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Antibacterial, Biochemical Composition and FTIR Analysis of *Chicerous ramosus* from Kanyakumari Coast

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ABSTRACT

Four different solvent extracts of tissue of marine gastropod *Chicerous ramosus* were taken and their activity against human and fish pathogens were tested using standard well diffusion method. The maximum zone of inhibition of about 12mm was observed against *Proteus vulgaris* in the crude ethanol extract followed by 8mm against *Salmonella paratyphi* at the concentration of 50µl. Minimum inhibition zone of 2mm was obtained by ethanol extract against *Streptococcus mutants* and by methanol extract against *Salmonella dysentriae*. In case of fish pathogens a maximum zone of 6mm was observed by acetone extract against *V. harveyi* and a minimum zone of 2mm was observed by ethanol extract against *V. alginolyticus* and by acetone extract against *V. vulnificus.* The proximate composition such as protein, carbohydrate and lipid were studied and the results showed high percentage of protein of about 24.42%, followed by the carbohydrate 10.50% and lipid 1.24%. The FTIR analysis represents the presence of alkyl halides, aliphatic amines, aromatic amines, alkanes,1 amines, alcohols and phenols compound of gastropod extracts. The above-observed result showed that the gastropod extracts were rich in antimicrobial compounds. Further research is necessary to study the full structural components of the antimicrobial compounds from marine mollusc by analytical techniques. Present study indicated that the marine mollusc collected from the Kanyakumari coast possessed several bioactive compounds of therapeutic interest. To our knowledge, this is the first report on antimicrobial, biochemical and FTIR studies of *C. ramosus* of Kanyakumari coast. Further studies are going on to isolate specific compounds and to study the biological activities present in them.

INTRODUCTION

Modern medicine is dependent on chemotherapeutic agents. The use of antimicrobial drugs to control infectious diseases must be among the greatest achievements in medicine in the last century (Allsop, 1998). After many clinically useful antibiotics like streptomycin, chloramphenicol, chlortetracycline, neomycin, oxytetracycline, erythromycin etc. were discovered, most bacterial infections seemed to be conquered. However, a decade after the spread of antibiotic therapy a number of species of *Staphylococcus*, *Mycobacterium* and Gram-negative enteric bacteria had developed resistance to antibiotics (Oiwa, 1992). Antibiotics must be used in a concentration low enough to avoid undesirable damage to the host, which, at this stage, is not possible with many of the currently used synthetic antibiotics. The highest level of antibiotic resistance was determined in bacteria inhabiting seafood, compared to water and sediment isolates (Ashok kumar *et al*., 2009).

Aquaculture has been the world's fastest growing food production system for the past decade (Muir, 1995 and Tacon, 1997). In 1997, over 30% of food consumed by humans was provided by aquaculture (Gesamp, 2001). In Asia, shrimp viral diseases caused farmers about 1 billion-dollar loss every year since 1994 (Tibbetts, 2001). The disease was initially controlled almost exclusively by the use of antimicrobial drugs. The increasing intensity of shrimp farming was inevitably paralleled by the increasing incidence of diseases in the farming systems. Pathogens such as *Vibrios* are the members of the normal bacterial flora of shrimps and act as primary and secondary invaders of shrimps in the culture system (Lightner *et al*., 1992).

In the past 20 years, the pharmacological industry has been relatively successful in containing problems due to single resistance determinants. However, the advent of multiple resistance mechanism has severely reduced the use of currently used antibiotics. There is a growing concern about the use and particularly the abuse of antimicrobial drugs not only in human medicine and agriculture but also in aquaculture. Hence, the need of the hour is a search for novel antibacterial compounds with therapeutic potential for which the pathogens may not have resistance (Patil *et al*., 2001). The search for new antibiotics is a continuous process. Approximately 2,500 new metabolites were reported from a variety of marine organisms during the decade from 1977-1987 (Ireland *et al*., 1993).

Marine organisms have been a rich source of novel chemical compounds (Faulkner, 1996), which have boosted the development of marine natural products chemistry for about three decades. Among the marine organisms, molluscs are one of the most successful forms of animal life and they have conquered almost every habitat and exist in all the oceans (from shallow tidal pools to the deepest trenches). Many studies on bioactive compounds from mollusc exhibiting antibacterial, antitumour, antileukemic and antiviral activities have been reported worldwide (Hochlowski *et al*.,1983, Faulkner *et al*.,1983, Faulkner.,1988 and Prem Anand *et al*.,1997). The bioactive substance was isolated from gastropod *Drupa margariticola* (Faulkner.,1995 and Faulkner.,1996). So far *C.ramosus* study has been done on many other coasts, antibacterial activity of the same species may vary with the coast based on the nutritive availability, change in temperature and seasons.In the current study area work has not been done on *C.ramosus*. So the present study was carried out on *C.ramosus* from Kanyakumari coast.

MATERIALS AND METHODS

2.1. Collection of sample and preparation of tissue extract

The animals were collected from Kanyakumari coast of Tamil Nadu and were brought to the laboratory in an ice box. The samples were washed with tap water until the sand and mud were removed from the shells. After that, the shells were broken using a hammer to remove the soft body tissue. The removed tissues were rinsed with sterile distilled water, cut into small pieces and kept in petri dishes and dried at a constant temperature at 50°C for 24 hours in a hot air oven. The dried material was powdered thoroughly for solvent extraction (Vimal a*t al*., 2012). Approximately 5g of powder was immersed separately into ethanol, methanol, acetone and butanol solvents and they were cold steeped at -18°C. The extracts from each solvent were filtered twice using Whatman No.1 filter paper and used for the experimental work.

2.2. Bacterial cultures

Five species of human pathogens; *Bacillus cereus, Proteus vulgaris, Shigella dysenteriae, Salmonella paratyphi*, and *Streptococcus mutants* were obtained from the Christian Medical College Hospital, Vellore. Five fish pathogens; *Vibrio harveyi, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio alginolyticus* and *Vibrio anguillarum* were obtained from Fisheries Department, Cochin.

2.3. Antibacterial screening

The antibacterial screening was investigated against five human and fish pathogens by agar well diffusion method following the method of (Ramasamy *et al*., 2011). Twenty four hours old nutrient broth cultures of test bacteria was aseptically swabbed on sterile Mueller Hinton agar plates. Wells of 5mm in diameter were made aseptically using well cutter, and 50 µL of four different solvent extracts of tissues were inoculated. The results were calculated by measuring the zone of inhibition in millimeters. Each concentration tested, duplicates were maintained for the confirmation of activity.

2.4. Biochemical composition

2.4.1. Estimation of total protein

The Folin - Ciocalteu phenol method (Lowry *et al*., 1951) was adopted for the estimation of total protein in the tissue.

2.4.2. Estimation of total carbohydrate

The total carbohydrate content was estimated by using the procedure of Dubois *et al*., (1956) with phenol - sulphuric acid.

2.4.3. Estimation of total lipid

The chloroform-methanol extraction procedure of Folch *et al*., (1956) was used for extracting lipid from the tissue.

2.5. FT-IR spectrophotometry (Fourier Transform- Infra Red spectrum analysis)

The crude samples of *C. ramosus* (10mg) was mixed with 100mg of dried potassium bromide (KBr) and compressed to prepare as a salt disc. The disc was then read spectrophotometrically (Bio- Rad FTIR-40- model, USA). The frequencies of different components present in each sample were analyzed.

3. RESULTS

3.1. Antibacterial Screening

The antimicrobial activity of crude tissue sample of gastropod *C. ramosus* was evaluated with human and fish pathogens using solvents such as ethanol, methanol, acetone and butanol at the concentrations of 50 µl by well diffusion method. The crude ethanol extracts were more active exhibiting broad spectrum of antimicrobial activity than the crude methanol, acetone and butanol extracts. The maximum zone of inhibition of 12mm was observed for bacteria *Proteus vulgaris* in the crude ethanol extract followed by 8mm against *Salmonella paratyphi*. Minimum zone of 2mm was obtained by ethanol extract against *Streptococcus mutants* and by methanol extract against *Salmonella dysentriae* (Fig.1). In case of fish pathogens, a maximum zone of 6 mm was observed by acetone extract against *V.harveyi* and a minimum zone of 2mm was observed by ethanol extract against *V. alginolyticus* and by acetone extract against *V. vulnificus*. (Fig. 2).

3.2. Biochemical composition

The proximate compositions such as protein, lipid and carbohydrates contents of *C. ramosus* tissue were estimated. The results of the present study revealed that the protein composition was high 24.42% followed by carbohydrate 10.50% and lipid 1.14%.

3.3. FT-IR spectrophotometry (Fourier Transform- Infra Red spectrum analysis)

FTIR spectrum of the sample *C. ramosus* showed that 11 major peaks that were shown at 3442.70,3419.56,2981.74,2933.53,1647.10,1446.51,1367.44,1280.65,1072.35,1043.42,742.54 cm^{-1} whereas all the remaining peaks were very close and at 3917.16, 3895.94, 3885.08, 3813.01, 3724.29, 2858.31, 2356..85, 2131.19, 1583.45,1390.58,1330.70, 1217.00, 1157.21, 1014.49, 960.48, 846.89 and 700.11cm^{-1}. (Figure.3).

4. DISCUSSION

Marine natural products have drawn the attention of researchers in recent years due to their pharmacological value. Extensive studies have been carried out with many marine plants and animals. As the marine plants and animals get adapted to different types of habitats in the marine environment they show several adaptations, to overcome the difficulties they face in an ecosystem such as predation, surface fouling, space, reproduction (Ramasamy *et al*., 2011). Defensive mechanism is one such adaptation which is well studied in most of the invertebrates. The antibacterial assay have been reported long back in several molluscan species such as *Crassostrea virginica* (oyster), *Mytilus edulis* and *Geukensia demissa* (mussel) (Anderson *et al*., 2001), *Dicathais orbita* (muricid mollusks) (Benkendorff *et al*., 2001), *Dolabella auricularia* (sea hare) (Vennila *et al*., 2011 and Constantine, 1975), 21 species of *Dorid nudibranchs* tissues exhibited significant antibacterial and antifungal properties (Gunthorpe *et al*.,1987). The studies carried out with marine natural products during the last decades have uncovered many substances with biomedical potential, which has raised the significance of many research groups toward this ecosystem as a source of new drugs (Ramasamy *et al*., 2011).

In the present study, four different solvent extracts of *Chicerous ramosus* were screened for their activity against human and fish pathogens using standard well diffusion method, the maximum inhibiting zone of 12mm was observed for human pathogen *Proteus vulgaris* in the crude ethanol extract followed by 8mm against *Salmonella paratyphi* at the concentration of 50 µl. Minimum zone of 2mm was obtained by ethanol extract against *Streptococcus mutants* and by methanol extract against *Salmonella dysentriae*. In case of fish pathogens, a maximum zone of 6mm was observed by acetone extract against *V. harveyi* and a minimum zone of 2mm was observed by ethanol extract against *V. alginolyticus* and by acetone extract against *V. vulnificus*. Suresh *et al*., (2012) also reported that the maximum inhibition zone of 10.13mm against *Klebsiella pneumonia* in the ethanol extract of *Babylonia zeylanica* and the minimum inhibition zone (1mm) was observed against *V. cholera.* In the case of *Harpa conoidalis*, the maximum inhibition zone (9.16mm) was observed against *S. paratyphi* in ethanol extract and the minimum inhibition zone (1.03mm) was noticed against *Proteus mirabilis***.** In a similar way *Babylonia spirata* exhibited the antibacterial activity of ethanol, acetone, methanol, chloroform and water extracts; the maximum inhibition zone (12mm) was observed against *Pseudomonas aeruginosa* in the crude ethanol extract and the minimum inhibition zone (2mm) (Periyasamy *et al*.,2012). Present work reveals that the gastropod *C. ramosus* has potential antimicrobial activity

Foods from the sea are utilized for hundreds of years been a source of high-quality protein. In the five basic groups seafood belongs to the same category as meat, poultry, eggs, dried beans and peas. Protein is essential for the sustenance of life and exists in largest quantity of all nutrients as a component of the human body (Okuzumi *et al*., 2001). Present investigation revealed that the maximum level of protein content in *C. ramosus* was 24.42%. Babu *et al*., (2010) assessed the percentage of protein ranged from 19.25 to 27.9% in the Mesogastropod, (*Bursa spinosa).* The protein content of gastropods varied from 18.71 to 29.81% (Shanmugam *et al*., 2001). Arularasan (2009) contributed the percentage of protein ranged from 47.86 to 70.18% in males and from 49.64 to 72.21% in females of *Strombus canarium* gastropods of Gulf of Mannar**.** The protein content of the present study was similar to that of the previous studies

Carbohydrates are a group of organic compounds including sugars, starches and fiber, which is a major source of energy for animals. In the present study, the percentage of carbohydrates in the body tissue was 10.50%. Thivakaran (1988) estimated maximum levels (5.31%) of carbohydrate in *L. quadricentus* and (4.69%) in *N. pyramidalis*. Shanmugam (1987) reported the carbohydrate in *Pythia plicata* values from 0.84% to 3.04%. In *D. cuneatus*, generally the carbohydrate content was found to be high when compared to other molluscs. The carbohydrate content of the present study was higher when compared to the previous studies.

The lipids are highly efficient as source of energy, in that they contain more than twice the energy of carbohydrate and proteins. In the present study lipid content of tissue was 1.14%. Rajkumar (1995) reported lipid content in *Rapana rapiformis* which ranged from 0.85-2.12% in male and 0.95 - 2.96% in female gastropod. Nirmal (1995) found the highest level of lipid 10.38% in *Babylonia zeylanica* and 1.97% in *Pleuroploca trapezium* respectively.

The FTIR spectrum of the *C. ramosus* sample recorded the number of peaks lying between 700.11 cm^{-1} and 3917.16 $cm^{-1}$and it showed 11 major peaks. The previous research showed that the *B. spirata* (thazhagunda southeast coast of India) sample also recorded the number of peaks lying between 465.75cm^{-1} to 3388.75cm^{-1} (Periyasamy *et al*., 2011).

Conflict of interest statement

We declare that we have no conflict of interest.

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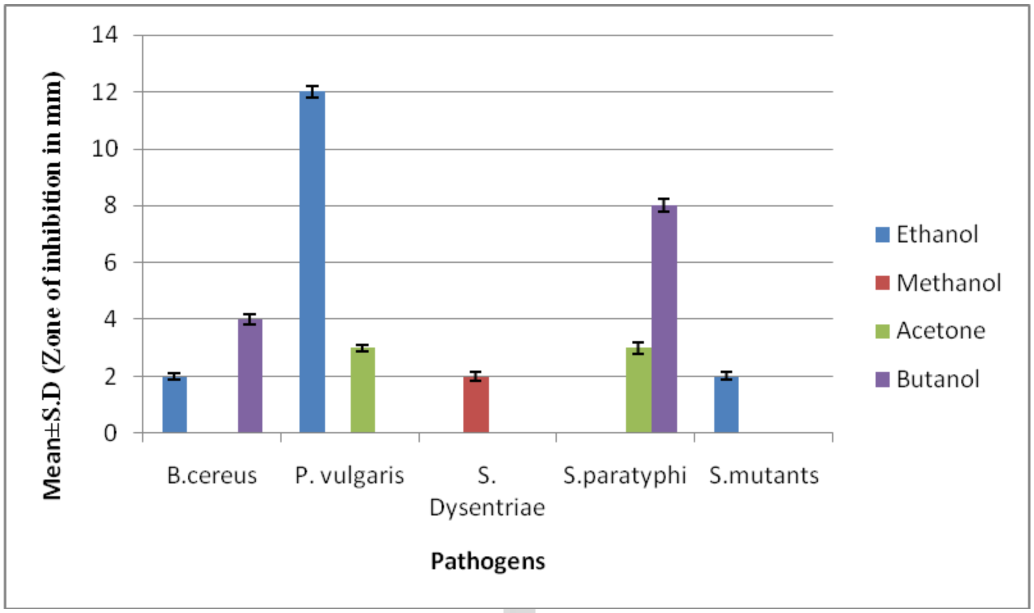

Fig. 1. Antibacterial activity of tissue extract of *C. ramosus* against human pathogens

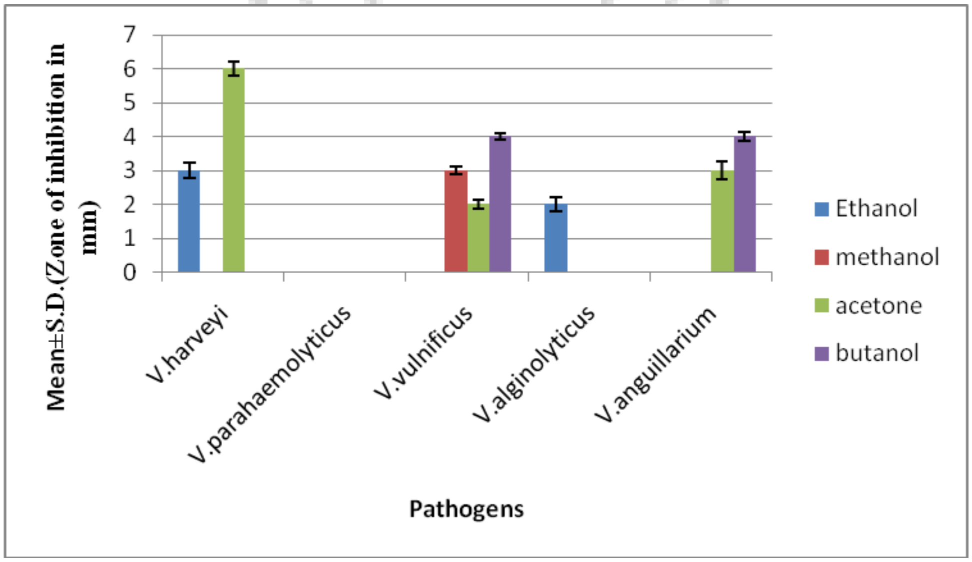

Fig. 2. Antibacterial activity of tissue extract of *C. ramosus* against fish pathogen

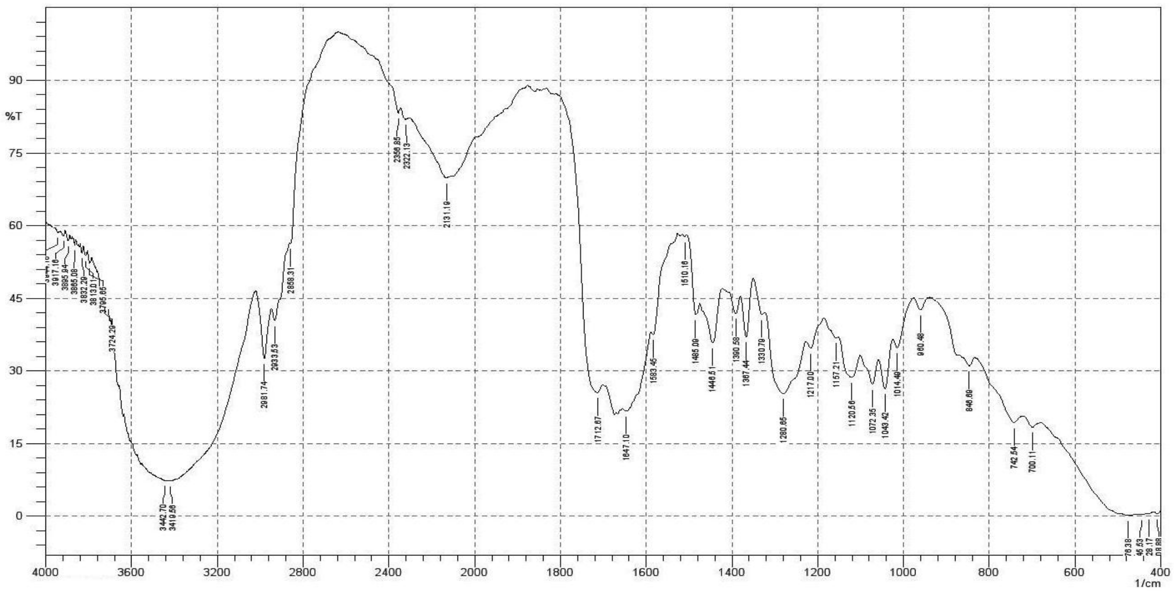

Fig. 3. FTIR spectrum of the sample of *C. ramosus*