Asian Fisheries Science ***25*** *(2012):158-169*

ISSN 0116-6514

Asian Fisheries Science
The Journal of the Asian Fisheries Society

Isolation and Biomedical Screening of the Tissue Extracts of Two Marine Gastropods *Hemifusus pugilinus* (Born, 1778) and *Natica didyma* (Roding, 1798)

C. RAVI[1*], A. KARTHIGA[1] and V. VENKATESAN[2]

[1]Department of Zoology, Thiagarajar College (Autonomous), Madurai – 625 009, India.
[2]Central Marine Fisheries Research Institute, Cochin, India.

Abstract

The *in vitro* antibacterial activity of the acetone and methanol extracts of the marine gastropods *Hemifusus pugilinus* (Born, 1778) and *Natica didyma* (Roding, 1798) were tested against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Enterobacter aerogenes* following standard disc diffusion method. Highest activity (19.3±0.39 mm) was observed in the methanol extract of *N. didyma* against *E. coli*. The extracts were also subjected to anti-inflammatory activity using human red blood cell (HRBC) membrane stabilisation method. All the extracts exhibited anti-inflammatory activity, and was high in the acetone extract of *H. pugilinus* (72.58±1.66%) at 1mL concentration. However, the control drug hydrocortisone exhibited significantly higher activity than the tissue extracts of both the animals. The extracts were screened for their alpha amylase inhibitory activity also and all the extracts inhibited the alpha amylase enzyme activity. Among the mollusc extracts, maximum inhibition was observed in *H. pugilinus* acetone extract at 50 µL concentration (72.23±0.44%). But, the control drug (Acarbose) had significantly higher inhibitory action than the tissue extracts of both the animals. The amount of protein was comparatively high in the tissue of both *H. pugilinus* (1.34±5.63 µgmL^{-1}) and *N. didyma* (0.90±4.37 µgmL^{-1}) than carbohydrates and lipids.

Introduction

Many microbial pathogens that infect human beings developed resistance to most of the available antibiotics; hence the need for new drugs becomes mandatory (Anbuselvi et al. 2009). Chemical drugs may lead to adverse effects and recent researchers now focus on pharmacologically active compounds from natural sources. Marine organisms contain much undiscovered bioactive compounds; the number of new active compounds isolated from marine organisms are estimated at 10,000 (Kelecom, 2002). Inflammation occurs when bacteria or other pathogens enter the body and it may be caused by other environmental factors also (Jongyoon et al. 2009). Commonly used steroidal and non-steroidal anti-inflammatory drugs causes severe side effects in patients hence the search for novel anti-inflammatory agents is essential. Manoalide, pseudopterosins, topsentins, scytonemin and debromohymenialdisine are some of the compounds isolated from molluscs that exhibited anti-inflammatory activity (Chellaram and Edward, 2009a).

The deficiency of insulin leads to diabetes mellitus (DM). The World Health Organization reported that 5.0% of global population has been affected by DM and at present, there is no drug to

*Corresponding author. E-mail address: ravi_cyril@yahoo.co.in

give permanent remedy to DM (Geethalakshmi et al. 2010). Glycosidase and alpha amylase are the key hydrolysing enzymes involved in the digestion of carbohydrates (Ali et al. 2006). Inhibition of these enzymes resulted in decreasing the rate of digestion of carbohydrate, prolonged the time of the carbohydrate digestion and thereby reduced the amount of glucose level in blood. The anti diabetic agent which was isolated from molluscs and studied in rat models showed significant hypoglycemic effect (Tiwari et al. 2008).

Molluscs are considered as one of the important sources to derive bioactive compounds that exhibit antitumor, antimicrobial, anti-inflammatory, and antioxidant activities (Anbuselvi et al. 2009; Chellaram and Edward, 2009b; Benkendorff et al. 2011). Molluscs also contain rich nutrients that are beneficial to people of all ages (Anand et al. 2010). In our body, oxidation process leads to cell damage, cancer and degenerative diseases; antioxidant molecules present in different molluscs prevent cell damage from oxidation reaction (Nagash et al. 2010). Compounds isolated from molluscs were also used in the treatment of rheumatoid arthritis and osteoarthritis (Chellaram and Edward 2009a). Mollusc extracts also exhibited antiviral and antibacterial activity against fish pathogenic bacteria, and the extract also may be applied in aquaculture (Defer et al. 2009).

Hemifusus pugilinus (Born, 1778) is a herbivorous gastropod that feeds on algae and other flora present abundantly in the Gulf of Mannar (Edward et al. 2008). It possesses in its body, a heparin like substance (Balamurugan et al. 2009) and immunomodulatory activity was studied in *Hemifusus* genus (Akerkar et al. 2009). *Hemifusus pugilinus* and *Natica didyma* (Roding, 1798) have a nutrient-rich meat and ornaments are being made from their shells; calcium carbonate is also separated from their shell for industrial purpose (Pillai and Menon, 2000). Literature survey revealed the urgent need to explore marine molluscs and not much work has been carried out in *H. pugilinus* and *N. didyma*. Hence, those two animals were chosen for the present study with well defined executable objectives.

Materials and Methods

Collection of molluscs

Fresh and live *H. pugilinus* and *N. didyma* were collected from the Mandapam coastal area (Lat. 09º17′11.3″ N and Long. 79º 09′17.1″ E), Ramanathapuram District, Tamil Nadu, India, during the morning hours (8.30 am - 9.30 am) with support from local fishermen. Collected samples were transported with the use of plastic containers and identified at the Central Marine Fisheries Research Institute (CMFRI), Mandapam camp and then transported to the Zoology Laboratory of Thiagarajar College, Madurai. The samples were then washed with tap water until the removal of sand and mud from the shells. After that, the shells were broken using a hammer to remove the soft tissue body. The removed tissues were rinsed with sterile distilled water and cut into small pieces, which were shade-dried for 48-96 hr at room temperature.

Preparation of solvent extracts

Four hundred milliliter acetone was taken in a conical flask and 100 g dried tissue of *H. pugilinus* were immersed in it. The mouth of the flask was covered with aluminum foil and kept in a shaker for 72–96 hr. The resultant material was filtered through Whatman No.1 filter paper. The solvent was distilled and the concentrated extract was stored in a plastic container. Similar methodology was followed for the preparation of other extracts.

Antibacterial assay

The bacterial strains used for the screening such as *Enterobacter aerogenes, Escherichia coli* (Gram negative), *Bacillus subtilis* and *Staphylococcus aureus* (Gram positive) were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India and maintained in the laboratory in slopes of nutrient agar at 4 °C. The antimicrobial activity of acetone and methanol extracts of *H. pugilinus* and *N. didyma* were tested against the above-said organisms following the standard disc diffusion method (Hussain and Ananthan, 2009). Active cultures for experiments were prepared by transferring a loopful of culture to test tubes containing Muller Hinton Broth that was incubated without agitation for 24 hr at 37 °C. Muller Hinton Agar plates were prepared and the inoculum (24 hr old broth culture) of the bacteria was spread on them using sterile swab. The extract (20 µL) loaded 6 mm disc was placed on the plates with the use of sterile forceps. The discs loaded with the respective solvent and gentamycin served as controls and they were also placed on the same plate. The plates were then incubated at 37 °C for 24 hr and observed for clear zone of inhibition and the inhibition zone was measured in mm. Six replications were maintained for each category.

Anti-inflammatory activity

The human red blood cell (HRBC) membrane stabilisation has been used as a method to study the anti-inflammatory activity (Nirmaladevi and Periyanayagam, 2010). Blood was collected from healthy human volunteers and the collected blood was mixed with equal volume of sterilised Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3,000 rpm and the packed cells were washed with isosaline (0.85%, pH 7.2). A 10% (v/v) HRBC suspension was made with isosaline. Two types of media such as hypo saline and distilled water were used in the present experiment. In hyposaline medium, the assay mixture contained 1 mL phosphate buffer (0.15M, pH 7.4), 2 mL hyposaline (0.36%) and 0.5 mL HRBC suspension and hydrocortisone was used as the reference drug. Instead of hyposaline, 2 mL of distilled water were used as the control in the distilled water medium. For the experiments, instead of hydrocortisone, different concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mL) of mollusc extracts (*H. pugilinus* and *N. didyma*) were added. All the assay mixtures were incubated at 37 °C for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated using a colorimeter at 560 nm. The percentage of haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane

stabilisation or protection was calculated using the following formula. Six replications were maintained for each category.

$$\% \text{ protection} = 100 - \frac{\text{OD of drug treated sample}}{\text{OD of control}} \times 100$$

Alpha amylase inhibition assay

The alpha-amylase inhibitory activity for each extract was determined based on the colorimetric assay (Nickavar and Mosazadeh, 2009). Starch solution was obtained by stirring and boiling 0.25 g of soluble potato starch in 50 mL of distilled water for 15 min. The enzyme solution (0.5 unit·mL^{-1}) was prepared by mixing 0.001 g of alpha amylase in 100 mL of 20 mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. Various concentrations (10, 20, 30, 40, 50 mg·mL^{-1}) of extracts were dissolved in 1 mL of dimethylsulfaoxide (DMSO) to give concentrations ranging from 10 to 50 mg·mL^{-1}. The colour reagent was a solution containing 96 mM 3, 5-dinitrosalicyllic acid (20 mL), 5.31 M sodium potassium tartarate in 2 M sodium hydroxide (8 mL) and distilled water (12 mL).

One mL of each mollusc extract (*H. pugilinus* and *N. didyma*) and one mL enzyme solution were mixed in a test tube and incubated at 25 °C for 30 min. After that, 1 mL starch solution was added and incubated at 25 °C for 30 min. Then, 1 mL colour reagent was added and the closed tube was placed in a 85 °C water bath. After 15 min, the reaction mixture was removed from the water bath, cooled and diluted with 9 mL distilled water. The absorbance value was determined at 540 nm. For correcting the background absorbance, individual blanks were prepared. In that case, the colour reagent solution was added prior to the addition of starch solution and then the test tube was placed in the water bath. In the control, instead of extracts, 1 mL DMSO was added. The commonly used alpha amylase inhibitory drug acarbose acted as control. Six replications were maintained for each category. The inhibition percentage of alpha amylase was determined by the following formula:

$$\text{Inhibition of alpha amylase (\%)} = 100 \times (\Delta A_{\text{control}} - \Delta A_{\text{sample}} / \Delta A_{\text{control}})$$

$$\Delta A_{\text{control}} = A_{\text{test}} - A_{\text{blank}}$$

$$\Delta A_{\text{sample}} = A_{\text{test}} - A_{\text{blank}}$$

Quantitative estimation of biomolecules

Standard methodology was followed for the estimation of carbohydrates (Anthrone reagent method), protein (Biuret method) and lipid (Potassium dichromate reagent method) in the tissue samples of *H. pugilinus* and *N. didyma* respectively.

Statistics

All the statistics were performed using the Statistical Package for Social Sciences (SPSS) software.

Results

Anti bacterial activity

In *H. pugilinus*, the zone of inhibition produced by the acetone extract against *E. aerogenes, B. subtilis, S. aureus* and *E. coli* were 18.21±2.42, 10.32±1.65, 14.0±1.78 and 15.24±0.28 mm, respectively. For methanol extract, it was 19.21±4.52, 12.24±0.64, 14.32± 0.75 and 18.32±0.94 mm, respectively. Among the two extracts of *H. pugilinus*, the methanol extract exhibited significant inhibitory effect on all the pathogens and it was highest against *E. aerogenes* (19.21±4.52 mm). In *N. didyma*, the zone of inhibition produced by the acetone extract against *E. aerogenes, B. subtilis, S. aureus* and *E. coli* were 4.30±0.42, 9.54±1.12, 8.53±1.45 and 4.12±1.28 mm, respectively. For the methanol extract, it was 19.33±4.52, 19.12±0.64, 18.62±0.75 and 17.41±0.28 mm, respectively. *Natica didyma* exhibited high inhibitory activity against all the bacterial strains and maximum activity was observed in *E. aerogenes* (19.33±4.52 mm). The results are presented in Fig. 1.

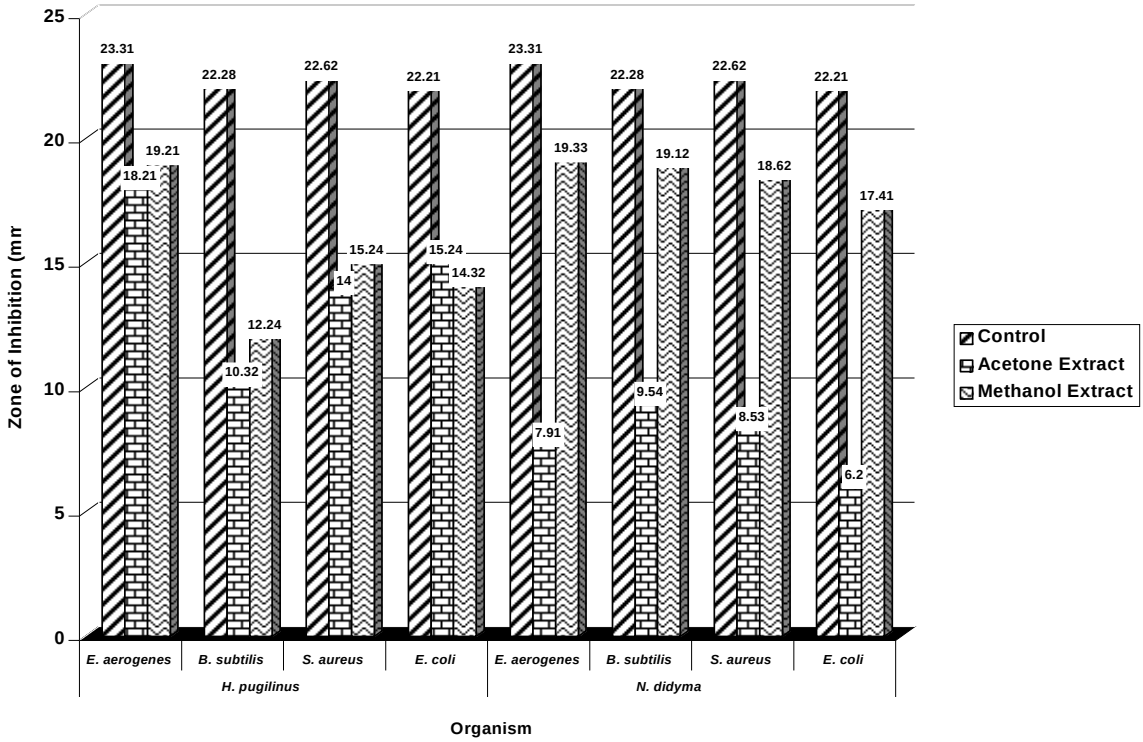

Fig. 1. Zone of inhibition produced by the tissue extracts of *H. pugilinus* and *N. didyma* on the tested bacterial strains.

Anti inflammatory activity

Anti inflammatory activity was found in all the extracts. The concentration and the activity were directly proportional to each other, i.e. when the concentration increased, the activity also increased and the results are presented in Table 1 and 2. In *H. pugilinus*, the highest activity was exhibited by the acetone extract both in the hyposaline medium (72.58±1.66%) and in the distilled water medium (57.19±9.09%) (Table1). Similarly, for *N. didyma*, highest activity was exhibited by the acetone extract both in the hyposaline medium (68.21±1.66%) and in the distilled water medium (49.32±9.09%). However, in all the categories the control drug hydrocortisone exhibited significantly higher activity than the extracts (P = 3.649 and 3.886 for *H. pugilinus* and *N. didyma* in the hyposaline medium and P = 0.00037 and 1.21 in the distilled water medium respectively).

Table 1. Anti inflammatory activity (in percentage) of various solvent extracts of *H. pugilinus*.

Sl. No	Medium	Concentration of the mollusc extract (mL)	Control (Hydrocortisone)	Methanol Extract	Acetone Extract
		0.2	70.25±2.10	42.25±2.16	62.21±0.18
		0.4	74.35±1.57	46.21±7.44	64.52± 0.85
1.	Hyposaline	0.6	78.16±1.66	51.46±2.94	66.21±1.8
		0.8	83.16±0.89	55.54±1.66	70.24±6.14
		1.0	87.56±1.66	58.25±5.14	72.58±1.66
		0.2	52.13±2.10	32.15±3.51	42.0±1.66
2.	Distilled	0.4	55.24±0.89	35.15±5.20	45.48±0.014
	water	0.6	57.18±0.89	38.13±0.94	48.17±1.27
		0.8	62.23±0.18	44.42±2.90	52.34±8.18
		1.0	65.25±1.66	46.23±5.90	57.19±9.09

Table 2. Anti inflammatory activity (in percentage) of various solvent extracts of *N. didyma*.

Sl. No	Medium	Concentration of the mollusc extract (mL)	Control (Hydrocortisone)	Methanol Extract	Acetone Extract
		0.2	70.25±2.10	44.22±2.16	58.25±0.18
		0.4	74.35±1.57	48.15±7.44	61.52±0.85
1.	Hyposaline	0.6	78.16±1.66	52.85±2.94	63.21±1.8
		0.8	83.16±0.89	56.83±1.66	65.32±6.14
		1.0	87.56±1.66	59.25±5.14	68.21±1.66
		0.2	52.13±2.10	28.22±3.51	34.12±1.66
2.	Distilled	0.4	55.24±0.89	30.15±5.20	38.12±0.014
	water	0.6	57.18±0.89	35.85±0.94	42.23±1.27
		0.8	62.23±0.18	37.83±2.90	45.15±8.18
		1.0	65.25±1.66	40.25±5.90	49.32±9.09

Alpha-amylase inhibition assay

Solvent extracts of *H. pugilinus* and *N. didyma* were also assayed for their α-amylase inhibitory activity. Both extracts were found to exhibit the inhibitory action on α-amylase enzyme, but the control drug acarbose exhibited more inhibition than the extracts. They were statistically significant (P = 1.452 and P = 6.957 for *H. pugilinus* and *N. didyma* respectively) and the percentage of inhibition varied and are shown in Fig. 2. In *H. pugilinus*, high inhibition was observed in the acetone extract (72.23±0.44 %) at 50 µL concentration and in *N. didyma*, high inhibitory effect was exhibited by acetone extract (51.23 ± 0.44 %) at 50 µL concentration.

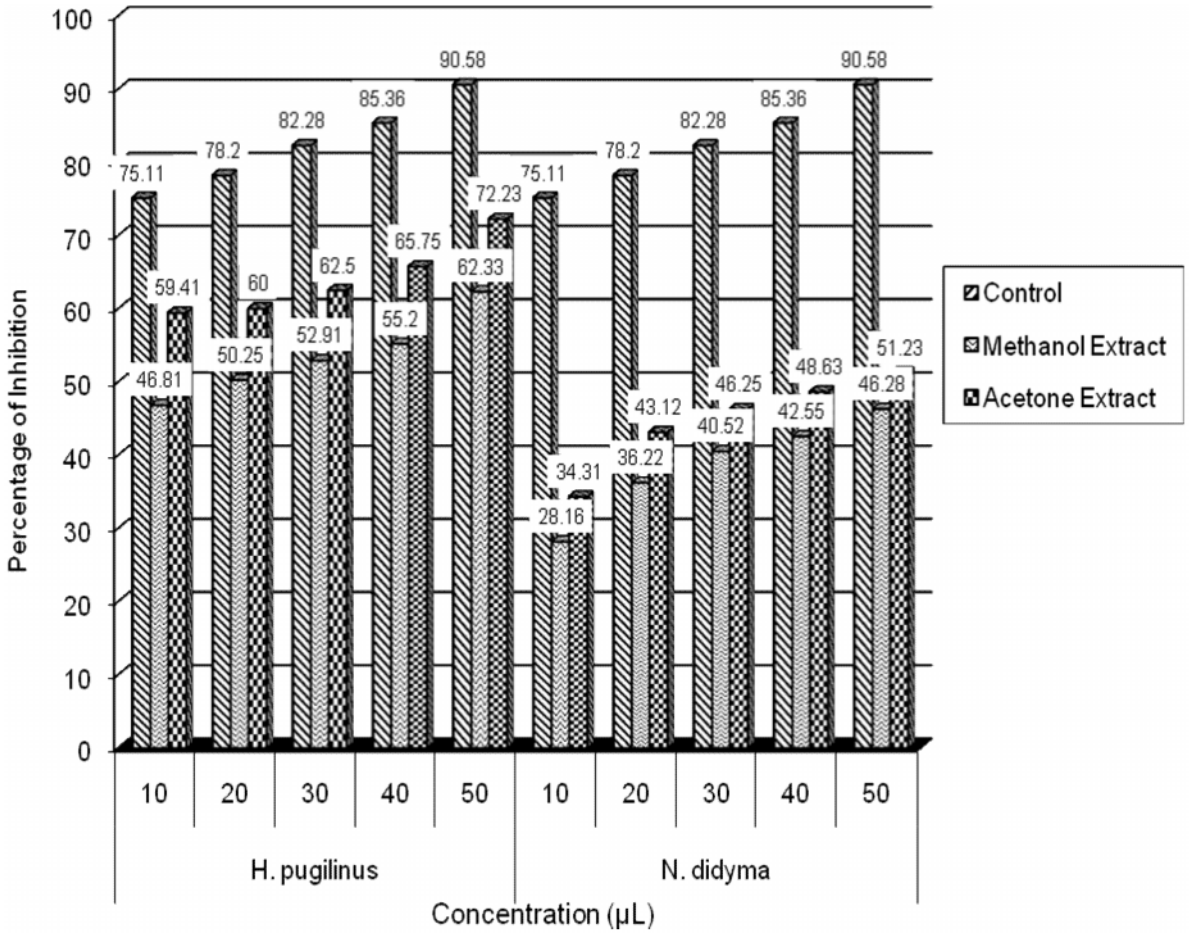

Fig.2. Alpha amylase inhibitory activity (in %) of the various tissue extracts of *H. pugilinus* and *N. didyma*.

Quantitative estimation of biomolecules

The estimated amount of protein, carbohydrate and lipid present in the tissue of *H. pugilinus* are 1.34±5.63, 1.22±8.25 and 0.57±2.14 µg·mL^{-1}, respectively. For *N. didyma* it was 0.90±4.37, 0.77±0.89 and 0.49±1.45 µg·mL^{-1}, respectively (Fig. 3).

Discussion

The tissue extracts of two marine gastropods, namely *H. pugilinus* and *N. didyma*, were tested for its antibacterial assay against *E. aerogenes*, *E. coli*, *B. subtilis* and *S. aureus*, if any, in the *in vitro* conditions. The study revealed that the acetone and methanol extract of both the animals inhibited the growth of the tested pathogenic microbial strains. Anand and Edward (2002) reported the antibacterial activity of methanol extract of *Cypraea errones* Linnaeus, 1758 and the acetone phases of *Pteria chinensis* Leach, 1814 showed inhibition in the fish pathogenic strains (Mayer et al. 2004). Although different species and experimental procedure were followed in different studies,

they indicated the high degree of antimicrobial activity in marine molluscs. These results encourage the idea that marine molluscs are potent sources for drug development (Defer et al. 2009). The present study showed that *H. pugilinus and N. didyma* contain compounds with broad antibacterial activity. However, further investigations involving application of the extracts as drugs for humans need more research.

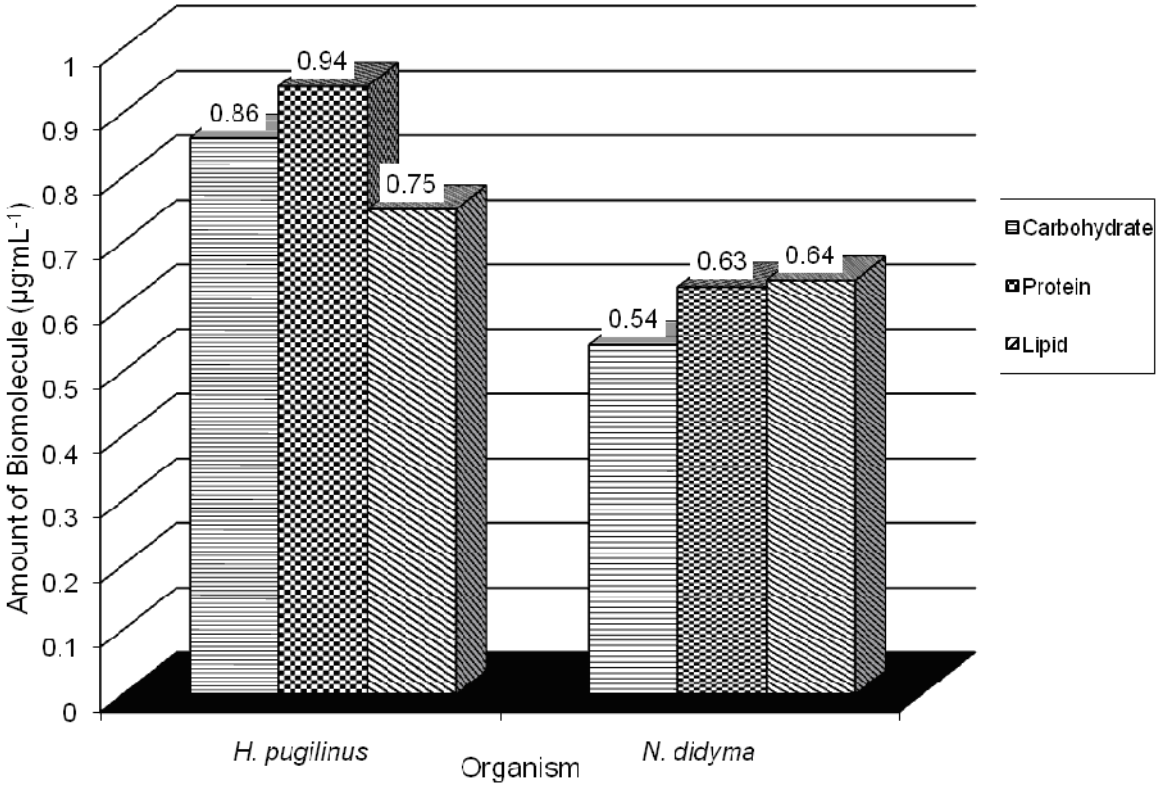

Fig. 3. Quantitative estimation of biomolecules in the experimental animals *H. pugilinus* and *N. didyma*.

Inflammation is the primary physiological defense mechanism in our body system; chronic diseases cause uncontrolled and persistent inflammation. Inflammation may be caused by several biochemical and pharmacological compounds and by the environment (Arunachalam et al. 2011). The imbalanced secretion of soluble mediators known as cytokines also cause adverse inflammation effect (Yoon et al. 2009). In the present experiment, all the tested mollusc extracts exhibited anti-inflammatory activity but the control drug exerted higher activity than the extracts. Generally, the standard drugs may exhibit more activity than the experimental compounds and Shreedhara et al. (2009) and Yeshwante et al. (2009) also observed high activity in the analgin and indomethacin reference drugs respectively. The *in vivo* anti inflammatory activity was studied in acetone column purified extracts of *Trochus tentorium* Gmelin, 1791 and *Drupa margariticola* (Borderip 1832) in the Swiss mice and albino rat models. The bioactive compounds manolide, pseudopterosins, topsentins and scytonemia isolated from molluscs are responsible for the anti inflammatory activity (Chellaram and Edward, 2009a).

Diabetes mellitus (DM) is one of the most serious endocrine disorders caused by the deficiency of insulin production (Type 1 DM) or combined resistance to insulin action and the insulin secretory response (Type 2 DM). One of the therapeutic approaches for decreasing post-prandial hyperglycemia is to prevent absorption of glucose by the inhibition of carbohydrate-hydrolysing enzymes such as α-glucosidase and α-amylase. To slow down the action of both the enzymes by inhibitors might be one of the valuable approaches to control type 2 DM (Sungkim et al. 2011). A hundred and seventy one million people were affected by DM in 2000; it may well increase to at least 366 million by 2030 (Ali et al. 2006). Acarbose and voglibose is the currently used drug for α-glucosidase and α-amylase inhibitors in treating the non-insulin-dependent DM but they cause side effects such as abdominal distension, bloating, flatulence and diarrhea. These are all caused by the excessive inhibition of pancreatic α-amylase, leading to bacterial fermentation of undigested carbohydrate in the colon (Jaiyesimi et al. 2009). The anti hyperglycemic activity of the crude extract of *Scapharca inaequivalvis* (Bruguière, 1789) was studied in rat models (Tiwari et al. 2008); results indicated that the mollusc extract possessed anti-diabetic activity. Both acetone and methanol extracts of *H. pugilinus* and *N. didyma* exhibited the alpha amylase inhibition in the *in vitro* condition.

The estimated amount of protein, carbohydrate and lipid present in the tissue of *H. pugilinus* are 1.34±5.63, 1.22±8.25 and 0.57±2.14 $\mu g \cdot mL^{-1}$, respectively. For *N. didyma*, it was 0.90±4.37, 0.77±0.89 and 0.49±1.45 $\mu g \cdot mL^{-1}$, respectively (Fig. 3). Alkaloids, saponins, sterols, poly phenols, flavonoids and sesquiterpene lactones are important common biomolecules present in the marine gastropods. The analyses of secondary metabolites from molluscs are necessary to find out the novel compounds with newer mode of action for future drug discovery (Sreejamole and Radhakrishnan, 2010).

Conclusion

The tissue of both *H. pugilinus* and *N. didyma* possess bioactive compounds that have potent antibacterial, anti-inflammatory and α-amylase inhibitory activities. Further studies are essential to explore those bioactive compounds and to convert them into usable drugs. Since both molluscs are edible, they could be included in the diet as prophylaxis.

Acknowledgements

The authors are thankful to the authorities of Thiagarajar College, Madurai for the institutional facilities.

References

Akerkar, A.S., C.A. Ponkshe and M.M. Indap. 2009. Evaluation of immuno modulatory activity of extracts from marine animals. Indian Journal of Marine Sciences 38 :22-27.

Ali, H., P.J. Houghton and A. Soumyanath. 2006. α-amylase inhibitory activity of some Malaysian plants used to treat diabetes, with particular reference to *Phyllanthus amarus*. Journal of Ethno Pharmacology 107:449-455.

Anand, P.T., C. Chellaram, R. Kumaran and C. F. Shanthini. 2010. Biochemical composition and antioxidant activity of *Pleuroploca trapezium* meat. Journal of Chemistry and Pharmaceutical Research 2:526-535.

Anand, T.P. and J.K.P. Edward. 2002. Antimicrobial activity in the tissue extracts of five species of cowries *Cypraea* spp. (Mollusca: Gastropoda) and an ascidian *Dideminum psammathodes* (Tunicata: Didemnidae). Indian Journal of Marine Sciences 31:239-242.

Anbuselvi, S., C. Chellaram, S. Jonesh, A. Jayanthi and J.K.P. Edward. 2009. Bioactive potential of coral associated gastropod, *Trochus tentorium* of Gulf of Mannar, southeastern India. Journal of Medicinal Science 9:240-244.

Arunachalam, K., T. Parimelazhagan and S. Manian. 2011. Analgesic and anti-inflammatory effects of *Merremia tridentate*. International Journal of Pharmacy and Pharmaceutical Sciences 3:75-79.

Balamurugan, K., V. Kalaichelvan and A. Dey. 2009. Isolation and identification of heparin like substances from the body tissue of *Conus musicus.* International Journal of PharmTech Research 1:1650–1653.

Benkendorff, K., C.M. McIver and C.A. Abott. 2011. Bioactivity of the murex homeopathic remedy and of extracts from an Australian murcid mollusc against human cancer cells. Evidence-Based Complementary and Alternative Medicine. Article Id:879585-doi.10.1093/ecam/nep042 1.vol. 2011, 12 pp.

Chellaram, C. and J.K.P. Edward. 2009a. *In vivo* anti-inflammatory bustle of reef associated mollusc, *Trochus tentorium*. Advanced Biotech (June) 9:32 – 34.

Chellaram, C. and J.K.P. Edward. 2009b. Antinociceptive assets of coral associated Gastropod, *Drupa margariticola*. International Journal of Pharmacology 5:236-239.

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

Edward, J.K.P., G. Mathews, J. Patterson, R. Ramkumar, D. Wilhelmsson, J. Tamelander and O. Linden. 2008. Status of coral reefs of the Gulf of Mannar, Southeastern India. In: Ten years after bleaching - facing the consequences of climate change in the Indian Ocean. Oceans Research and Development in the Indian Ocean (CORDIO) Status Report (eds. D. O. Obura, J. Tamelander and O. Linden), pp 45-60. Mombasa.

Geethalakshmi, R., D.V.L. Sarada, P. Marimuthu and K. Ramasamy. 2010. α-amylase inhibitory activity of *Trianthema decandra* L. International Journal of Biotechnology and Biochemistry 6 :369-376.

Hussain, M.S. and G. Ananthan. 2009. Antimicrobial activity of crude compound ascidians, *Didemnum candidum* and *Didemnum psammathodes* (Tunicata: Didemnidae) from Mandapam (South east coast of India). Current Research Journal of Biological Sciences 1:168–171.

Jaiyesumi, A.F., K. Abo and W. Richard. 2009. α-amylase inhibitory effect of 3β- olean- 12-en-3-yl(9Z)-hexadec-enoate isolated from *Spondias mombin* leaf. Food Chemistry 116:285 - 288.

Jongyoon, W., Y.M. Ham, K.N. Kim, S.Y. Park, N.H. Lee, C.G. Hyun and W.J. Lee. 2009. Antiinflammatory activity of brown alga *Dictyota dichotoma* in murine macrophage RAW 264.7 cells. Journal of Medicinal Plants Research 3:1-8.

Kelecom, A., 2002. Secondary metabolites from marine microorganisms. Annals of the Brazilian Academy of Sciences 74:151–170.

Mayer, M.S.A., R. Kirk and R. Gustafson. 2004. Marine pharmacology in 2001-2, anti-tumor and cytotoxic compounds. European Journal of Cancer 40:2676-2704.

Nagash, Y.S., R.A Nazeer, and N.S. Sampath Kumar. 2010. *In vitro* antioxidant activity of solvent extracts of molluscs (*Loligo duvauceli* and *Donax strateus*) from India. World Journal of Fish and Marine Sciences 2:240-245.

Nickavar, B. and G. Mosazadeh. 2009. Influence of three morus species extracts on α-amylase activity. Iranian Journal of Pharmaceutical Research 8:115-119.

Nirmaladevi, K. and K. Periyanayagam. 2010. *In vitro* anti inflammatory activity of *Plectranthus amboinicus* (Lour) spreng by HRBC membrane stabilization. International Journal of Pharmaceutical Studies and Research 1:26–29.

Pillai, V. N. and N.G. Menon. 2000. Edible and ornamental resource gastropods. Marine Fisheries Research and Management 34:525–535.

Shreedhara, C.S., V.P. Vaidya, H.M. Vagdevi, K.P. Latha, K.S. Muralikrishna and A.M. Krupanidhi, 2009. Screening of *Bauhinia purpurea* Linn. for analgesic and anti inflammatory activities. Indian Journal of Pharmacology 41:75 -79.

Sreejamole, K.L. and C.K. Radhakrishnan. 2010. Preliminary qualitative chemical evalution of the extracts from mussel *Perna viridis.* International Journal of Pharmaceutical Sciences Review and Research 5:38-42.

Sungkim, J.S., T.K. Hyun and M. Jokim. 2011. The inhibitory effects of ethanol extracts from sorghum, foxtail millet and proso millet on α-glucosidase and α-amylase activities. Food Chemistry 124:1647-1651.

Tiwari, P., N. Rahuja, R. Kumar, V. Lakshmi., M.N. Srivastava, S.C. Agarwal , R. Raghubir, and A.K. Srivastava. 2008. Search for antihyperglycemic activity in few marine flora and fauna. Indian Journal of Science and Technology 5:1–5.

Yeshwante, S. B., A.R. Juvekar, D.M. Nagmoti, S.S. Wankhede, A.S. Shah, R.B. Pimprikar, and D.S. Saindane. 2009. Anti-inflammatory activity of methanolic extracts of *Dillenia indica* L. leaves. Pharmacology 1:6 -66.

Yoon, W.J., Y.M. Ham, K.M. Kim, S. Yeungpark, N.H. Lee and C.G. Hyun. 2009. Anti-inflammatory activity of brown alga *Dictyota dichotoma* in murine macrophage RAW 264.7 cells.Journal of Medicinal Plants Research 3:1-8.

Received: 03.08.2011; Accepted: 25/03/2012 (MS11-62).