International Journal of Zoology and Applied Biosciences
Volume 1, Issue 6, pp: 262-266, 2016
https://doi.org/10.5281/zenodo.1310792
Research Article

ISSN: 2455-9571
http://www.ijzab.com

POTENTIAL ANTIBACTERIAL ACTIVITY OF THE LAND SNAIL *CRYPTOZONA BISTRIALIS* IN NAGAPATTINAM, TAMIL NADU, INDIA

***Valarmathi, V.**

P.G and Research Department of Zoology, A.D.M. College (Autonomous), Nagapattinam-611001, Tamil Nadu, India

Article History: Received 20th October 2016; Accepted 16th November 2016; Published 31st December 2016

ABSTRACT

Antibacterial screening was conducted by Agar well-diffusion method to find out the antibacterial property of the soft body tissues of the land snail *Cryptozona bistrialis* against the human pathogenic bacteria species namely *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aerogense* and *Salmonella typhi*. The tissue extract of *C. bistrialis* exhibited the highest significant activity against *Staphylococcus aureus* (30mm), *Escherichia coli* (25 mm) and *Klebsiella pneumonia* (25 mm) and showed the minimum inhibitory against the *Pseudomonas aerogense* (10 mm) and Salmonella typhi (10 mm). Hence the study reveals the presence of antibacterial factors in the whole body of the land snail *C. bistrialis.*

Keywords: Antibacterial activity, Land snail, *Cryptozona bistrialis*, Human pathogenic bacteria.

INTRODUCTION

The molluscs are the very good source for biometrically important products. Many of these organisms are known to possess compounds as a common means of defense (Indap and Pathare, 1998). Snails are efficient producers of meat which is protein rich nutrient for malnutrition.

The study of natural products that exhibit biological activity, derived from plants and animals has long been showing significant biomedical value and crude products isolated from marine organisms have served as source of many drugs (Kamboj, 1999). In the most of the publications concerning antimicrobial activity in Mollusca, either single body compartment alone, like haemolymph and egg masses, or extracts of whole bodies have been tested for activity (Haug *et al.*, 2003).

Studies of bioactive compounds from plant and animals are of great interest because of their potential therapeutic use (Bansemir *et al.,* 2006). Pharmaceutical industries are continuously finding ways not only to combat ailments due to pathogenic microorganisms but also due to the advent of multiple resistant mechanism. The demand for effective and non-toxic antibacterial therapeutics has become even greater with the increased incidence of bacterial infections. Hence, there is a need for new antibiotic drugs with lesser environmental and toxicological risks. Studies on bioactive compounds from mollusc may be useful in antimicrobial therapy.

The land snails have high medicinal value-they are used in the prevention and care of diseases like hypertension. Based from local reports, the snails have been utilized as folk medicine for several ailments such as asthma, cough, allergies, wound treatment and malaise. On the other hand, the soft tissue body of the snail has become part of a home cooked viand and is usually eaten to treat respiratory related ailments. The bioactive compounds isolated from the gastropods are considered to have a role in the chemical defense of the animals against their predators (Avila, 1995). Many studies on bioactive compounds from molluscs exhibiting antibacterial and antiviral activities have been reported worldwide (Fenical, 1997).

Snails have high medicinal values that help in the prevention and curing of cardiovascular diseases such as hypertension, kidney diseases, tuberculosis, anemia, diabetes, asthma, curing cancer, hemorrhoid and to restore virility and vitality in men apart from its richness in nutritional value (Odunnaiya, 1991**).**

A perusal of literature indicated that works on the land snail *Cryptozona bistrialis* is very much limited in

*Corresponding Author: Dr. V. Valarmathi, Head and Associate Professor, P.G and Research Department of Zoology, A.D.M.College (Autonomous), Nagapattinam-611001, Tamil Nadu, India, Email: mathivalar.k@gmail.com.

Nagapattinam area. Hence this research aimed to verify the therapeutic properties of snails through antibacterial screening. In addition, this present investigation was undertaken to ascertain the antimicrobial capability of the tissue extracts from the soft body of *C. bistrialis* against human pathogens. Specifically, this study was aimed to measure the zone of inhibition from the composite against five bacterial species *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Salmonella typhi*.

MATERIAL AND METHODS

Snail collection

Specimens of *C.bistrialis* were collected from Kadambadi area of Nagapattinam. About 50 snails were collected at regular interval for the period of 2 months (February 2016 and March 2016). The freshly collected snails were brought to the laboratory. The snails were reared in the wooden cage filled with gravel and garden soil about 6-8 cm height. Cold water was sprinkled to moisten the soil from time to time and to keep the snails in active condition. The snails were fed every morning with carrot, chowchow, cucumber, cabbage and radish. Special care was taken to clean the cage by removing fecal pellets, leftover food material daily. The snails thrived well when provided with the temperature of 28^{o}C ± 1°C and optimum relative humidity by periodical spray of cold water.

Antibacterial screening

Collection and preparation of culture inoculums: The stock cultures of bacteria used for the screening were *E. coli, S. aureus, P. aeruginosa, K. pneumonia* and *S. typhi* purchased Doctor's Diagnostic Center, Thillai nagar, Tiruchirappalli. All the cultures were tested for purity and maintained on nutrient agar slants at 4°C. Inoculums was prepared by suspending a loop full of bacterial cultures into 10 ml of nutrient broth and was incubated at 37°C ± 2°C for 24 to 48 hours with the aim of increasing their life span.

Preparation of solvent extracts: About 25 snails were kept for one day in the plastic container in order to allow them to empty their guts and the soft body tissue from the shell was removed. Then the soft tissue body was homogenized with solvent using the blender. The resulting solution was transferred to a flask and subjected to solvent extraction using a rotavap.

Determination of antibacterial activity

Agar well-diffusion method: Agar well-diffusion method (Perez *et al.,* 1990) was followed to determine the antibacterial activity. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 24 hours cultures of *E. coli, S. aureus, P. aeruginosa, K. pneumonia* and *S. typhi*. Agar wells (5 mm diameter) were made in each of these plates using sterile cork borer. The extract of fresh snail poured into the respective wells and plates were left for 1 hour to allow a period of pre incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions. The plates were incubated in an upright position at 37°C ± 2°C for 24 h for. Antibacterial activity of the soft tissue body was determined by measuring the zone of inhibition (diameters of the zones in mm) against each test bacteria was using a diameter measurement scale. The experiment was carried out in triplicates and the mean values of the results were recorded for antimicrobial activity.

RESULTS

Antibacterial activity was tested against five bacterial pathogens with respect to extract of the soft body tissues of the land snail *C. bistrialis*. The antibacterial activity is shown in Table 1 and Figures 1-5.

Zone of inhibitions

Present findings showed that the extracts of the soft body tissues of *C.bistrialis* all were able to inhibit the growth of the tested bacteria species. Table 1 shows the zone of inhibition produced by the soft body tissue extracts of *C.bistrialis* against the following bacteria species namely *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aerogense* and *Salmonella typhi*.

In the tissue extract, the zone of inhibition exhibited by the *Staphylococcus* aureus (30mm) was significantly higher than the other bacterial species. However, the result of the tissue extract from *Escherichia coli* (25mm) and *Klebsiella pneumonia* (25 mm) showed a higher zone of inhibition comparatively.

The present study also showed the minimum inhibitory zone of the soft body tissue extract against the microbial strain Pseudomonas *aerogense* and *Salmonella typhi*.This finding also indicated that the extracts of the soft body tissues of the *C.bistrialis* showed the strong antibacterial activity against gram positive organisms such as *Staphylococcus aureus*. The gram negative organism like *Escherichia coli* and *Klebsiella pneumonia* showed higher activity but the *Pseudomonas aerogense* and *Salmonella typhi* showed their narrow spectrum of activity.

Table 1. Antibacterial activity of the soft body of *C. bistrialis*.

S. No.	Organism	Zone of inhibition (mm)
1	*Escherichia coli*	25
2	*Staphylococcus aureus*	30
3	*Klebsiella pneumoniae*	25
4	*Pseudomonas aerogense*	10
5	*Salmonella typhi*	10

Each value is mean of 5 isolates.
Zone of inhibition was acceptable as sensitive.

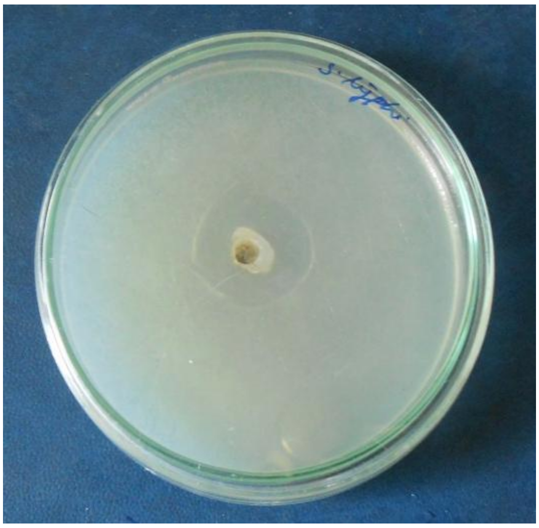

Figure 1. Zone of inhibition developed against Salmonella typhi.

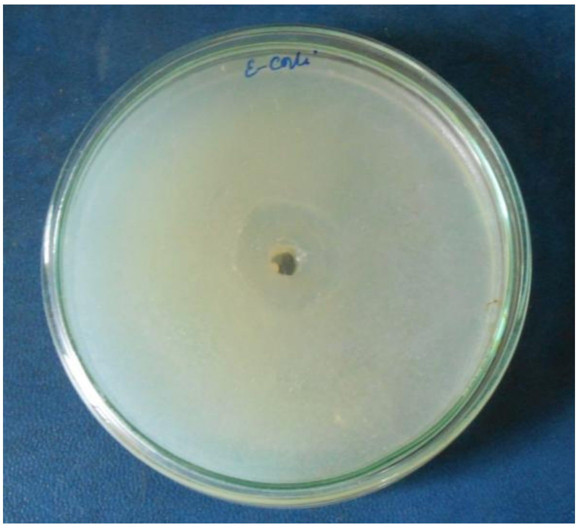

Figure 2. Zone of inhibition developed against E.coli.

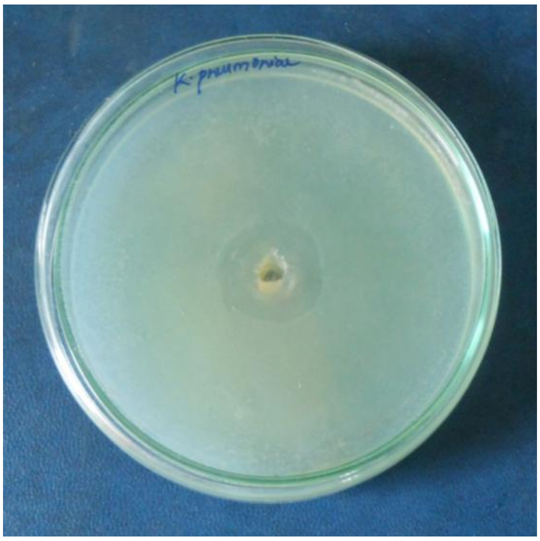

Figure 3. Zone of inhibition developed against Klebsiella pneumonia.

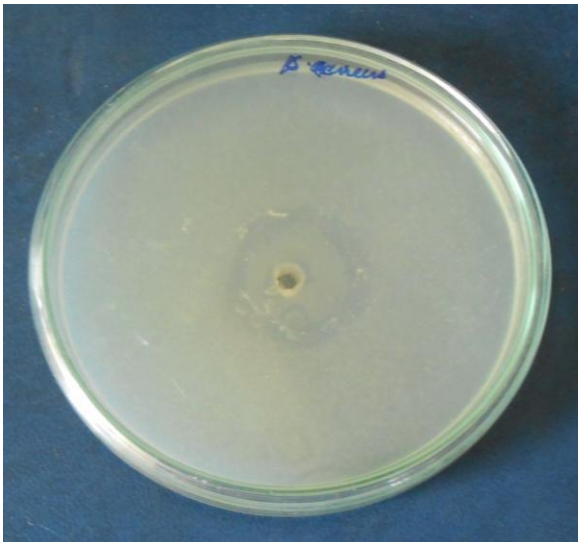

Figure 4. Zone of inhibition developed against Staphylococcus aureus.

Figure 5. Zone of inhibition developed against *Pseudomonas aerogense.*

DISCUSSION

Molluscs are considered as one of the important natural sources to derive many bioactive compounds that exhibit antitumor,antimicrobial, anti-inflammatory and antioxidant activities (Benkendorff *et al.*, 2011). Numerous pathogenic microorganisms have developed their resistance to commonly available antibiotics; hence the need for developing new virulent drugs against these harmful pathogens becomes more important. Chemical drugs may lead to adverse effects and recent researchers have focused on pharmacologically active compounds from natural sources. Compounds isolated from marine molluscs were also used in the treatment of rheumatoid arthritis and steoarthritis (Chellaram and Edward, 2009). Marine mollusc extracts also exhibited antibacterial and antiviral activity against fish pathogenic bacteria and the extract also may be applied in aquaculture (Defer *et al*., 2009).

Antibacterial activity in marine gastropods was reported by Rajaganapathi *et al*. (2001). The antibacterial activity of the extracts of four bivalves against few pathogens like *K. oxytoca* and *B. subtilis* showed significant activity (Jayaseeli *et al*., 2001). Anand and Edward (2001) studied the antibacterial activities in ethanol extracts of gastropod species Babylonia spirata and *T. brunneus* and observed highest activity against *E. coli*, *K. pneumoniae*, *P. vulgar*is and *S. typhi*. Diane *et al*. (2009) reported the antibacterial and antiviral activities on three bivalves and two gastropods species.

Land snail *Achatina fulica* able to produce a glycoproteic secretion which has been demonstrated to present some biological effects, such as antibacterial properties against gram positive and negative microorganisms (Martins *et al.,* 2003; Lorenzi and Martins, 2006). In this study, the mucous secretion of *A. fulica* inhibited the bacterial growth of both *Staphylococcus aureus* and *Staphylococus epidermidis*. Other studies attesting the antibacterial properties of the mucus of invertebrate snails have been previously reported (Iguchi *et al*., 2006).

The products from many mollusks have been said to very useful in medical practice. It has been proposed that this mucous secretion is rich in lysozyme, opsonines, and other antibacterial and antiviral factors (Fuchino and Barrett, 1992).

The molluscs are the treatment of infectious diseases. Moreover, cost of potential source of bioactive substances. The bioactive production of synthetic drugs is also high and they because compounds isolated from the gastropods are considered adverse effect when compared to bioactive naturally to have a role in the chemical defense of the animals derived drugs. Hence, intense research is under against their predators. Molluscs in the land are progress towards the search for natural remedies with common sight and are virtually untapped resource for the potent biological activities from terrestrial organisms. Discovery of novel compounds.

The shell and tissue composite extracts from *H. daphnis* were tested for its antibacterial property against *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtilis* showed that both the tissue composite and shell methanolic extracts were able to inhibit the growth of the four tested bacterial species. The study on the mucus from the common brown garden snail, *H. aspersa* has a demonstrable antimicrobial activity against several strains of *P. aeruginosa*. The antibacterial factor might be functioning to protect the wet-skinned animal from external infection.

The present study indicates the whole body extraction of *C. bistrialis* would be a good source of antibacterial agents and would replace the existing inadequate and cost effective antibiotics. In the present investigation, distinct antibacterial activity was observed against almost of the five human pathogenic bacteria. The soft body tissue extracts of *C. bistrialis* showed highest inhibitory activity against *S. aureus* (30 mm), *E. coli* (25 mm) and *K. pneumonia* (25 mm). Lowest activity was observed against *P. aerogense* and *S. typhi*.

CONCLUSION

The present findings encourage that the land snails are the potent sources for antibacterial drug development. Hence the present study reveals the presence of antibacterial factors in the whole body of the land snail *C. bistrialis*. The present study was recommended to carry out in detail on the mechanism of the growth inhibition by the antibacterial factor in this snail.

ACKNOWLEDGEMENTS

The authors express sincere thanks to the Principal, A.D.M. College (Autonomous), Nagapattinam for necessary facilities provided to carry out this research.

REFERENCES

Anand, T.P. and Edward, J.K.P., 2001. Screening for antibacterial activity in the opercula of gastropods. *Puket. Mar. Biol. Centre Spl. Pub.,* 25: 215-217.

Avila, C., 1995. Natural products of opisthobranch molluscs: a biological review. *Oceanogr. Mar. Biol.,* 33, 487-559.

Bansemir, A., Blume, M., Schroder, S. and Lindequist, U., 2006. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *J. Aquacul.,* 252, 79-84.

Benkendorff, K., McIver, C.M. and Abott, C.A., 2011. Bioactivity of the murex homeopathic remedy and ofextracts from an Australian murcid mollusc against human cancer cells. *Evid-Based Compl. Altern. Med.*, 1, 12-16.

Chellaram, C. and Edward, J.K., 2009. In vivo anti-inflammatory bustle of reef associated mollusc, *Trochus tentorium. Adv Biotech.,* 8(12), 32-34.

Defer, D., Bourgnon, N. and Fleury, Y., 2009. Screening for antibacterial and antiviral activities in three bivalve and two gastropod marine molluscs. *Aquaculture*, 293(1-2), 1-7.

Diane, D., Nathalie, B. and Yannick, F., 2009. Screening for antibacterial and antiviral activities in three bivalve and two gastropod marine mollusks. *Aquaculture,* 293, 1-7.

Fenical, W. and Fenical, W., 1997. New pharmaceuticals from marine mollusc organisms. Trend. Biotechnol., 15, 339-341.

Fuchino, G.M. and Barrett, S.S.H., 1992. Introduction a l'ethnomedecine. In: Medicine traditionelle et couverture des soins de sante (R.H. Bannerman, Ed.). Genebra, Suica, OMS, pp.17-24.

Haug, T., Stensverg, K., Olsen, O., Sandsdalen, E. and Styrovld, O.B., 2003. Antibacterial activities in various tissue of the horse mussel *Modiolus modiolus. Invertebr. Pathol.,* 85, 112-119.

Iguchi, S.M.M., Aikawa, T. and Matsumoto, J.J., 1982. Atividade antibacteriana do muco mucina de caracóis. *Comp. Biochem. Physiol.*, 27(3), 571-574.

Indap, M.M. and Pithare, S.P., 1998. Cytotoxicity and bioactivity of some marine animals. Indian. *J. Mar. Sci.,* 27, 433-437.

Jayaseeli, A.A., Prem, T.A. and Murugan, A., 2001. Antibacterial activity of four bivalves from Gulf of Mannar. *Phuket. Mar. Biol. Cent. Spec. Pub.,* 25(1), 215-217.

Kamboj, V.P., 1999. Bioactive agent from the Ocean Biota. In: Ocean Science: Trends and Future Directions (B.L.K. Somayajulu, Ed.). Indian National Science Academy. New Delhi, India, pp. 197-227.

Lorenzi A.T. and Martins, M.F., 2006. Analise colorimetrica eespectroscopica do muco de caracois terrestres *Achatina spalimentados* com racao diferenciada. *R. Bras. Zootec.,* 37(3), 572-579.

Martins, M.F., Caetano, F.A.M. and Sirio, O.J., 2003. Avaliacao do reparode lesoes de pele de coelhos tratadas com secrecao mucoglicoproteica do escargot Achatina. *Braz. J. Vet. Res. Anim. Sci.,* 1(1), 1-11.

Odunnaiya, O., 1991. Studies on the Growth Rate of a marginata Fed Pawpaw Leaf, Water- leaf and Maize chaff. B. Agric. Thesis, Ogun State University, Ago-Iwoye.

Perez, C., Pauli, M. and Bazerque, P., 1990. An antibiotic assay by the agar-well diffusion method. *J. Actabiol.*, 15, 113-115.

Rajaganapathi, J., Kathiresan, K. and Singh, T.P., 2001. Purification of anti- HIV protein from purple fluid of the sea hare *Bursatella leachii* de Blainville. *Mar. Biotechnol.*, 4(5), 447-453.