Journal of Applied and Natural Science 2 (2): 266-268 (2010)

JANS

A study on presence of bioactive compounds in snail *Achantina fulica*

G. V. Zodape

Department of Zoology, S. S. and L.S. Patkar College of Arts and Science and V. P. Varde College of Commerce and Economics S.V. Road, Goregaon (West), Mumbai- 400 062 (Maharashtra), INDIA

E-mail: gautamvz5@yahoo.com

Abstract: The bioactivity study of the crude extract as well as the pure compounds isolated by preparative TLC, of the snail were carried on bacteria, *Escherichia coli PBR 322, Vibrio cholerae* and cockroaches *Periplanata americana*. The growth of the bacteria was found to be inhibited and the cockroaches in moribund condition were found to be dead after a period of 24 hrs of incubation. The results concluded that the extract of the snail contained the bioactive compounds.

Keywords: Snail, Bioactive compound, *Escherichia coli, Vibrio cholerae, Periplanata americana, Achantina fulica*

INTRODUCTION

Natural products, whether based on microbial, plant, terrestrial animals, marine organisms or other origins have prone to be a rich source of lead compounds in drug discovery Faulkner (2002). Venomous animals offer an enormous additional potential. Currently, more than 100000 venomous animal species are reported, covering almost all phyla (snakes, scorpions, spiders, cone snails, hymepters, sea anemones, jellyfishes and even some lizards, birds and fishes) each of them is characterized by a unique venom compound of hundreds of bioactive molecules (Sonja *et al.,* 1995 and Clifford *et al.,* 2003). These components have evolved and are optimized by nature through million of years to catch and digest preys or for protection against predators Halvorson (1998). The collection, identification and evaluation of bioactive compounds from terrestrial organisms are one of the active fields of research in biological study at present (Carte *et al.,* 1994). The experimental approach consists of a systematic investigation about the action of crude or purified extract on physiological preparations or whole animals to outline the profile of their biological activities and mechanism of action**.** The present work has been undertaken for isolation and purification of bioactive compounds and action of the extracts of the snail for its biological activity on bacteria and cockroach *Periplanata americana.*

MATERIALS AND METHODS

Snail *Achantina fulica*, were collected from the garden area of Patkar College Goregaon (West), Mumbai during the monsoon season and acclimatized for 2 days at room temperature before use. Pure culture of bacteria, *Escherichia coli PBR 322, Vibrio cholerae*, were collected from Bhavans National Research Center, Andhari, Mumbai and stored in a refrigerator at 2-8^{0}C till their use. Cockroaches *Periplanata americana* were collected from the drain of Goregaon, near Patkar College Goregaon (west), Mumbai, and acclimatized at room temperature for 2hrs before their use by using the standard methods (Pelczar *et al.,* 1993 and APHA, 1999).

The nutrient media was prepared by dissolving 5g of peptone, 3g beef extract, 8g sodium chloride and 150g agar in about 800ml of water and adjusting the pH of the solutions to 7.3 by drop wise addition of 1N sodium hydroxide. The solution was heated 2-3 minutes, cooled and diluted to 1litre with distilled water. All the apparatus such as syringes, pipettes, conical flasks, Petri dishes and the nutrient media were sterilized in an autoclave before their use.

Chem. Tech. TLC model of HPTLC available at Ancrom test lab. Mulund, Mumbai was used for the analysis of the samples. In this system stationary phase was precoated with aluminum plate containing silica gel ($^{60}F_{254}$), where as the mobile phase a mixture was containing butanol: methanol: water (3:1:1 v/v). The development of the sample spots were done using twin trough chamber. The Deutorium lamp at 254 nm was used for densitometric scanning of the samples.

All chemicals and solvents used were of analytical grade supplied by M/S S.D. fine chemicals, Thane, (India).

Preparation of crude extract: Finely crushed snail was homogenized with a mixture of 80% methanol and 1% acetic acid by heating in water bath at 60^{0}C for half an hrs. The process was repeated with more amount of methanol-acetic acid mixture (5ml) thrice. The supernatant solution was decanted of and centrifuged at 3000 rpm for 20 minutes.The residue settled if any of rejected and the

clear supernatant solution was placed in a separating funnel and extracted with dichloromethane to defated the solution. The upper clear defated aqueous solution was taken in a beaker and heated on a water bath 40-45^{0}C till solid obtained. This solid was weighed and dissolved in 1% aqueous Tween- 80 solution such that the concentration of the solution corresponds to 1mg/ml and stored in screw capped vials in a refrigerator at -20^{0}C till further use (Vankateshwaran, 1997).

Isolation of compounds from the crude extract: 5ml of the crude extract was spotted on a TLC plate coated with silica gel (thickness 0.5mm),dried and kept in the saturated twin trough chamber containing butanol :methanol: water in the ratio of 3:1:1(v/v) as a mobile phase and developed up to 9cm length . The plate was removed and dried. The development of the sample spots were done using twin trough chamber. The Deutorium lamp at 254 nm was used for densitometric scanning of the samples. The two spots were obtained. The spots were scrapped off and dissolved in methanol and filtered and evaporated to dryness to get pure compounds.

RESULTS AND DISCUSSION

The species of snail *Achantina fulica*, were collected from the garden area of Patkar College Goregaon (West), Mumbai were found to belong to gastropoda class.

Effect on bacteria: 10-12 ml of sterilized nutrient medium was placed in each of the sterile petridishes. The pure culture of bacteria *Escherichia coli PBR 322, Vibrio cholerae* were transferred in to the petridishes by streaking method using a nichrome wire loop. By disc diffusion method 10 µl of the crude methanolic extract solution and each of the two compounds isolated on TLC was added separately to the respective petridishes and placed in inoculation chamber adjusted at 37^{0}C. The growth of bacteria was obtained after 24 hrs of incubation. It was found that the growth of bacteria *Escherichia coli PBR 322, Vibrio cholerae* was inhibited due to the presence of some bioactive compounds in the crude extract as well as with the compounds isolated on TLC. (Table 1) It was found that *Escherichia coli PBR 322* shows more zone of inhibition, than the *Vibrio cholerae* in crude extract as well as the compound isolated on TLC from the snail.

The similar results were found by (Yasushi *et al.*, 1985). The antibacterial factor from the body surface of the African giant snail, *Achatina fulica* Ferussac, was isolated by DEAE-Toyopearl 650M ion exchange chromatography. The isolated preparation exhibited highly positive antibacterial activity both for the Gram-positive bacteria. *Bacillus subtilis* and *Staphylococcus aureus* and for the Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, but it lost such activity when heated at 75°C for 5 min.

Effect on cockroaches *Periplaneta americana*: Each of 10 cockroaches was taken in four different chambers of size (22X12X9cms). 10µl of the extract / methanolic solution of the compounds isolated on TLC were injected in the thorax of the each cockroach in the first three glass chambers, the fourth glass chamber was kept as control and no extract was injected in it. The mouth of all the chambers were closed by mosquito net and mortality was noted till 24hrs of injection.

Cockroaches showed cleaning behavior at the initial stages of poisoning by rubbing their bodies with their legs or sides of the cages. Marentric movements of the mouth parts were also observed and at the later stages of poisoning tremors, incoordination and convulsion leading to paralysis were noted. Cockroaches in moribid condition were found to be dead after 24hrs.

More than 2600 scientific studies have been carried out over the last 20 years testify to the important contribution of toxins extracted from gastropod snails to medicine and cellular biology (Pickrell, 2003). A team from the University of Melborne extracted the conotoxin from a cone-shell snail. They found that it not only inhibits pain as being 10000 times more powerful than morphine, but also accelerates the recovery of injured nerves (Holmes, 2002). The conus species have evolved deadly nerve toxins and small, conformationally constrained peptides of 10-30 amino acids. The conotoxin extracted from cons snail have valuable probes in physiological and pharmacological studies (Myers *et al.*, 1993). Dolastatin, a cytotoxin peptide from *Dolabell auricularia* is an antineoplastic substance (Pettit *et al.*, 1989). Ulapualida-A, a sponge derived macrolide isolated from the nudibranch *Hexabranchus Sanguineus* exhibits cytotoxic activity against L 1210 murine leukemia and cells and antifungal activity, which exceeds that of clinically useful amphotericin-B (Rorsener and Scheuer, 1986). Chromodorolida-A isolated from *Chromocloris*

Table 1. Effect of crude extract and the compound isolated on TLC (Fraction one and two) showing zone of inhibition (in millimeters).

	Control	***Escherichia coli PBR 322***	***Vibrio cholerae***
		Zone of inhibition	
Crude extract	00 mm	21 mm	16mm
TLC Fraction -1	00 mm	28 mm	18 mm
TLC Fraction -2	00 mm	24 mm	23 mm

cavae exhibit in *vitro* antimicrobial and cytotoxic activities.

Conclusion

The species of snail *Achantina fulica*, contained two compounds. The extract of the whole snail in 80% methanol and 1% acetic acid and the methanolic solution of each of the two compounds isolated on TLC showed an inhibition of growth of bacteria *Escherichia coli PBR 322, Vibrio cholerae* indicating the presence of antibacterial compounds. The percent mortality of the cockroaches was found to be 100% when the cockroaches were fed with the extract of the snail and the methanolic solution of the compounds indicating the presence of bioactive compounds in the snail.

REFERENCES

APHA (1999). Standard methods of the examination of water and west water, 20th Edn, Washington, D.C.; American Public Health Association / American Water Works Association / Water Environment Federation.

Carte, B. K., Chan, G., Freyer, A., Hemling. M.E., Compagone, R.S. and Faulkner, D.J. (1994). Pentatheipins and trithianes from two *Lissoclinum* species and a *Eudistoma* sp.; Inhibitors of protein kinase c. *Tetrahedron*, 50: 12785-12792.

Clifford Kavan T., Gross Liaini, Johnson Kwame, Martin Khali J, Shaheen Nagma and Harrington Melissa, A. (2003). Slime-trail tracking in the predatory snail, Euglandina rosea. *Behavioral Neuroscience*, 117 (5): 1086-1095

Faulkner, D.J. (2002). Marine natural products, *Nat. Prod. Res.* , 19, 1-48.

Halvorson, H.O. (1998). Aquaculture, marine sciences and oceanography: A confluence connection. *New Engl.J.Higher Ed. E con. Dev.,* 13 : 28-42

Holmes, I. (2002). Snail toxin could ease chronic pain.Nature Science Update. March 29.(http://www.manandmollusc.net/links_medicine.html)

Vankateshwaran, K. (1997). Microchemolytic assay P-41-42. In: Laboratory manual on advanced Techniques in marine Biotoxinology (Venka K. and Pani Prasad K. Ed) CAS in Fishery science. CIFE Mumbai India: p-76.

Pelczar, M. J.(1993). Text book of microbiology. 5th edition, Tata McWell Hill publication.

Myers, P.A., Cruz,L.Z., Rivier,J.E. and Olivera,B.M.(1993). Conus peptide as chemical probes for receptors and ion channels. *Chem.Rev.,* 93:1923-1936.

Pettit,G.R.,Singh,S.B.,Hogan,F.,Loyd-Williams,P. Herald,C.L., Burbett, D.D. and Clewlow,P.J.(1989).The absolute configuration and synthesis of natural(-)-dolostatinio. *J. Am Chem. Soc.,* 70,5463-5465.

Pickrell, J. (2003). Wonder Drug Snails face threats, Expers warn. *National Geographic News*. 1-2 (http://news.nationalgeographic.com/news/2003/10/1016_031016_conesnail.html.

Rorsener, J.A. and Scheuer, P.J. (1986).Ulapualids A and B,extraordinary antitumor macrolides from nudibranch egg masses. *J. Am Chem Soc*., 108:846-847

Sonja Hesbacher, Bruno Baur, Anette Baur and Peter Proksch (1995). Sequesterian of lichen compounds by three species of terrestrial snails: *Journal of Chemical Ecology.* 21 (2): 21

Yasushi Kubota, Youichi Watanabe, Hisako Otsuka, Toru Tamiya, Takahide Tsuchiya and Juichiro J. Matsumoto; (1985), Purification and characterization of an antibacterial factor from snail mucus. *Comparative Pharmacology*. 82 (2) : 345-348.