administration need more research.

Table-1 Activity of Components identified in Ethyl acetate extract of F. nicobaricus by GC MS

No.	RT	Name of the compound	Molecular Formulae	MW	Peak Area %	Compound Nature	**Activity
1.	3.92	Cyclohexane, isothiocyanato-	C7H11NS	141	0.25	Sulfur compound	Antimicrobial
2.	7.17	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	206	0.13	Phenolic compound	Antimicrobial Anti-inflammatory Antioxidant
3.	10.23	Tetradecanoic acid	C14H28O2	228	0.38	Myristic acid	Antioxidant Cancer preventive Hypercholesterolemic
4.	14.52	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	1.99	Linoleic	Antiinflammatory, Hypocholesterolemic Cancer preventive, ,

						acid	Anticoronary,
5.	16.93	1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl-	C18H24N2	268	3.06	Amino compound	Antimicrobial Anti-inflammatory
6.	18.28	1-Hexadecanol, 2-methyl-	C17H36O	256	0.49	Alcoholic compound	Antimicrobial
7.	19.70	1,2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390	70.94	Plasticizer compound	Antimicrobial Antifouling
8.	24.95	1-Docosanol	C22H46O	326	1.05	Alcoholic compound	Antimicrobial
9.	26.23	Methoxyacetic acid, 2-pentadecyl ester	C18H36O3	300	1.86	Acetic acid compound	Antimicrobial
10.	27.03	Cholesterol	C27H46O	386	12.10	Steroid	Antimicrobial Anti-inflammatory Anticancer
11.	28.11	Ergost-5-en-3-ol, (3à)-	C28H48O	400	1.06	Steroid	Antimicrobial Anti-inflammatory Anticancer

**Source: - Dr.Duke's Phytochemical and Ethnobotanical Databases

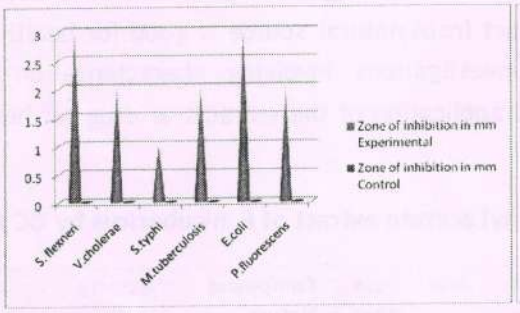

Figure-1

Antibacterial activity of Methanol extract of *F. nicobaricus*

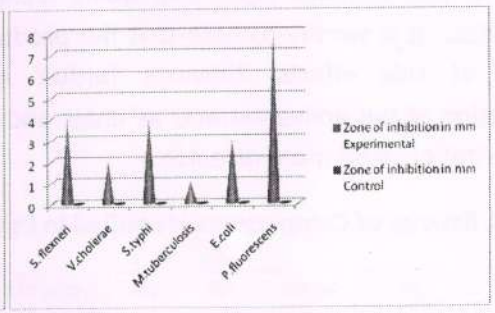

Figure-2

Antibacterial activity of Chloroform extract of *F. nicobaricus*

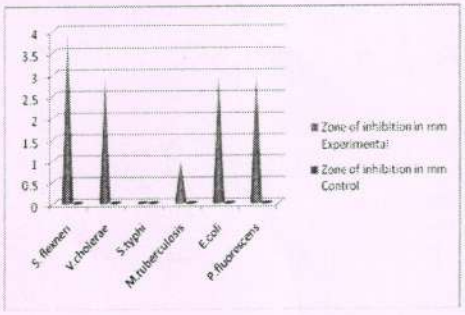

Figure-3

Antibacterial activity of Ethylacetate *F. nicobaricus*

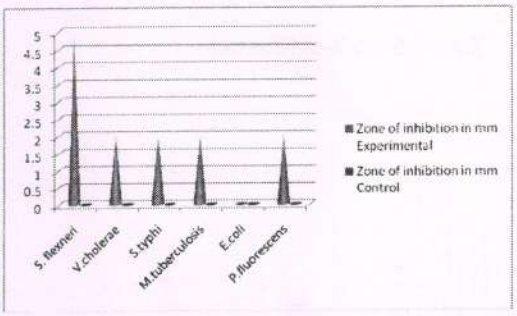

Figure-4

Antibacterial activity of Methonal and extract of Chloroform (1:1) extract of *F.nicobaricus*

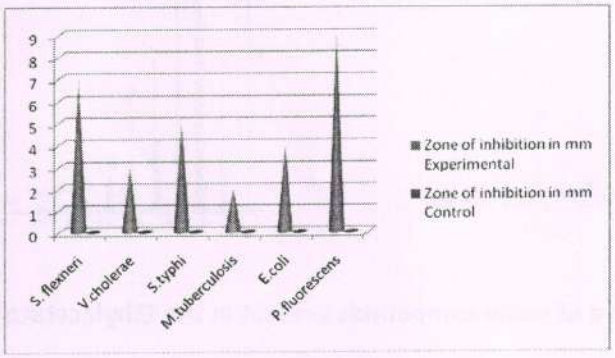

Figure-5

Antibacterial activity of Ethylacetate and Chloroform (1:1) extract of *F. nicobaricus* Phenol, 2,4-bis(1,1-dimethylethyl)-

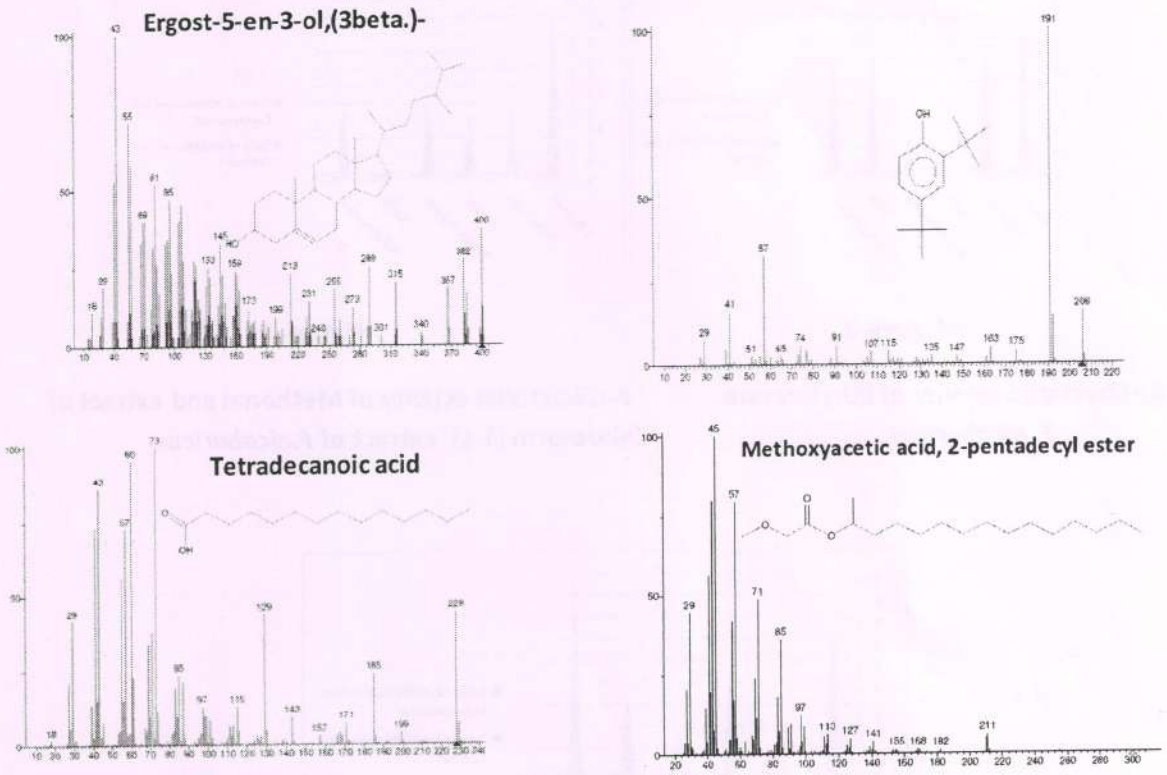

Figure- 6 GC-MS spectra of some compounds present in the Ethylacetate and Chloroform (1:1) extract of *F. nicobaricus*

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