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Antibacterial activity of the tissue extracts of *Conus betulinus* and *Conus inscriptus* Linnaeus, 1758 (Mollusca: Gastropoda) from Nagapattinam, Southeast coast of India

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ABSTRACT

Objective: The study of marine organisms for their bioactive potential, being an important part of marine ecosystem has picked up the rhythm in recent years with the growth recognition of their importance in human life as well as animals. **Methods:** In this present study ethanol, methanol and acetone extract of two gastropods *Conus betulinus* and *Conus inscriptus* were assayed for the antibacterial activity against three poultry bacterial pathogens. **Results:** The antibacterial activity of *Conus betulinus* tissue extract, showed maximum zone of inhibition (15mm) against *Salmonella* pullorum and minimum activity (4 mm) was observed in *Salmonella* typhimurium. In *Conus inscriptus*, the tissue extract showed the maximum zone of inhibition (12mm) against *S. enteritidis* and minimum activity (3 mm) was observed in *S. pullorum*. Molecular size of muscle protein was determined using Sodiumdodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). FTIR analysis reveals the presence of bioactive compounds signals at different ranges. **Conclusions:** Among the two gastropods, *Conus betulinus* showed more antibacterial activity than that of *Conus inscriptus*. The reveled in this research shows that gastropod *Conus betulinus* tissue is medicinal value due to high quality of antibacterial compounds.

1. Introduction

Salmonella can be found in virtually every part of the world and carried by an extremely wide variety of hosts including humans and other mammals, birds, reptiles, and insects [1, 2]. *Salmonella* are responsible for a variety of acute and chronic diseases in both poultry and humans. In humans, *Salmonella* are the cause of two diseases called salmonellosis: 1) enteric fever (*Salmonella* typhimurium is the strain that causes typhoid fever) resulting from bacterial invasion of the bloodstream, and 2) acute gastroenteritis, resulting from a food-borne infection/intoxication. *Salmonella* spp. infections continue to plague the poultry industry and cause substantial losses in productivity. Infected poultry products are among important sources for food-borne outbreaks in humans. *Salmonella* spp., among S. typhimurium, have long been major causative agents of food-borne infection and has been a concern of the poultry industry. *Salmonella* spp. has been focus of numerous scientific investigations aimed at eliminating the bacterium [3]. Antimicrobial intervention in poultry is milestone in a total disease prevention programme that emphasizes preventive disease management. Use of antimicrobials in poultry industry has been fundamental intervention strategy since the I960's [4]. There are antibiotics approved for use as injections in day-old chickens and turkeys to control omphalitis or yolk sac infections. This procedure has been performed for over 30 years in the poultry industry. Marek's disease in-ovo vaccination technique provided a window of opportunity for an injection of antibiotic for effective control of the yolk sac infection by placing the antibiotic at the point of contamination, when the yolk sac is withdrawn into the body cavity. The growing global concern over antibiotic resistance and the stigma of *Salmonella* spp. associated with poultry has resulted in increased research efforts designed to eliminate *Salmonella* spp. infections.

Many studies on bioactive compounds from molluscs

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exhibiting antitumor, antileukemic and antiviral activities have been reported worldwide [5, 6, 7]. Among the Gastropod, several families of carnivorous marine snails are known to produce toxic venoms, which are used in the capture of prey [8]. The bioactive substance was isolated from gastropod Drupa margariticola [9,10] elucidated the venom from Conus figulinus (a vermivore) which contain antiarrhythmic property through pharmacological experiments on isolated guinea-pig left atria. Another vermivore cone Conus lorosii has proved to contain in its venom, the cardiotonic principle like digoxin [11]. The molecular biological approach has proven more powerful than earlier protein/peptide based technique tor the detection of novel conotoxins. The marine gastropods are very good source for bioactive compounds. Therefore, the aim of the present study was to evaluate the antibacterial activity of the tissue extracts of two gastropods *Conus betulinus* and *Conus inscriptus* against different poultry pathogenic bacterial strains.

2. Materials and Methods

2.1. Extraction of antibacterial compounds from gastropod

Live specimens of *Conus betulinus* and *Conus inscriptus* (Family: conidea) were collected from Nagapattinam (Lat. 11 ° 15 N and Long. 79 ° 46 E), Southeast coast of India. The specimens were brought to the laboratory and their soft bodies were removed by breaking the shells. The body tissue of the two samples (30 g) was cut into small pieces and air-dried for 24h at room temperature before extraction with solvents. Then the tissues were rinsed with sterile distilled water and the tissue sample was used for extraction using different solvents such as ethanol, methanol and acetone. The extracts were cold steeped over night at -18 0C and filtered with Whatman No. 1 filter paper. The filtrate was poured in previously weighted Petri plate and evaporated to dryness in rotary evaporator [12, 13]. The dried crude extracts were used for antibacterial assay against poultry pathogens (*S. pullorum, S. enteritidis* and *S. typhimurium*). All the poultry pathogenic bacterial strains were obtained from Veterinary College and Research Institute, Namakkal.

2.2. Antibacterial activity of gastropods extracts

Three species of pathogenic bacteria namely *Salmonella pullorum*, *Salmonella enteritidis* and *Salmonella typhimurium* were used to screen the antibacterial activity of the gastropod extracts. Pathogenic bacterial strains were inoculated in sterile nutrient broth and incubated at 37 ℃ for 24h. Pathogens were swabbed on the surface of the Mullar Hinton agar plates and discs (Whatman No.1 filter paper 6mm diameter) impregnated with the 50 μl of gastropod extracts were placed on the surface. Control discs were placed with antibiotics and solvents to asses the effect of antibiotics and solvents on pathogens. The plates were incubated at 37 °C for 24h and the antibacterial activity was measured accordingly based on the inhibition zone around the disc impregnated with gastropod extracts.

2.3. Molecular size of muscle protein SDS PAGE

Molecular size of muscle protein was determined using SDS PAGE gel following the procedure [14]. Glass plates were assembled and 20 mL of 15% resolving gel was prepared and poured immediately to the notch plate. It was over laid with butanol, after polymerization was completed over lay was poured off and washed the top layer with deionized water. Then 8ml of stack gel was over laid. Approximate volume of 1XSDS gel loading buffer and sample was taken. Heated it at 100 ℃ for 3 min. Assembly was fixed in electrophoresis apparatus then 15 μL of sample and marker (2- 97KDa) was loaded respectively in the well, run the gel and stain with coomassie brilliant blue.

2.4. FT-IR spectro photometry (Fourier Transform- Infra Red spectrum analysis)

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

3. Results

3.1. Antimicrobial Assay

The zone of inhibition in different bacterial strains against *Conus betulinus* tissue extraction is shown in (Fig .1). Among the various strains maximum zone of inhibition (15mm) was recorded in *Salmonella* pullorum strain and minimum zone of inhibition (4mm) was observed in *Salmonella* typhimurium strain. The positive control (oxytetracycline hydrocholride) was observed activity against all the bacterial strains tested. The maximum activity against *Salmonella* pullorum (20mm) and the minimum activity were observed against *Salmonella* enteritidis (18mm).

The antibacterial activity of the *Conus inscriptus* tissue extraction shows maximum activity in *Salmonella* enteritidis (12 mm) and minimum activity was recorded in *Salmonella* pullorum (3mm) is shown in (Fig. 2). The positive control (oxytetracycline hydrocholride) was showed activity against all the bacterial strains tested. The maximum activity against *Salmonella* typhimurium (20mm) and the minimum activity were observed against *Salmonella* enteritidis (18mm).

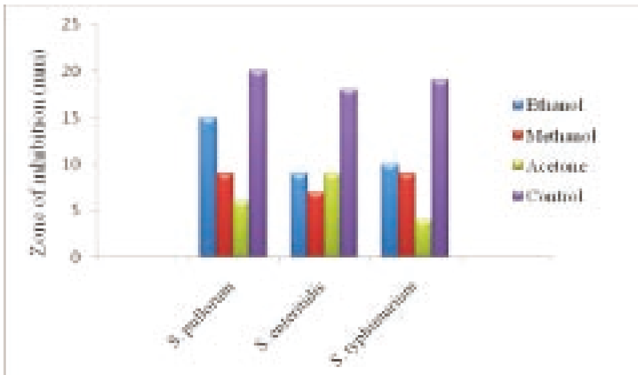

Figure 1. Antibacterial activity of *C. betulinus*

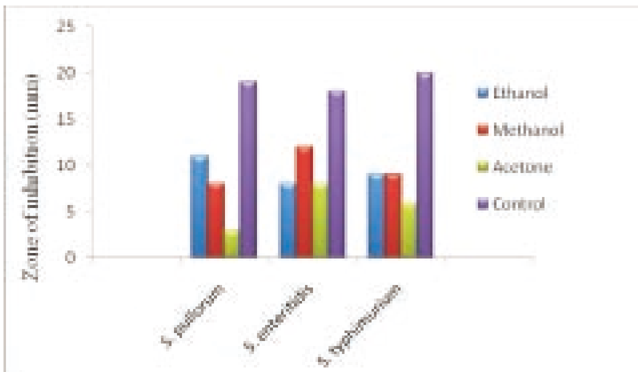

Figure 2. Antibacterial activity of *C. inscriptus*

3.2. SDS PAGE

The tissue extraction samples showed antibacterial activity was subjected to SDS-PAGE to estimate the molecular weight of proteins present in it. Different standard were used to determine the molecular weight of tissue extract proteins. The stained gel revealed that the tissue extract contained a simple population of proteins. There is different molecular weight marker proteins were used (*C. inscriptus* 97, 63, 61, 42 KDa, *C. betulinus* 93, 61, 42, 40 KDa) band was detected in the gel that represented protein of 97- 2 KDa shown in (Fig. 3).

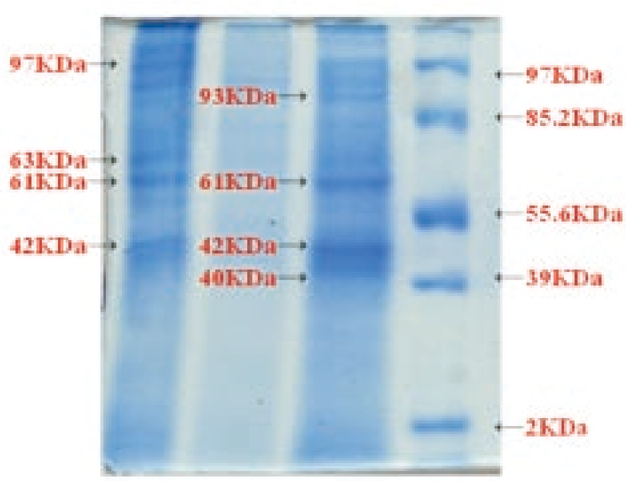

Figure 3. SDS-PAGE, Molecular weight of crude proteins in *C. betulinus* and *C. inscriptus*

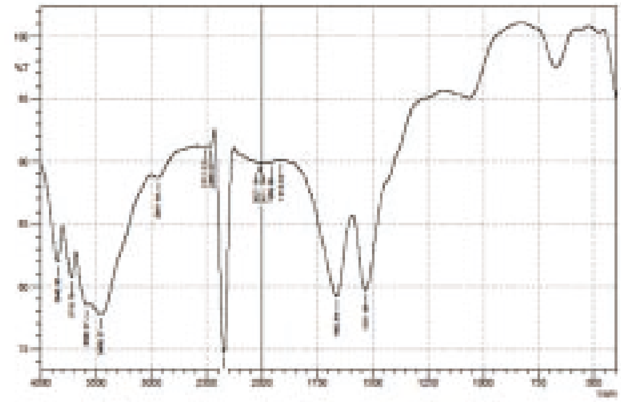

Figure 4. The FTIR spectrum of crude sample in *C. betulinus*

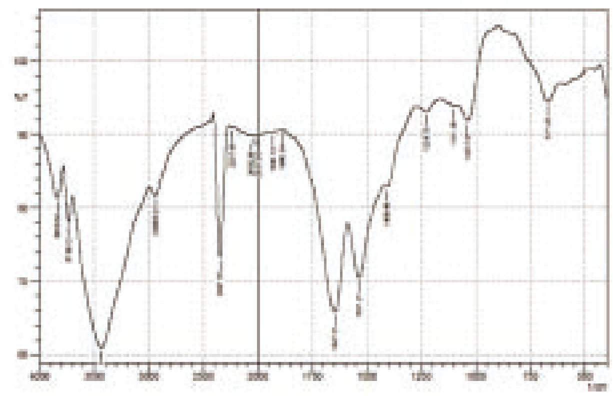

Figure 5. The FTIR spectrum of crude sample in *C. inscriptus*

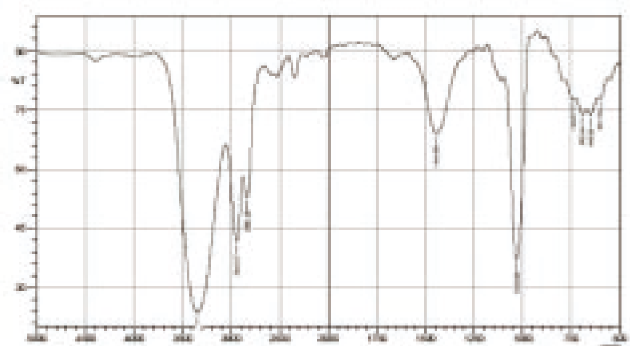

Figure 6. The FTIR spectrum of standard

3.3. FT-IR spectrum analysis

IR spectrum of the crude extracts of *C. betulinus* and *C. inscriptus* was compared with standard (Antibiotic). The IR spectrum of the standard depicted five major peaks at 3344.57, 2943.37, 2835.36, 1444.68 1029.99 cm-1(Fig. 6). Fourier Transform Infra Red FTIR spectrum of the lyophilized *C. betulinus* sample the IR spectrum of the 5 major peaks at 3585.67, 3458.37, 2343.51, 1662.64 and 1531.48 cm-1. Whereas the spectrum of the sample of *C. betulinus* showed the remaining all peaks were very close value (Fig.4). The lyophilized *C. inscriptus* sample the IR spectrum of the 4 major peaks at 3437.15, 2347.37, 1647.21 and 1537.27 cm-1. Where the spectrum shows of the sample of *C. inscriptus* showed and remaining all peaks are very close value (Fig.5).

4. Discussion

Antimicrobial agents are essential drugs for human and animal health and welfare. Antimicrobial resistance is a global public and animal health concern that is influenced by both human and non-human antimicrobial usage. The human, animal and plant sectors have a shared responsibility to prevent or minimise antimicrobial resistance selection pressures on both human and non-human pathogens. Analysis of patterns of antibiotic resistance was further performed by separating serotype Typhimurium and other serotypes because of peculiar features due to the prominent role of the 'DT104 complex' and its resistance pheno-genotype in the epidemiological environment of such a serotype [15]. The antimicrobial property of thyme has been shown to be attributable to the essential oil fraction. Some researchers have demonstrated the antimicrobial activity of the most common terpene compounds, such as thymol, carvacrol, linalool, eugenol, "-pinene, and $-pinene in this medicinal herb against several microbial strains[16].

In recent years, development of multidrug resistance in the pathogenic bacteria and parasites has created major clinical problems in the treatment of infectious diseases [1,37] reported that the Isolation and identification of pathogenic bacteria, with special reference to Ornithobacterium rhinotracheale associated with respiratory diseases. Developed countries have used for a long time systems of surveillance of food safety problems. In the present investigation distinct antibacterial activity was observed against almost all the poultry pathogenic bacteria. Ethanol extracts of *Conus betulinus* showed highest activity against *S. pullorum* and control showed highest activity against *S. pullorum*, respectively and other acetone extracts showed lowest activity against *S. typhimurium*. The methanol extract of *Conus inscriptus* exhibited highest activity against *S. enteritidis*. Ethanol and acetone extract displayed highest activity against *S. pullorum*, *S. typhimurium* and *S. enteritidis* and other acetone extracts showed lowest activity against *S. pullorum*. [18] reported the root extract of *Cymodocea serrulata* was tested for the antimicrobial sensitivity against the poultry pathogens viz. *Klebsiella*, *E. coli*, *Staphylococcus* sp and *Salmonella* sp. The antibacterial activity of the tissue extract of *C. betulinus* and *C. inscriptus* against the poultry pathogen might be due to the presence of proteins. Tannins [19] form irreversible complexes with proline rich proteins, resulting in the inhibition of cell protein synthesis of bacteria [20]. Flavonoids are phenolic structure containing one carbonyl group complexes with extra cellular and soluble protein and with bacterial cell wall [21,22] also confirmed the inhibitory activity of this species against 64.2% of the evaluated bacteria, including *S. Choleraesuis*. The addition of sub-therapeutic levels of antibiotics to broiler feed causes an increase in weight gain [23,39] reported that the essential oils and their major monoterpene alcohols were tested against nine bacterial strains and the essential oils with high phenolic contents were the most effective antimicrobials.[38] Major antibiotic resistance have been reported in various *Salmonella* remains a public health and economic regions throughout the world and antibiotic resistant problem in developing countries. Isolation of various pathogenic bacteria probably indicates that frequent indiscriminate uncontrolled use of antibiotics in layer poultry farms might result in resistance to antimicrobial agents among the pathogenic bacteria, particularly for *E. coli* [24,25] reported that the antibacterial activity of some fruits; Berries and Medicinal Herb Extracts against poultry strains of *Salmonella*. [26] reported the in vitro antibacterial activity of 21 hydroethanolic vegetable extracts was assessed against 20 serovars of *Salmonella*. Regarding the tested extracts, 85.7% of them presented antibacterial activity. [40] The interaction (synergy, antagonism or addition) between two compounds depends on the concentrations of the single component. Marine molluscs are highly delicious seafood and also very good source of bioactive compounds.

In the present investigation tissue extraction that showed antimicrobial activity was subjected to SDS-PAGE to estimate the number and molecular weight of proteins present. After electrophoresis clear band were detected in the gel which represented proteins of molecular weight (*C. inscriptus* 97, 63, 61, 42 KDa, *C. betulinus* 40, 42, 61, 93 KDa). [27] observed that unclear bands ranging from 14 KDa and 29 KDa in marine bivalves *M. casta* and *P. viridis*.[28] reported that the crude proteins showed 5 to 6 bands ranging from 45 to 261 KDa on Meretrix meretrix and Meretrix casta. In the present study indicates that the tissue extraction of *C. inscriptus* and *C. betulinus* would be a good source of antibacterial compounds and would replace the existing inadequate and cost effective antibiotics. IR spectrum of the crude antibacterial extracts of *C. betulinus* and *C. inscriptus* was compared with standard antibiotic. The IR spectrum of the standard depicted five major peaks at 3344.57, 2943.37, 2835.36, 1444.68 1029.99 cm-1. Whereas the spectrum of the crude antibacterial compound of *C. betulinus* and *C. inscriptus* also showed the few number of major peaks with very close values at 3585.67, 3458.37, 2343.51, 1662.64, 1531.48 cm-1 and 3437.15, 2347.37, 1647.21, 1537.27 cm-1 respectively. The antibacterial compounds from whole body tissue of *C. betulinus* and *C. inscriptus* crude showed major peaks which is said to be responsible for the chemical groups. The band at 1656 cm-1, which occurs at similar wavelength in polyamides and proteins, is commonly assigned to stretching of the C=O group hydrogen bonded to N-H of the neighboring infra sheet chain [29], The acetyl amino group was represented by a band at 1411 cm^{-1}, the carboxyl group at 1654 cm^{-1}, 1418 cm^{-1} CH_2 bending & CH_3 deformation, 1116 cm^{-1} asymmetric in phase ring stretching mode, 690 cm^{-1} OH-Out-of plane bending, 896 cm^{-1} Ring stretching [30]. Bands in the 820-850 cm^{-1} spectral region were attributed

C-O-S stretching based on the results of [31], as observed by [32] the sample showed the absorption band for the carboxylic group at 1654 cm^{-1} and acetyl amino group at 1400 cm^{-1} which were also reported by [33] that 1615 cm^{-1} (carboxylic group) and 1375 cm^{-1} (acetyl amino group) in the sulfated mucopolysacharides isolated from the skin of chimaera sp. FTIR analysis reveals the presence of antimicrobial compound signals at different ranges. The research the gastropods *C. inscriptus* and *C. betulinus* muscle is value medicinal due to high quality of antimicrobial compounds.

The first attempt to locate antimicrobial activity in the marine organism was initiated around 1950's has reported by [34]. Organic substances produced by marine plants and animals have been shown to affect bacterial behavior was reported by [35]. The extent to which secondary metabolites function as an antibacterial chemical defense however, has not been demonstrated [36]. Crude products isolated from marine organisms have served as a source of drugs and starting materials for synthesis of useful drugs. In addition, because of the differences in the environmental conditions, new or unusual biochemical entity having biological activity can be evolved by marine organisms. So it is believed that the studies of new and unique compounds derived from marine organisms will continue to increase our basic knowledge with respect to pharmacology and medicine. The results in this research show that gastropod *Conus betulinus* and *Conus inscriptus* body tissue of crude sample is value drug due to high quantity bioactive compound, well-balanced antibacterial activity. In conclusion in the present study indicates that the active fractions of ethanol, methanol and acetone tissue extracts from *Conus betulinus* and *Conus inscriptus* could be effectively used as alternative bioactive compounds to rectify the problem of unknown diseases disseminated from the poultry borne food products.

The present study was revealed that two species *Conus betulinus* and *Conus inscriptus* showed antimicrobial activities against the poultry pathogenic microbial forms. They represent potential pharmacological leads perhaps possessing novel and uncharacterized mechanisms of action that might ultimately benefit the ongoing global search for clinically useful antimicrobial agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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