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RESEARCH ARTICLE

IDENTIFICATION OF BIOACTIVE COMPOUNDS AND ANTIBACTERIAL ACTIVITY OF MARINE GASTROPOD CONUS ACHATINUS GMELIN, 1791

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Abstract

The aim of the present study is to screen a molluscan gastropod, *Conus achatinus* to find out the possible bioactive compounds present in the whole body tissues of experimental animal. The antibacterial activity was assayed against ten bacterial pathogens by Disc Diffusion Technique at three different concentrations. The crude methanolic extract showed significant antibacterial activity with minimum inhibitory zone of 2 mm against *Pseudomonas aeruginosa* and maximum 18 mm against *Klebsiella pneumoniae* at 100mg/ml concentration. It was further fractionated into four fractions using the different solvent system. Of all the fractions tested, (F2) and (F3) showed the potent activities. The fraction F2 showed minimum inhibition zone of 9 mm against *Pseudomonas aeruginosa* and *Proteus vulgaris* and the maximum of 14 mm against *Streptococcus pyogenes* and F3 showed the minimum inhibition zone of 12 mm against *Klebsiella pneumoniae* and 20 mm against *Staphylococcus aureus* at 100 mg/ml concentration. The GC-MS analysis of the whole body tissue extract of *Conus achatinus* revealed the presence of alkaloid, amino, nitrogen, aldehyde, alcoholic, sulphur, nitrogen and steroid compounds which might be responsible for the antibacterial activity.

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Introduction:-

Oceans offers a large biodiversity of fauna and flora which is estimated to be over 5,00,000 species and more than double that of land. The marine environment is a huge source for of natural products and most of them yet to be discovered. Apart from human medicines, the research on marine natural products in the last three decades has also brought to the discoveries of many chemically and biologically interesting molecules, that have become indispensable tools in biochemical research and played significant roles in the recent advancement of life sciences. Tincu and Tailor (2004) reported that sessile and slow moving benthic marine organisms are the rich source of bioactive metabolites. These metabolites can be considered as a kind of autogenic protection and are proved to be the timely alternative natural medicine to human beings. Generally, the ocean meets the food requirements of human, a far better profitability is obtained by producing human consumables and the highest profitability is currently expected from bioactive compounds (Je *et al.*, 2005; Jeon and Kim, 2002; Kim *et al.*, 2001). There are more than thousands of bioactive compounds discovered in molluscs. So far, many bioactive compounds like peptides, sterols, terpenes, polypropionates, nitrogenous compounds, macrolides, prostaglandins, fatty acids derivatives, alkaloids etc., showing specific type of activities have been identified and isolated from molluscs (Mactoob and Ronald., 1997; Balcazer *et al.*, 2006 and Blunt *et al.*, 2006).

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Members of many classes of molluscs possessing bioactive compounds having antitumour, antileukemic, and antibacterial properties have been reported worldwide (Anand *et al.*, 1997; Ilangedone *et al.*, 1999; Rajaganapathy *et al.*, 2000 and Chellaram *et al.*, 2004). Conversely, most medicines are derived from shelled gastropods and bivalves (Kirsten Benkendorf, 2010). The bioactive compounds isolated from these shelled gastropods are considered a role as chemical defense against their predators. Today, the great majority of bacterial infections are easily controlled with natural or synthetic drugs. Antibacterial activity has been previously proved in a wide range of molluscan species (Mitta *et al.*, 2000; Rajaganapathy *et al.*, 2000; Prem Anand and Patterson Edward, 2002; Prema., 2006; Annamalai *et al.*, 2007; Maripandi *et al.*, 2010 and Subavathy, 2012). We are still in need of safer, cheaper and effective drugs. Marine molluscs, are the source of biologically active products is largely unexplored in India. Hence, a broad based screening of marine molluscs is necessary to explore the bioactive compounds.

Due to the above facts, the present study has been undertaken to test the whole body tissue of marine molluscan extract against bacteria and to isolate and characterize the possible antibacterial compound from the test mollusc *Conus achatinus*.

Material And Methods:-

Conus achatinus were collected from the Gulf of Mannar in Thoothukudi coastal region during low tides from the sea in its natural habitat. The freshly collected samples were cleaned and washed with fresh seawater to remove all impurities. The shells were broken and the tissues were made into small pieces with aseptic scissors and then dried in hot air oven at 56°C for 48 hours and used for further study.

Preparation Of Extracts:-

Methanol extract of the body tissue was prepared by the technique given by Thilaga (2005) which was slightly modified. Air dried materials were taken and immersed in 100% A.R. grade methanol for 10 days in room temperature. The extract from the solvent was filtered using Whatman No.1 filter paper. The filtrate was poured into petriplates and evaporated to dryness. The dried substance was used for further studies.

Fractionation:-

Crude methanol extract was fractionated by silica gel column chromatography with four different solvent systems. Elutions with Hexane : Chloroform (F1), Chloroform (F2), Benzene : Methanol (F3) and Distilled water (F4) in the order of their polarity afforded four fractions viz., F1, F2, F3 and F4. A known amount of extracts was taken and their organic solvents were removed by vacuum evaporation. Solids were dissolved in deionized water and concentration series of 1mg/ml, 10mg/ml and 100mg/ml were prepared and used.

Antibacterial Assay:-

Antibacterial assay of both crude and column fractionated extracts of *Conus achatinus* were determined against ten bacterial pathogens through disc diffusion technique. *Pseudomonas aeruginosa*, *Bacillus cereus*, *Shigella flexneri*, *Vibrio cholerae*, *Salmonella typhi*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus aureus* which were obtained from the Microbiology Department of Sri Paramakalyani College, Alwarthurichi. These bacterial strains were inoculated in sterile nutrient broths respectively and incubated at $28 \pm 2^\circ\text{C}$ for 24 hours. The impregnated discs along with the Control were kept at the centre of the agar plates, seeded with test bacterial cultures. The plates were incubated at 37°C for 24 hours and the inhibition zone was measured from the outer side of the discs to the inner side of the inhibition zone. The extract showing broad spectrum antibacterial activity was examined by testing different concentrations viz., 1mg/ml, 10mg/ml and 100mg/ml. Each activity was tested thrice for confirmation of activity. The most potent fraction was characterized to know the functional groups through GC-MS study. GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system at Indian Institute of Crop Processing Technology, Tanjore.

Identification Of Compounds:-

Interpretation on mass spectrum GC-MS was conducted using the data base of National Institute of Standard Technology (NIST) having more than 62,000 patterns. The unknown components with electrons impact fragmentation patterns of the mass spectra of column fractionated test mollusc was matched with the spectrum of the known components stored in NIST ver.21 National Institute of Standard Technology, the mass spectra library and predicted from Dr. Duke's Phytochemical and Ethnobotanical Database.

Results:-

In the present study, antibacterial activity of whole body tissue extract of *Conus achatinus* was tested and presented in figures (1-3) and plates (1-3). The extract showed more pronounced antibacterial activities against some human pathogenic bacterial strains. The crude methanolic extract of *Conus achatinus* showed the range of activity from 2mm to 18mm. At 100mg/ml concentration, the minimum inhibitory zone of 2mm against *Pseudomonas aeruginosa* and maximum of 18 mm against *Klebsiella pneumoniae* were recorded. Of all the fractions tested, (F2) and (F3) showed the most potent activities. F2 developed minimum inhibitory zone of 9 mm against *Pseudomonas aeruginosa* and *Proteus vulgaris* and maximum 14 mm against *Streptococcus pyogenes*. F3 developed inhibition zones which varied from 12 mm and 20 mm. It showed the minimum inhibitory activity of 12 mm against *Klebsiella pneumoniae* and 20 mm against *Staphylococcus aureus* at 100 mg/ml concentration. These promising results revealed, the presence of alkaloid, amino, nitrogen, aldehyde, alcoholic, sulphur, nitrogen and steroid compounds by GC-MS analysis of whole body tissue of *Conus achatinus*, which might be responsible for the antibacterial activity.

Fig. 1:-Antibacterial activity of crude methanol extract of *Conus achatinus*

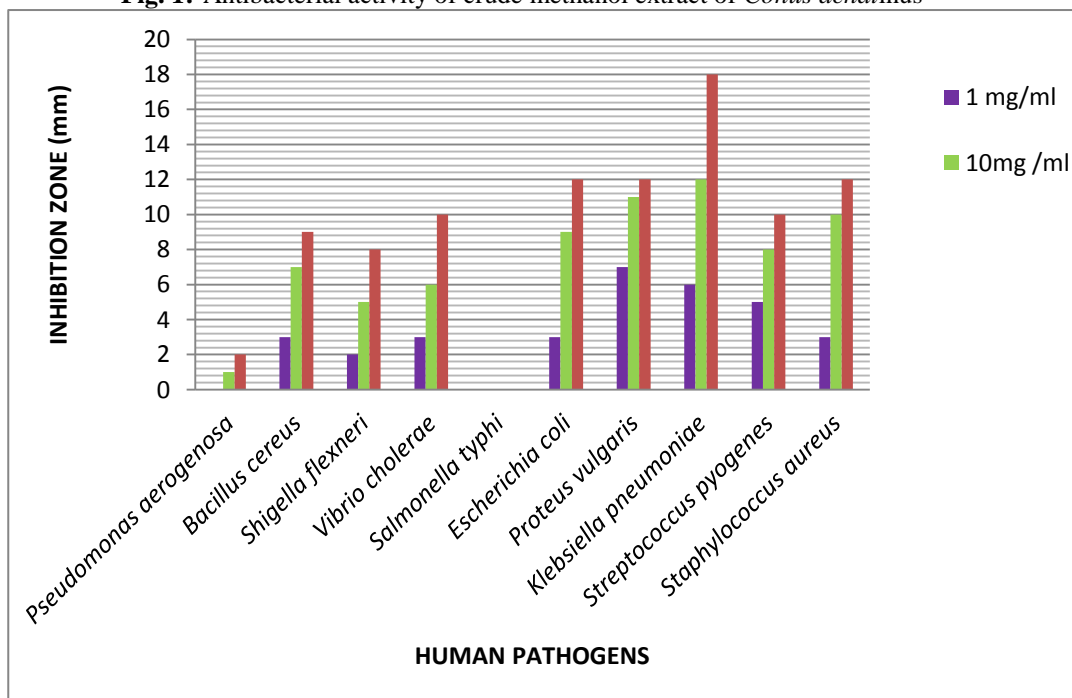


Fig. 2:- Antibacterial activity of F2 fraction (Chloroform) of *Conus achatinus*

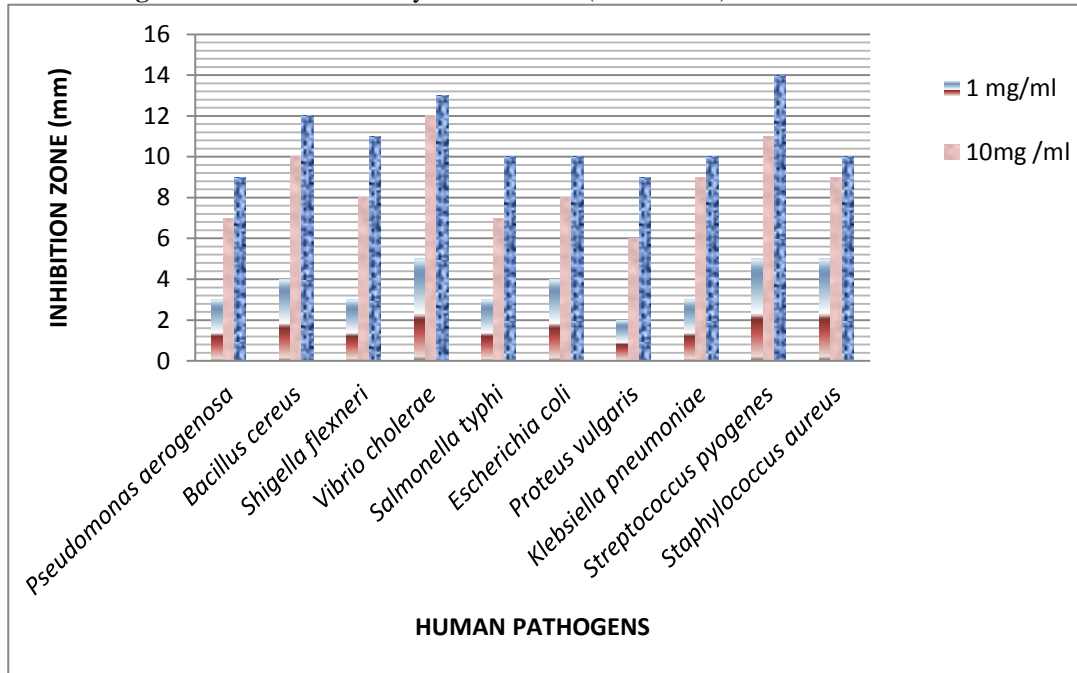


Fig. 3:- Antibacterial activity of F3 fraction (Benzene:Methanol) of *Conus achatinus*

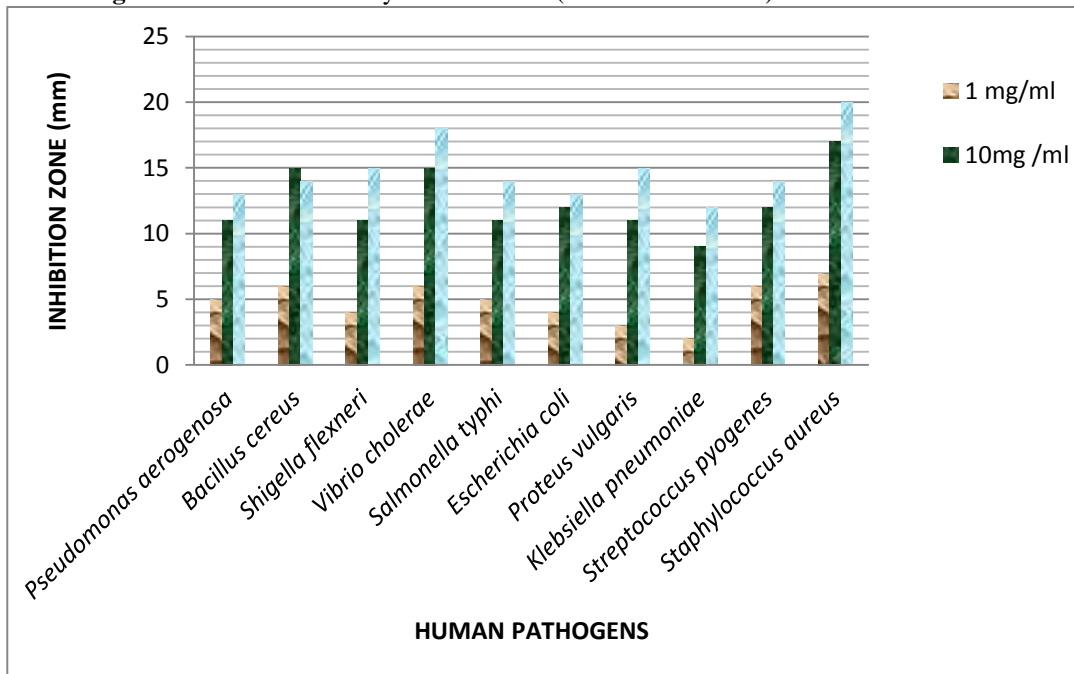
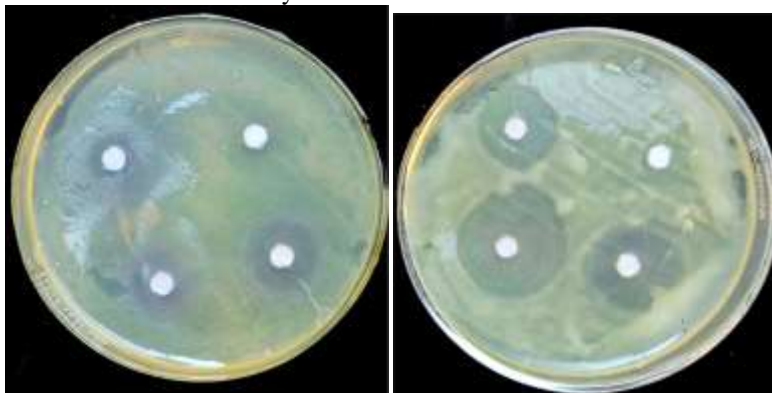
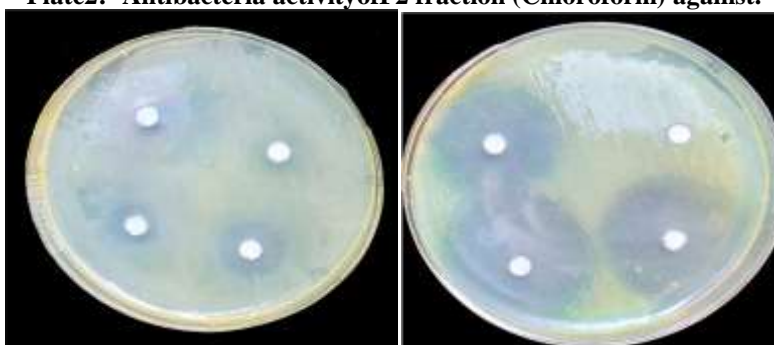
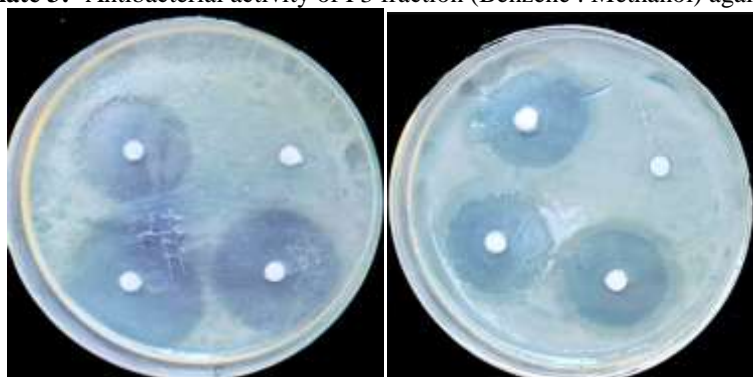


Plate 1:- Antibacterial activity of crude methanol extracts of *Conus achatinus**Pseudomonas aeruginosa**Klebsiella pneumoniae***Plate2:-** Antibacteria activity of F2 fraction (Chloroform) against.*Pseudomonas aeruginosa**Streptococcus pyogenes***Plate 3:-** Antibacterial activity of F3 fraction (Benzene : Methanol) against*Klebsiella pneumoniae**Staphylococcus aureus***Table 1:-** Activity of components identified in *Conus achatinus* by GC-MS

S. No	RT	Name of the compound	Peak Area %	Compound Nature	**Reported activity
1	3.15	Aziridine, 1-methyl-	4.75	Alkaloid	Antimicrobial Antiinflammatory
2	10.37	5-Methyl-2-hexanone oxime	0.33	Ketone compound	No activity reported
3	10.85	Cyclohexanol,2-amino-,trans-	1.73	Amino compound	Antimicrobial

4	11.41	Azocine, octahydro-	0.85	Nitrogen compound	Antimicrobial
5	12.59	Butanal, O-methyloxime	7.29	Aldehyde compound	Antimicrobial
6	13.2	L-Homocitrulline	22.39	Nitrogen compound	Antimicrobial
7	14.35	Pentanal, oxime	0.88	Aldehyde compound	Antimicrobial
8	14.76	E-2-Tetradecen-1-ol	1.77	Alcoholic compound	Antimicrobial
9	15.47	Z-10-Pentadecen-1-ol	1.62	Alcoholic compound	Antimicrobial
10	15.71	Lysine	1.73	Amino acid	Nutrient
11	15.97	Trimethylamine, compd. with borane (11)	0.59	Amino compound	Antimicrobial
12	16.89	1,1-Cyclopropane dicarbonitrile, 2,2-dimethyl-	1.18	Nitrogen compound	Antimicrobial
13	17.46	Cyclopentanol, 2-(aminomethyl)-, cis-	0.41	Amino compound	Antimicrobial
14	19.8	Tricyclo[4.2.1.1(2,5)]decan-3-ol	0.77	Alcoholic compound	Antimicrobial
15	20.44	n-Nonanoylmorpholine	0.63	Nitrogen compound	Insect repellent
16	21.01	Thiomorpholine	0.88	Sulphur compound	Antimicrobial used in Pharmaceuticals
17	21.31	Semioxamazide		Amino compound	Antimicrobial
18	28.85	Cholan-24-oic acid, 3-oxo-, methyl ester, (5á)-	50.06	Steroid	Antimicrobial Antiinflammatory Anticancer Antiasthma Diuretic

Discussion:-

In the present study, the whole body tissue extract of the marine gastropod *Conus achatinus* showed significant antibacterial activity against ten bacterial pathogens. The crude extract showed more pronounced antibacterial activities against some human pathogenic bacterial strains. It could be explained that the bioactive compounds present in the experimental organism might have been developed as chemical defense of animals against their predators (Chellaram and Edward, 2009) living in their specific environment. Anand and Edward (2001) noted that the crude methanol extract of *Cypraea errones* exhibited promising antibacterial activity. Chellaram *et al.*, (2004) reported that chloroform and acetone extracts of *Ptera chinensis* showed broad spectral antibacterial activity against eight fish pathogens tested. Similarly Annamalai *et al.*, (2007) reported that *Perna viridis* showed maximum inhibition against *E. coli* and *Staphylococcus aureus*. Anbuselvi (2009) found that acetone column purified fractions of *Trochus tentorium* showed higher antibacterial activity. Santhi (2011) reported antibacterial activity of the whole body tissue extract of *Purpura persica* and the results showed wide spectral activity against some pathogens. Antibacterial activity has been previously proved in a wide range of molluscan species (Mitta *et al.*, 2000; Rajaganapathy *et al.*, 2000; Prem Anand and Patterson Edward, 2002; Prema., 2006; Annamalai *et al.*, 2007; Maripandi *et al.*, 2010 and Subavathy, 2012). This could be attributed to the fact that the test extract might contain secondary metabolites which inhibited the growth of bacteria.

Apart from the crude extract, the fractionated extract of *Conus achatinus* inhibited *Klebsiella pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus aureus* showed promising results in all the pathogens. F2 (Chloroform) fraction showed minimum inhibitory zone of 9 mm against *Pseudomonas aeruginosa* and *Proteus vulgaris* and maximum of 14 mm against *Streptococcus pyogenes*. F3 fraction (Benzene:Methanol) of *Conus achatinus* showed wide spectrum activity by developing maximum inhibition zone of maximum 20mm against *S. aerues* and minimum 12 mm against *K. pneumoniae*. The present finding is a supportive evidence of Rajaganapathy *et al.*,

(2000) who have reported that the methanol extract from the whole body tissue of *Hemifuses pugilinus* exhibited activity against *B. subtilis*, *E. coli* and *K. pneumonia*. Santhana Ramasamy and Murugan (2003) reported that methanolic extract of *Chicoreus virgineus* and *Chicoreus ramosus* showed broad spectrum antibacterial activity of the body tissue extract. Dhinakaran *et al.*, (2011) observed that ethanol and methanol extract of marine gastropod *H. pugilinus* showed maximum antibacterial activity against few pathogens. The above findings corroborate with the observations of the present study.

Antibacterial activity of marine animals and their bioactive products at molecular level have been studied by various authors and the molecules responsible for antibacterial activity have been identified and characterized. The GC-MS analysis of the extract of *Conus achatinus* revealed eighteen compounds (Table 1). Of the 18 compounds, the following 15 compounds viz., Aziridine, I-methyl, Cyclohexanol, 2-aminotrans, Azocine, octahydro-, Butanal, O-methylxime, L-Homocitrulline, Pentanal oxime, E-2-Tetradecen-1-ol, Z-10-pentadecen-1-ol, Trimethylamine compound with borane, 1,1-cyclopropane decarbonitrile, 2,2-dimethyl, Cyclopentanol, 2, (aminomethyl)- cis-, Tricyclo [4.2.1.1 (2,5)] decan-3-ol, Thiomorpholine, Semioxamazine and cholan-24-oic acid, 3-oxo-methyl ester (5a)- could be responsible for the antibacterial activity of the present study.

To conclude, the present study clearly showed that among all the fractions, F2 and F3 fractions of the study animal *Conus achatinus* showed more activity. This may be due to the presence of above mentioned compounds in the body extracts. So, further studies are needed to isolate and characterize these compounds to study their mode of action in pharmacological field.

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