EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 10
212-216
Year: 2019

GC MS ANALYSIS AND *IN VITRO* ANTI- INFLAMMATORY ACTIVITY OF PURPLE INK SECRETED BY *BURSATELLA LEACHII*

M. R. Kokane[1,2], V. Anuradha[1]* and K. Revathi[3]

[1&1*]PG & Research Department of Biochemistry, Mohamed Sathak Arts and Science College, Sholinganallur, Chennai-119.
[2]Central Institutes of Fisheries Nautical and Engineering Training, Royapuram, Chennai-13.
[3]Former Principal and Head, Department of Zoology, Ethiraj College for Women, Chennai-8.

***Corresponding Author: V. Anuradha**
PG & Research Department of Biochemistry, Mohamed Sathak Arts and Science College, Sholinganallur, Chennai-119.

Article Received on 09/07/2019 **Article Revised on 30/07/2019** **Article Accepted on 19/08/2019**

ABSTRACT
Bursatella leachii purple ink was tested for its anti- inflammatory activity by invitro methods. Standard drug Aspirin, Diclofenac sodium was used as positive control. In vitro anti inflammatory activity assay methods such as inhibition of albumin denaturation, heat induced hemolysis method, proteinase inhibitory action and HRBC membrane stabilization were carried out at different concentrations. The activity increased exponentially with the concentration of the sample. The results proved that for all the tested methods, the sample showed potent anti-inflammatory activity.

KEYWORDS: Anti- inflammatory, Albumin denaturation, membrane stabilization, HRBC method.

INTRODUCTION
Bursatella leachii, a shell less marine mollusk falls under Nudibranchs group commonly called as "Sea hare" and best known for fascinating range of colour and body forms. *Bursatella leachii* is grayish green to white tan brown blotches and has spots throughout the body covered with numerous long, branching fleshy papillae giving the animal its ragged appearance. The Ragged Seahare, *Bursatella leachii* is circum tropical, found worldwide in warm temperate to tropical marine environments (Rudman 1998), First record of ragged sea hare *Bursatella leachii* was reported by Sethi, Kokane *et al* (2015) in pulicat lake.

Bursatella leachii has two long, retractile olfactory tentacles called rhinophores lie on the head, and also two fleshy enrolled oral tentacles appears at each side of the mouth. *Bursatella leachii* purple ink contain high source of bioactive molecules. When the animal is irritated by touching, it starts secreting a purple ink as a mechanism of self- defense. As the animal inhabits a variety of intertidal habitat, they are exposed to several different predators. As a consequence, they produce a high amount of diverse secondary metabolites, such as steroids, terpenoids, quinines and brominated compounds that provide chemical defense and also allow them to cope with abiotic and biotic habitat condition.(Grkovic *et al*., 2005; Suarez- Jimenez *et al*., 2012). In the present study, Purple ink was assayed for in vitro anti inflammatory activity by different methods such as inhibition of albumin denaturation, membrane stabilization test using heat induced hemolysis, protein inhibitory action and HRBC membrane stabilization method.

MATERIAL AND METHODS
Isolation of Purple ink from Ink gland of *Bursatella leachii*
Bursatella leachii species was collected from Pulicat lake and kept in sea water container in live condition. After *Bursatella leachii* acclimatization with the lab condition, the purple fluid was obtained by disturbing the animal and it was diluted to 100ml with distilled water. All aqueous ink samples were centrifuged at 15,000g for 15 min as described by Vennila R., *et al*., (2011) and the supernatant was taken and lyophilized to extract a purple reside using a lyophilizer and stored for further use at 4^0C.

GC MS Analysis
The GC–MS analysis of the bioactive compounds was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). The spectrums of the components were compared with the database of spectrum of known components stored in the NIST library.

***In vitro* anti- inflammatory activity**

In vitro Anti-inflammatory activity of *Bursatella leachii* purple ink was carried out with Aspirin and Diclofenac as standard drug. The various invitro methods include Inhibition of albumin denaturation, Proteinase inhibitory action, HRBC membrane stabilization and Heat induced haemolysis. Concentration in the range of 100-500 µg/ml was fixed for both test samples and standard drug (Suganya V *et al.*, 2017).

% of inhibition = (Abs control – Abs sample) X 100 / Abs control

RESULT AND DISCUSSION

GC MS Analysis

The compounds identified by GC coupled mass spectral analysis is given in Table 1 and Fig.1. The compounds reported were 7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione and Dioxigenin Acetate. The presence of similar compounds in purple ink of same species was not reported yet. An anti-HIV protein, bursatellanin-P, has been isolated from the purple ink secretion of the species and reported (Rajaganapathi *et al*. 2002, Avilla 2006).

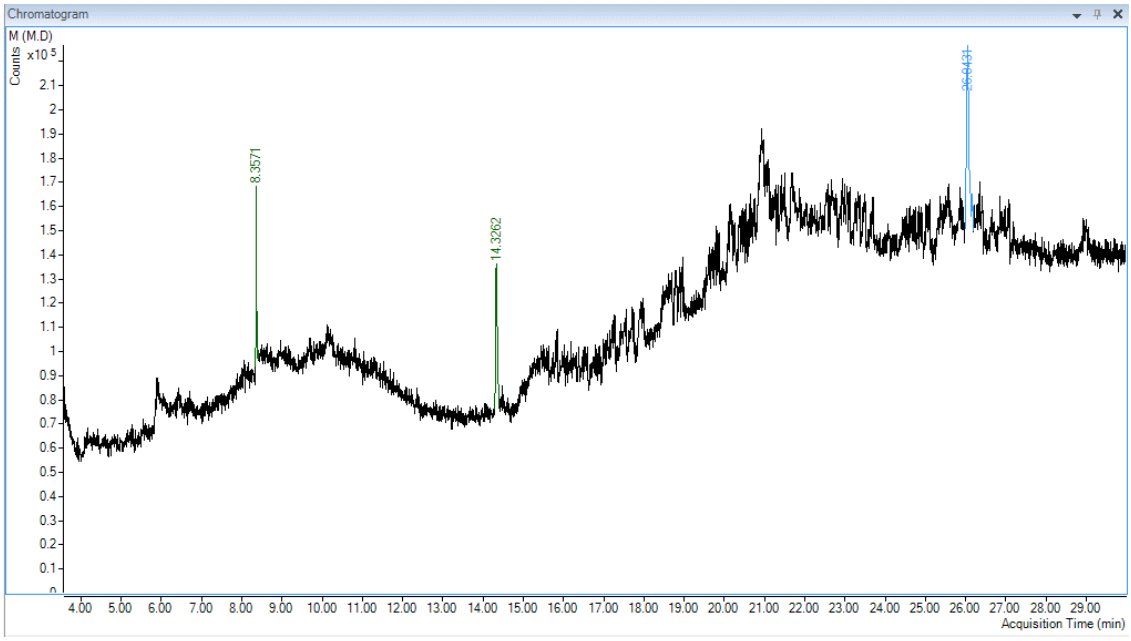

Fig 1: GC MS Spectrum with Three prominent peaks.

Table 1: Compounds Corresponding to GC MS Peak.

Compound RT	Name	Mol. Weight	Formula	Area %
8.3571	Cyclohexene, 1-(3-ethoxy-1-propenyl)-, (Z)-	166.136	C11H18O	3.724
14.3262	7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	276.173	C17H24O3	7.082
26.0431	Spirost-5-en-3-ol, acetate, (3.beta.,25R)-	456.324	C29H44O4	13.547

***In vitro* anti-inflammatory activity**

The purple color of the ink comes from red algal derived pigment the most abundant being phycoerythrobilin a chromophore of the photosynthetic pigments phycoerythrin; the biological activity of these pigments is uncertain (Chapmanand Fox 1969; MacColl *et al*., 1990; Prince *et al*., 1998; Coelho *et al*., 1998). This suggest that fluid may contain bioactive factors that act against potential enemies since the defense mechanisms of the sea hares are well developed, comparable to those of highly developed vertebrates (Cooper, 1980) . Ink also contains high concentration of amino acids and related nitrogenous compounds that act against crustacean predators through phago mimicry and sensory disruption. Lysosomal hydrolytic enzymes are release during inflammation which causes damages of the surrounding organelles and tissues with variety of disorders (Sadique *et al*, 1989). The search for new biological active compounds has focused on marine natural products. This focus is driven by the diversity of marine organisms and their production of secondary metabolites with extensive array of bioactive. Molluscs in particular, are well- known for production of valuable secondary metabolites and have used in the traditional medicine systems for many cultures to treat inflammatory conditions (Ahamad T.B 2017).

1. Inhibition of albumin denaturation

The denaturation of proteins is a well-documented cause of inflammation. Phenylbutazone, salicylic acid, flufenamic acid (anti- inflammatory drugs) etc, have shown dose dependent ability to thermally induced

protein denaturation (Mizushima *et al* 1968). In the present study, inhibition of albumin denaturation by purple ink extract was studied. The test sample showed effective inhibition of heat induce albumin denaturation when compared with standard Diclofenac.

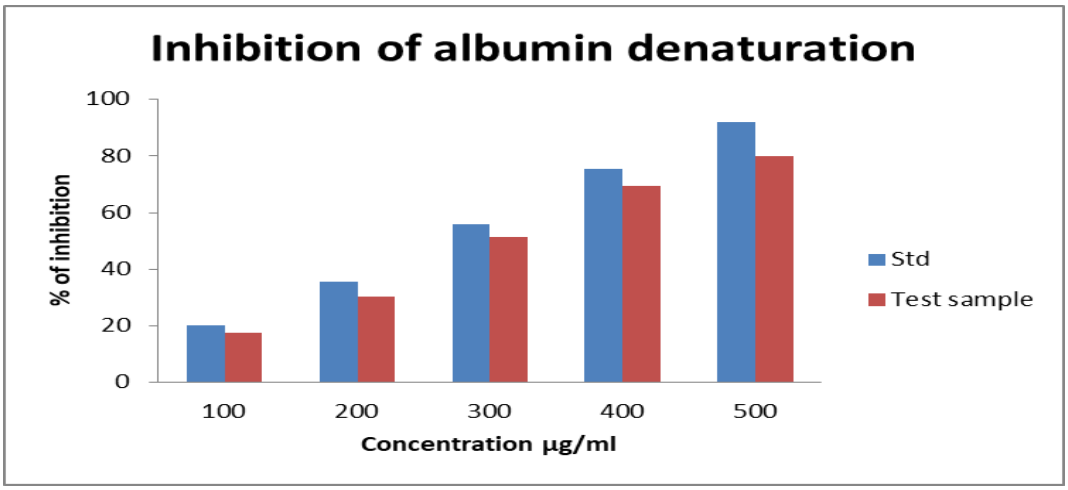

Fig 2: Inhibition of albumin denaturation.

The sample was tested for five different concentrations i.e 100 ug/ml to 500 ug/ml. The test samples showed 17.60 % inhibition at concentration of 100ug/ml followed by 30.48 %, 51.33 %, 69.42 % and 80.04 % for 200 ug/ml, 300ug/ml, 400ug/ml and 500ug/ml respectively **(Fig.2)**. It was compared with positive Standard Diclofenac sodium which showed 20.16 % inhibition at concentration of 100ug/ml and 91.84 % at maximum concentration of 500ug/ml.

2. Membrane stabilization test : Heat induced hemolysis

The mechanism of anti-inflammatory activity was studied through stabilization of RBCs membrane. Stabilization of lysosomal membrane is important to control the inflammatory response by preventing lysosomal activated neutrophil enzymes and proteases (Yamada K *et al* (1997); Johnson, P.M (2002); Anitha Rani *et al*., 2014).

The test samples inhibited the heat induced hemolysis of RBCs at various concentrations i.e 100 ug/ml to 500 ug/ml. **Fig. 3** shows that the aspirin standard drug has maximum inhibition of 87.84 % at concentration of 500 ug/ml whereas test sample showed 84.13 % for the same concentration. IC 50 value lies for standard OD is 256.716 µg/ml but for the test sample it is having 10% higher value.

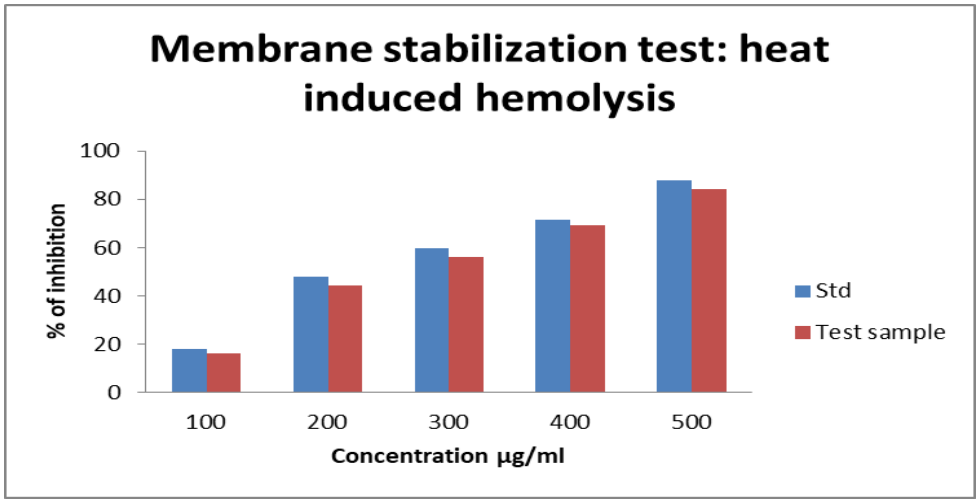

Fig 3: Membrane stabilization test: Inhibition of Heat induced hemolysis by *Bursatella leachii* purple ink.

3. Protein inhibitory action

Protein inhibitory action at different concentration in the range of 100-500 ug/ml is shown in the **Fig. 4**. The result of present investigation revealed that purple fluid (i.e ink) extract exhibited significant anti- inflammatory property than standard drug with concentration dependent manner.

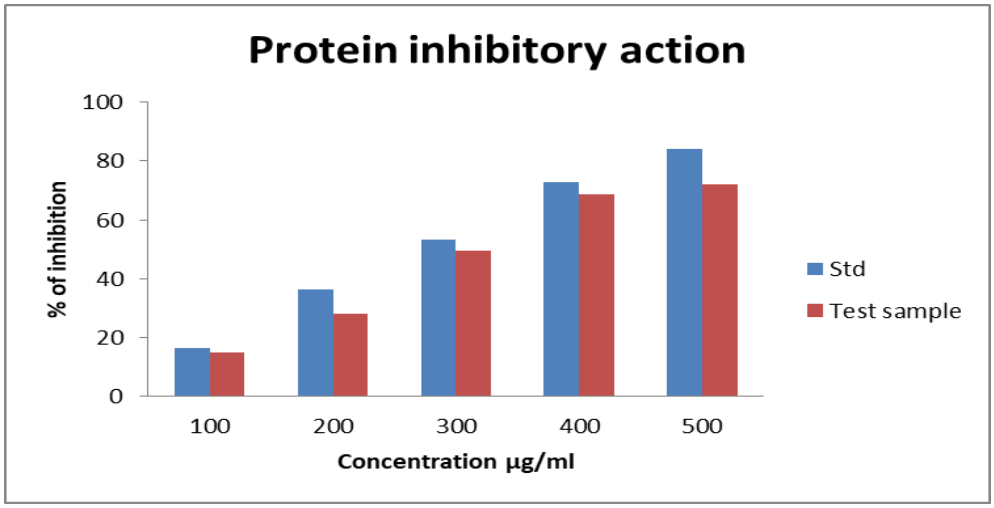

Fig 4: Protein Inhibitory action of *Bursatella leachii* Purple ink.

Percentage of protein inhibitory action at concentration of 500 ug/ml was found to be higher in standard drug (84.20 %) when compared with test sample (72.20%). The IC 50 value is more for the test sample in comparison with standard sample.

4. HRBC membrane stabilization method

Exposure of Red Blood Cells to injuries substances such as hypotonic medium heat, methyl salicylate or phenylhydrazine, results in the lysis of the membranes, accompanied by haemolysis and oxidation of haemoglobin (Feirrali *et al*. 1992). During inflammation, lysosomal enzymes produces a variety of disorder since Human Red blood cell (HRBC) are similar to lysosomal membrane component (Mounnissamy *et al*, 2008). The inhibition of hypotonicity and heat induced red blood cell membrane lysis was taken to measure the activity of anti- inflammatory**.**

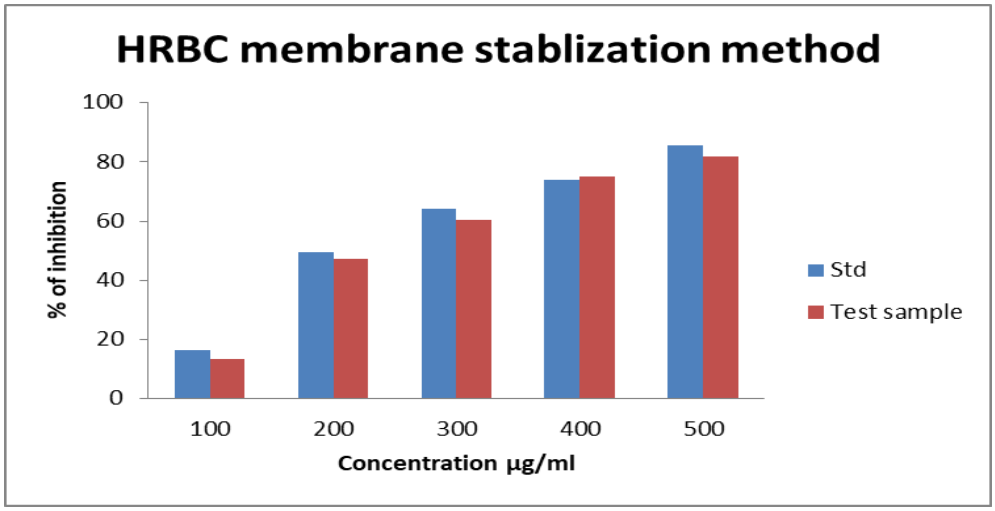

Fig 5: HRBC membrane stabilization Potential of *Bursatella leachii* purple ink.

It was observed by the HRBC membrane stabilization method that the standard and test samples showed 10 % difference in activity with higher IC 50 value shown by the test sample **(Fig. 5)**.

CONCLUSION

The present study shows that *Bursatella leachii* purple ink extract forms to be potent active drug for diseases associated with inflammatory disorders and in relieving the symptoms of pain; which was tested by four different *in vitro* methods. It is the initial stage of the study to focus on the efficiency of ink extract on inflammation.

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