Research Paper
Impact Factor: 3.567
Peer Reviewed Journal

IJMDRR
E- ISSN –2395-1885
ISSN -2395-1877

ANTIMICROBIAL COMPOUNDS FROM TROCHUS RADIATUS

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Abstract

The present study has been aimed to ascertain the antimicrobial activity of extracts from *Trochus radiatus* against various pathogenic bacterial and fungal strains using the agar disc diffusion method and the most probable antimicrobial compound by GC-MS study. Crude and eluted fractions were assayed for antimicrobial activity against six human bacterial pathogens viz *Vibrio cholerae, Salmonella typhi, Shigella flexneri, Escherichia coli, Mycobacterium tuberculosis* and *Pseudomonas fluorescens* and three fungal pathogens viz. *Candida albicans, Aspergilus flavus* and *Actinomyces* sp. Maximum effects of crude extracts of *T.radiatus* on fungi were exhibited by methanol extract. Maximum inhibition zone were obtained against, *V.cholerae, P. florescens, E.coli* and *S. typhi.* Of the five column chromatographic fractions, maximum number of bacterial pathogens was inhibited by F2 followed by F3and F5 and in fungal pathogens F2, F4 and F5 fractions respectively. The GC/MS study of *T.radiatus* revealed the probable antimicrobial compounds in F2 fraction viz. 2-Pyrrolidinone, 1-methyl-, Hydrocinnamic acid, Phenol, 2,4-bis(1,1-dimethylethyl)-, Benzenepropanoic acid, 4-hydroxy, and Cholesterol and in F2 fraction six antimicrobial compounds such as an alkaloid compound 2-Pyrrolidinone, 1-methyl, an aromatic compound Benzeneacetic acid, Acidic compound compound Hydrocinnamic acid, a Phosphorus compound Phosphoric acid, diethyl octyl ester and two steroid compounds Cholest-5-en-3-ol (3β)-, carbonochloridate, Cholesterol and Five antimicrobial compounds were detected in F4 fraction an alkaloid compound 2-Piperidinone, an aromatic compound Benzeneacetic acid, Acidic compound compound Hydrocinnamic acid, and two steroid compounds Cholest-5-en-3-ol (3β)-, carbonochloridate, Cholesterol .

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

Introduction

The knowledge acquired in the past two decades and the discovery of new groups of antimicrobial compounds makes natural antibiotics the basic element of a novel generation of drugs for the treatment of bacterial and fungal infections [1]. It is widely accepted among clinician medical workers, microbiologist and pharmacologist that antibiotic resistant will, in the near future, leave health care professional without effective therapies for bacterial and fungal infections. The critical events are the emergence of *Staphylococcus aureus* with decreased sensitivity to methicilin [2], worldwide resistance to penicillin in *Staphylococcus pneumonia* and multiple resistances to Mycobacterium tuberculosis. Consequently the priority for the next decades should be focused in the development of alternate drugs and as the recovery of natural molecules should be as natural as possible with a wide range of action over several pathogens easy to produce and not prone to induce resistant. A very different kind of substances have been obtained from marine organism among other reasons because they are living in a very exigent, competitive and aggressive surroundings very different in many aspects from the terrestrial environment, a situation that demands the production of quite specific and potent active molecules. Over the last 30 years many structurally novel antimicrobial metabolites have been isolated from marine organisms. Marine invertebrates offer a source of potential antimicrobial drugs [3]. Among the invertebrates, the molluscs are very good source for biomedical important products [4]. Many classes of molluscs with bioactive compounds like antitumour, antileukemic, antibacterial, cytotoxic, anti inflammatory and antiviral properties have been reported [5,6]. These reports suggest that molluscs are the rich source for discovering novel lead compounds for the possible development of new types of antibiotics for pharmaceutical use. Most marine animal produces bioactive metabolites in response to ecological pressures such as competition for space, deterrence of predation and the ability to reproduce successfully. In addition, marine animals have strategies to defend themselves against foreign organisms, by production of secondary metabolites that repel them. The need for discovery of new and novel antibiotics is imperative the microorganisms develop multidrug resistance by their peculiar pattern of adaptation behavior and problems of multi drug resistance in microorganisms are common in every field [7]. Keeping the importance of gastropods in terms of bioactive compounds with antimicrobial properties, and our continuous search of antimicrobial agents from natural source the present study has been undertaken to determine the antimicrobial activity of different extracts of marine mollusc whole body tissue extracts of *Trochus radiatus* against various human pathogenic microorganisms.

Materials and Methods

Collection and Preparation of Samples

The mollusc *T. radiatus* was collected from rocky shore of harbour area of Gulf of Mannar, near by Theraspuram Tuticorin, situated in the south east coast of India, between April 2015 and December 2015. 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 air-dried flesh was immersed in 100% A.R. Grade ethyl acetate, chloroform and methanol for 10 days at room temperature. The extract from the solvents was filtered by using Whatman no.1 filter paper and evaporated to dryness in rotary evaporator and the dried extract was stored at 0°C for further use.

Microbial strains used

Antimicrobial activity of tissue extracts were determined against six different bacterial pathogens viz., *Shigella flexneri, Vibrio cholerae, Salmonella typhi, Mycobacterium tuberculosis, Pseudomonas fluorescens and Escherichia coli* and three fungal stains viz., *Candida albicans, Aspergilus flavus* and *Actinomyces* sp.

Antibacterial susceptibility assay

In vitro antibacterial activity was assayed by the disc diffusion method [8]. A known amount of crude whole body 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 ethyl acetate 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. In vitro antifungal activity was determined using the techniques of Kelman *et al.*, 2001[9]. Pathogenic fungal strains were inoculated in potato dextrose agar medium and incubated at 48 hrs. Pathogens were swabbed 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 for antibacterial activity and 48hrs for fungal was measured accordingly based on the inhibition zone around the disc impregnated with gastropod extract. Antimicrobial 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. Same procedure was followed for chloroform and methanol crude and extracts.

Crude extract was fractionated and elusions were made with ethyl acetate (F1), ethyl acetate: chloroform (1:1) (F2), chloroform (F3), ethyl acetate: methanol (1:1) (F4) and methanol (F5). Eluted fractions were assayed for anti microbial activity following the above mentioned disc diffusion method.

Identification of compounds

The most potent crude extract of the test animal was subjected to GC-MS study which was carried out on a GC Clarus 500 Perkin Elmer system for the identification of probable antimicrobial different compounds.

Results

Antibacterial activity of extracts from *T. radiatus*

The crude ethyl acetate extract of *T. radiatus* with a range of activity varied from 2mm (*M. tuberculosis*) to 4mm (*V. cholerae ,S. typhi P. florescens & E. coli*) (Figure 1), in crude chloroform extract from 6mm (*S. typhi , & E. coli*) to 8mm (*V. cholerae , & M. tuberculae*) (Figure 2) and in methanol extract from 2mm (*S. typhi &M. tuberculae*) to 8mm (V. cholerae) , (Figure 3)respectively. Of the five column chromatographic fractions, maximum numbers of pathogens were inhibited by F2 fractions followed by F3 and F5 (Figures 5, 6 & 8). The highest activity of F2 fraction was exhibited against *V. cholerae,* followed by *E.coli ,S.typhi & M. tuberculae* (8mm) and the least against *S. flexneri* (4mm) (Fig.5). F5 fraction showed maximum activity against *M. tuberculae* (6mm) and the lowest against *S. flexneri* and *E. coli* (3mm) (Figure 8). Of the five fractions, the F2 fraction was active and the most susceptible pathogens in concern with the *T. radiatus* crude as well as in various fractions extract *were V. cholerae,P. florescens, , E.coli and S. typhi.*

Antifungal activity of extracts from T. radiatus

Maximum effect of inhibitory zone was obtained against *A. flavus* (10mm) followed by *C. albicans* by the crude ethyl acetate extract of *T. radiatus*, in chloroform *C. albicans* & *Actinomyces* sp. exhibited 8mm and by methanol extract *A. flavus* exhibited 10mm, *Actinomyces* sp. 11mm and *C. albicans* 9mm respectively (Figure 9, 10 & 11). F1 fraction exhibited maximum activity against *Actinomyces* sp. (11mm) followed by *A. flavus* (10mm) and *C. albicans* (9mm) (Figure 13). Highest inhibition zone was developed against *C. albicans* (10mm) and the lowest in *A. flavus* (8mm) in F2(Figure 14). Maximum inhibition zone was obtained against *C. albicans* (11mm) in F4 followed by *A. flavus* (10mm) and *Actinomyces* sp. (8mm) (Figure 15). Maximum activity was noticed against *C. albicans* (10mm) and minimum in *A. flavus* and *C. albicans* (7mm) in F5 fraction (Figure 16).

IJMDRR
E- ISSN –2395-1885
ISSN -2395-1877

GC-MS

The GC-MS study of the F2 fraction from *T. radiatus* revealed six antimicrobial compounds viz. 2-Pyrrolidinone, 1-methyl-, Hydrocinnamic acid, Phenol, 2,4-bis(1,1-dimethylethyl)-, Benzenepropanoic acid, 4-hydroxy, Cholest-5-en-3-ol (3β)-, carbonochloridate and (Table - 1). F2 fraction of *T. radiatus* revealed six antimicrobial compounds such as 2-Pyrrolidinone, 1-methyl, Benzeneacetic acid, Hydrocinnamic acid, Phosphoric acid, diethyl octyl ester and Cholest-5-en-3-ol (3β)-, carbonochloridate, Cholesterol (Table 2). Five antimicrobial compounds were detected in F4 fraction 2-Piperidinone, Benzeneacetic acid, Hydrocinnamic acid, and Cholest-5-en-3-ol (3β)-, carbonochloridate, Cholesterol (Table 3).

1 Antibacterial activity of crude Ethyl acetate extract of *T.radiatus* against pathogens

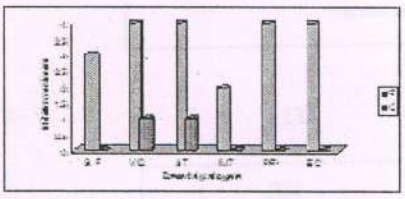


Figure 2 Antibacterial activity of crude Chloroform extract of *T.radiatus* against pathogens

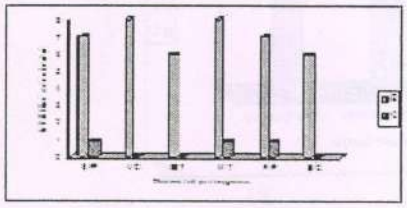


Figure 3 Antibacterial activity of crude Methanol extract of *T.radiatus* against pathogens

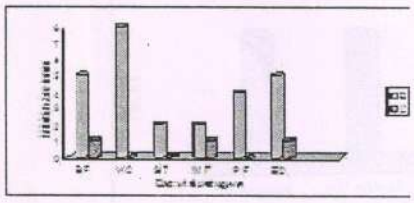


Figure 4 Antibacterial activity of various fractions of *T. radiatus* against pathogens F1

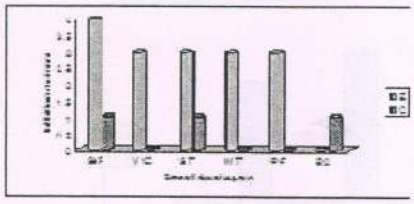


Figure 5 F2

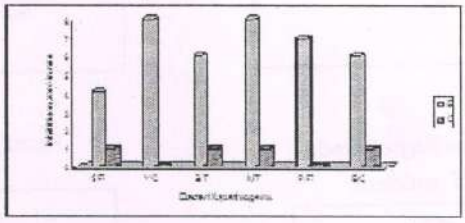


Figure 6 F3

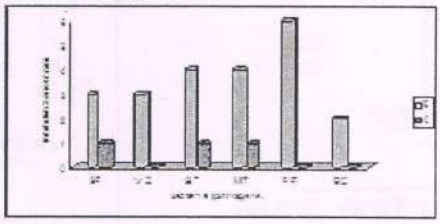


Figure 7 F4

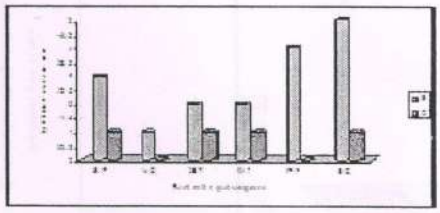
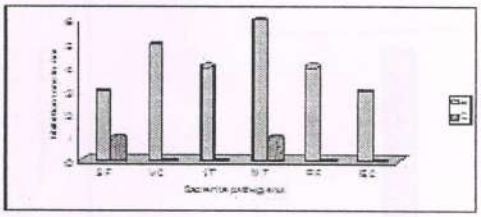


Figure 8 F5



IJMDRR
E- ISSN –2395-1885
ISSN -2395-1877

Figure 9 Antifungal activity of crude Ethyl acetate extract of *T.radiatus*

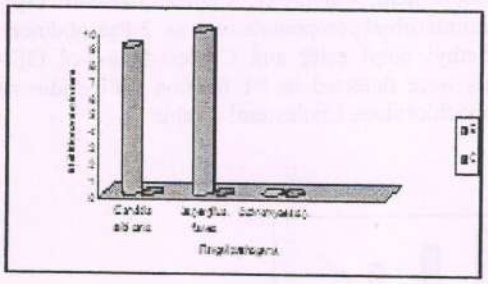


Figure 10 Antifungal activity of crude Chloroform extract of T.radiatus

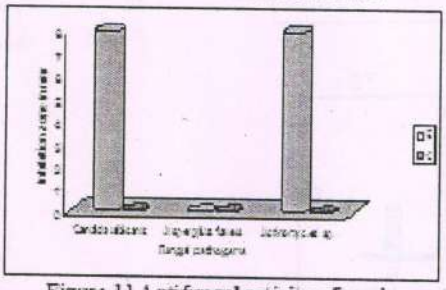


Figure 11 Antifungal activity of crude methanol extract of *T. radiates*

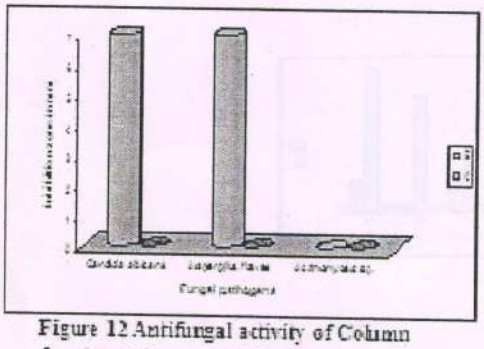


Figure 12 Antifungal activity of Column fractionated extract of *T. radiatus* F1

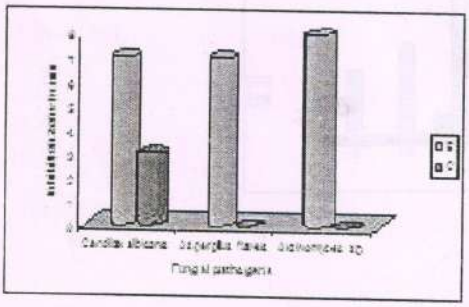


Figure 13 F2

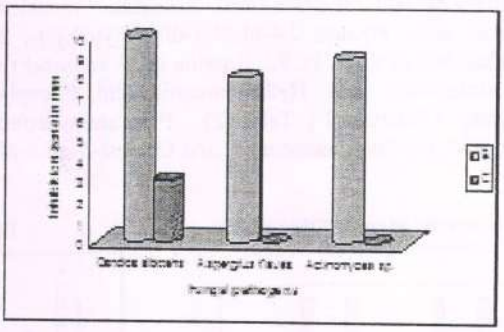


Figure 14 F3

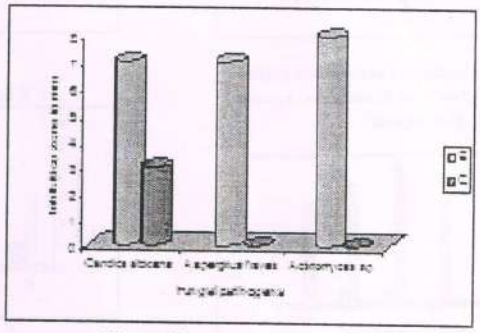


Figure 15 F4

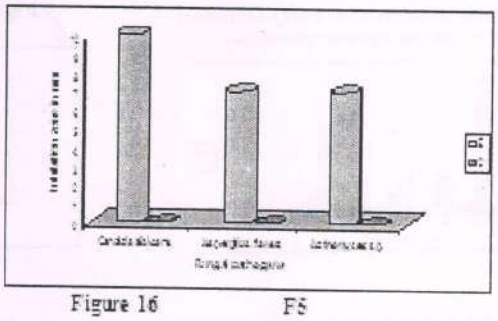
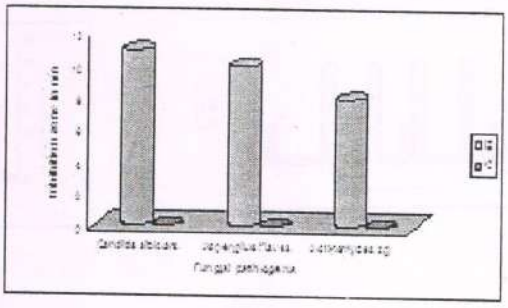


Figure 16 F5



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E- ISSN –2395-1885
ISSN -2395-1877

Table 1 Showing various activities of identified compounds by GC-MS in *T. radiatus*,

F1 **Ethyl acetate**

No.	RT	Name of the compound	Peak	Compound nature	Activity
1.	5.14	2-Pyrrolidinone, 1-methyl-	0.57	Alkaloid	Antimicrobial Anti-
2.	9.99	Phenol, 2,4-bis(1,1-dimethylethyl)-	0.36	Phenolic compound	Analgesic Antibacterial Anti-inflammatory
3.	23.83	Cholest-5-en-3-ol (3β)-, carbonochloridate	3.15	Steroid	Antimicrobial Anticancer Anti-inflammatory
4.	32.69	Cholesterol	35.41	Steroid	Antimicrobial Anticancer Anti-inflammatory

Table 2 **F2** **Ethyl acetate : Chloroform**

No.	RT	Name of the compound	Peak Area %	Compound nature	Activity
1.	5.16	2-Pyrrolidinone, 1-methyl-	0.67	Alkaloid	Antimicrobial Anti-inflammatory
2.	6.05	Benzeneacetic acid	0.94	Aromatic compound	Antimicrobial
3.	7.48	Hydrocinnamic acid	2.45	Acidic compound	Antimicrobial
4.	9.95	Phenol, 2,4-bis(1,1-dimethylethyl)-	0.30	Phenolic compound	Analgesic, Antibacterial Anti-inflammatory Antiviral Cancer preventive
5.	10.33	Phosphoric acid, diethyl octyl ester	0.55	Phosphorus compound	Antimicrobial
6.	13.05	Tetradecanoic acid	2.49	Myristic acid	Cancer preventive
7.	13.73	Oleic Acid	1.38	Oleic acid	Anti-inflammatory, Cancer preventive,
8.	23.77	Cholest-5-en-3-ol (3β)-, carbonochloridate	1.47	Steroid	Antimicrobial Anticancer Anti-inflammatory
9.	32.78	Cholesterol	13.73	Steroid	Antimicrobial,Anticancer Anti-inflammatory

Table 3, **F5** **Ethyl acetate : Methanol**

No.	RT	Name of the compound	Peak Area %	Compound nature	Activity
1.	5.23	2-Piperidinone	0.04	Alkaloid	AntimicrobialAnti-inflammatory
2.	6.09	Benzeneacetic acid	0.93	Aromatic compound	Antimicrobial
3.	7.60	Hydrocinnamic acid	9.41	Acidic compound	AntimicrobialAnti-inflammatory
4.	9.79	Phenol, 2,4-bis(1,1-dimethylethyl)-	0.09	Phenolic compound	Analgesic Antibacterial Anti-inflammatory, Cancer preventive

5.	11.60	Benzenepropanoic acid, 4-hydroxy-	7.15	Phenolic compound	Analgesic Antibacterial Anti-inflammatory Antiviral Cancer preventive
6.	23.74	Cholest-5-en-3-ol (3β)-, carbonochloridate	1.42	Steroid	Antimicrobial Anticancer Anti-inflammatory
7.	32.67	Cholesterol	20.43	Steroid	Antimicrobial Anticancer Anti-inflammatory

Discussion

Several marine molluscan extracts possessed broad spectrum antibacterial activities affecting the growth of bacteria,fungi and yeasts [10,11]. Antibacterial activity has previously been described in a wide range of molluscan species[12, 13]. The antibacterial activity of common marine molluscs from Parangipettai coast was studied and reported that the methanolic extract of molluscs exhibited significant activity against *Escherichia coli* [11]. This finding corroborate the results of the present study since methanol extract of *T. radiatus* showed pronounced activity against *E. coli.* The inhibitory action of the methanol fractions of *Perna viridis* was reported [14] against bacterial and fungal strains. Similar result was also reported in four bivalves against few pathogens and found that methanol extracts showed significant activity against *Bacillus subtilis*[15]. The crude methanol extracts of *Cypraea errones* exhibited promising results for antibacterial activity [11]. The methanol extract of *Didemnum candidum* maximumantibacterial activity was noted against *Salmonella typhi, Pseudomonas aeroginosa* and *Vibrio cholerae[16]*. Similar result was reported by Chellaram [17] in chloroform extract of *Pterai chinensis* which inhibited eight fish pathogens. Anbuselvi [18] found that acetone column purified fractions of *Trochus tentorium* shown highest antibacterial activity. The present study with test animal *P.persica* corroborates the earlier findings of suppressing the activity of *S.typhi*.

In the present investigation crude and column fractionated extracts of *T. radiatus* had distinct antifungal activity against both the pathogenic fungi tested. Moderate antifungal activity from the extract of various bivalve molluscs was reported [11]. The fungi are more resistant than the bacterial strains to the tested compound this could be leads to the nature of fungal cell wall made up of chitin which is relatively resistant including microbial decomposition [19]. Higher degree of inhibition by the column fractionated extracts in comparison to the crude could be concluded that the active compound may be modified during the fractionation process as reported by Cannell [20] The present findings of compounds identified by GC/MS are in agreement with Emiliano Manzo [21] . An antimicrobial peptide from the seminal plasma of the mud crab *Scylla serrata* was isolated [22]. A terpenoid caribenol A and B inhibited the growth of*Mycobacterium tuberculosi* [23]. Three antimicrobial linear ß-substituted sesterterpenes were isolated fromnudibranch *Hypselodoris capensis* [24]. A polypeptide type AMP (Antimicrobial peptide) isolated from the Chilean scallop *Argopecten purpuratus*, showed antifungal activity against F. *oxysporum* and *Saprolegnia parasitica* [25]. On account of their broad spectrum antimicrobial activity and the previous available literature *T. radiatus* was expected to be a new potential producer of new antibiotics.

Conclusion

Of the five fractions, the number of fractions active was F1, F4 and F5 respectively and *V. cholerae, P. florescens, E.coli and S. typhi* were the most susceptible pathogens in concern with the *T. radiatus* and the antimicrobial compounds identified by GC-MS were responsible for the inhibition of tested pathogens.

Acknowledgements

Thanks are due for the facilities provided by Jayaraj Annapackiam College, Periyakulam. The financial support from the University Grant Commission, Hyderabad is gratefully acknowledged.

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