Chapter 1

PHARMACOGNOSTICAL STUDIES OF *Stomopneustes variolaris*

(LAMARCK, 1816)

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years. Since the beginning of human civilization people sought natural compounds to cure diseases and enrich their health. Extracts from natural origins have served as a valuable source in many drug discovery programs. The World Health Organization estimates that approximately 80% of the world’s population relies primarily on traditional medicines for their primary health care (Farnsworth *et al*., 1985). Today 57% of the most prescribed drugs have their origin from natural products and 90% of the crude drugs are originated from plant sources.

The potentially available biodiversity on the Ocean realm is greater than that existing in the rainforests (Haefner, 2003). However, research in the field of marine derived natural product is comparably young. With the development of underwater technology and establishment of ecologically relevant bioassays, the systematic investigations of biologically active compounds from marine sources began intensively in the mid 1970s (McClintock and Baker, 2001). So far, over 20,000 natural products have been isolated and identified from various marine organisms, proving the constant and rapid growth of this field. Studies on the chemical composition of marine organisms is becoming more common today (Mayer *et al*.,
2011), but only less than 1% of the isolated compounds were examined so far for pharmacological activities.

The coastal and marine ecosystems have become the ultimate sink for discharges of domestic and industrial waste, spillage of oil and discharge of radioactive materials. For this reason, the benthic marine organisms present in these harsh environments produce chemicals for their own use in a diverse array of functions including self defense and signaling. Many of them have no precedence among structures of terrestrial origin and possess previously unknown pharmacological and toxicological properties (Selvin and Lipton, 2004).

Natural products are used throughout developed and developing countries because of their minimum side effects. However a key obstacle, which has limited acceptance of the alternative natural medicines, is the lack of documentation and stringent quality control. It is therefore essential to establish internationally recognized guidelines for assessing their quality (Rajesh et al., 2010). The process of standardization can be achieved by stepwise pharmacognostic studies such as selection of organism, biochemical, physicochemical studies, bioassay techniques, isolation procedures and structural elucidation.

The biochemical composition of marine organisms varied widely depending on several factors like species, size, sex, maturity, season and feeding regimes. Even though large numbers of marine animals are suitable for human consumption, our knowledge on its nutritional value is fragmentary. The fundamental biochemical component protein plays a vital role in every aspect of the structural and functional characteristics of the organism. The quality of protein is usually assessed by its
amino acid composition. Small peptides and amino acids present in the protein can contribute the food texture, taste and flavor (DeLaCruz et al., 2000). Carbohydrates exert a structural role in the cells and act as a major source of energy in human diets. Lipids are important energy reserves that can store more energy per unit volume than proteins or carbohydrates (Montero-Torreiro et al., 1998). In addition, lipids such as phospholipids, cholesterol and triglycerides are structural components of cells and subcellular membranes and it is important for the vital somatic growth (Takagi et al., 1980 and Liu et al., 2007). Lipids of marine origin are rich sources of Omega-3 polyunsaturated fatty acids and they have pronounced hypocholesterolemic effect when supplemented in human diet.

Literature reports indicate the importance of calcium, phosphorous, magnesium and chloride in human health. Some of these minerals serve as a major component in bone formation and repairs (Ardon et al., 1998 and Abulude et al., 2006). Physicochemical characteristics such as ash and moisture are used to estimate the impurities and also in detecting adulterations in the crude drug.

Scarcity of nutrient rich food and food supplements has been responsible for the recurring problems associated with malnutrition in developing countries. In India, 20 – 30% of the population does not get adequate nutrition. About 30% of the newborns in India are of low birth weight and about 36% adult women and 34% adult men suffer from chronic energy deficiency (UNDAF, 2013-2017). This problem can be overcome by the utilization of nutrient rich foods.

In recent years, there has been an increase in research on marine organisms, with particular interest on their proximate composition with desirable nutritive
properties (Lin et al., 2008; Babu et al., 2010; Diniz et al., 2012; Mathana et al., 2012 and Udo, 2012). But only fewer investigations has been carried out in echinoderms (Hu et al., 2010; Salarzadeh et al., 2012; Yahyavai et al., 2012 and Piccino et al., 2013) and most of these studies are restricted to only sea cucumbers and starfishes.

Because of health consciousness, many people are interested in seafood more in view of its nutritional superiority than all other sources of food. The sea urchin gonads are highly valued sea food commodities and are considered as culinary delicacies in many parts of the world (Liyana-Pathirana et al., 2002). About 100 g roe have 172 k Cal, 12 g of protein, 1.75 g of polyunsaturated fatty acids, 1.07 g of Omega 3 fatty acids and almost 3 g of fat (Pacific Urchin Harvesters Association, 2014).

Beyond their culinary attractions, sea urchin has a wide range of medical functions. Due to their peculiar high Mg-Calcite skeleton, echinoids exhibit a high fossilization potential. Since ancient times the fossils have been revered as objects of religious or superstitious power and the peoples believed that, it cures acidic stomach, kidney stones and sea sickness (Mcnamara, 2007). Traditional Chinese medicine recorded sea urchins cure heartache, otitits media, gastric and duodenal ulcers (Shang et al., 2014). A variety of sea urchin derived food and pharmaceutical products are available in many Asian countries. In India, sea urchins are not well known and they are not consumed as food. Although they are used by the coastal people to maintain fitness during long fishing travels and to prevent, reduce or cure several ailments like back pain, gastrointestinal tract problems and piles.
Several studies have been undertaken to determine the biochemical composition of sea urchins in different regions of the world (Fernandez, 1998 and Montero-Torreiro and Gracia-Martinez, 2003). However, there is only scarce information relating to most sea urchin species of India in terms of their proximate, mineral and amino acid composition. Gonads of the sea urchin *S. variolaris* are consumed in raw condition by the coastal people of Kanyakumari. However, hitherto no information is available on its nutrient composition. Therefore the present study has been designed to evaluate the pharmacognostic biochemical and physico-chemical characteristics of the slate pencil sea urchin *S. variolaris*. 
MATERIALS AND METHODS

SYSTEMATIC POSITION OF THE TEST ANIMAL

Kingdom : Animalia
Phylum : Echinodermata
Sub phylum : Echinozoa
Class : Echinoidea
Sub class : Euechinoidea
Infra class : Carinacea
Super order : Echinacea
Order : Stomopneustoida
Family : Stomopneustidae
Genus : Stomopneustes
Species : variolaris
Synonym : Echinus variolaris (Lamarck, 1816).
Common name : Slate pencil sea urchin (English), Moorai (Tamil).

SAMPLE COLLECTION AND PREPARATION

Live specimens of sea urchins were collected along the coast of Kanyakumari (Lat. N08°06´ 49´´; Long. E077°73´353´´), the south eastern boundary of Gulf of Mannar, India. S. variolaris was handpicked by divers during low tide time. The sea urchin was taxonomically identified and authenticated by scientists of
Central Marine Fisheries Research Institute (CMFRI), Vizhingam, Kerala. To avoid spawning, the urchins were brought to the laboratory immediately. All individuals were cleaned with tap water, dissected into two parts and the gonads were removed carefully (Plate 1.1). The exoskeleton was shade dried and the gonads were dried in hot air oven at 50-60°C for 24 hours. After complete drying all the samples were coarsely powdered using mortar and pestle. The powder was kept in separate airtight containers with proper labeling and stored at 4°C for further studies.

**ESTIMATION OF BIOCHEMICAL PARAMETERS**

1. **ESTIMATION OF TOTAL PROTEIN**

   The Folin-Ciocalteu phenol method of Lowry *et al.* (1951) was adopted for the estimation of total protein. The dry sample weighing 10 mg was homogenized with 1 ml of 10% TCA. It was allowed to stand for 30 minutes at room temperature and centrifuged at 3000 rpm for 15 minutes and the supernatant was discarded. The precipitate was dissolved in 1 ml of 0.1 N NaOH. An aliquot of the sample (1 ml) was mixed with 5.0 ml of alkaline copper reagent and was mixed well by vigorous shaking. After 10 minutes 0.5 ml of Folin-Ciocalteu reagent was added and mixed well. Bovine serum albumin was used as standard. A blank was prepared with 1 ml of 1N NaOH and was treated like the same way as mentioned earlier. All the test tubes were kept for 30 min at room temperature and the OD was calculated against the blank at 660 nm. The total protein was calculated by using the following formula:

   \[
   \text{Total protein} = \frac{\text{OD of standard} - \text{OD of sample}}{\text{Weight of the sample}} \times 100
   \]
2. ESTIMATION OF CARBOHYDRATE

The carbohydrate was estimated by anthrone method (Carrol \textit{et al.}, 1956). 0.01 mg of dry sample was thoroughly homogenized with 2 ml of 10% TCA. The homogenates were centrifuged at 3000 rpm for 15 minutes. An aliquot of the supernatant (0.2 ml) was mixed with 5 ml anthrone reagent and was kept in a boiling water bath for 15 minutes. The tubes were cooled and absorbance was measured at 620 nm in a spectrophotometer. D- Glucose was used as a standard. The system devoid of sample was used as the blank. The carbohydrate was calculated by the following formula:

\[
\text{Carbohydrate} = \frac{\text{OD of standard} - \text{OD of sample}}{\text{Weight of the sample}} \times 100
\]

3. ESTIMATION OF TOTAL CHOLESTEROL

The method of Zlatkis \textit{et al.} (1953) was used for the estimation of total cholesterol. 5 ml ethyl acetate: alcohol (1:1) mixture was added to 0.1 ml sample. The contents were vigorously shaken exactly for 2 minutes. The contents were centrifuged after 30 minutes and 1.0 ml supernatant was used for the development of colour. Then 2 ml of ferric chloride– acetic acid reagent (0.05 % solution of ferric chloride in glacial acetic acid) was added followed by 2 ml of concentrated $\text{H}_2\text{SO}_4$. 0.1 ml of cholesterol (2 mg cholesterol in 100 ml chloroform- Standard) and distilled water (blank) were simultaneously treated as above. After 30 minutes, the absorbance was read at 560 nm in a spectrophotometer.

\[
\text{Total cholesterol} = \frac{\text{OD of test} - \text{OD of blank}}{\text{OD of standard} - \text{OD of blank}} \times 1000 \text{ mg/ 100 ml}
\]
4. ESTIMATION OF TRIGLYCERIDES (Foster and Dunn, 1973)

To a 0.5 ml of sample 3.5 ml isopropanol was added followed by 1 ml of 0.08 N H$_2$SO$_4$ and 2 ml of heptane. The contents were then mixed thoroughly. An aliquot of the sample (0.2 ml) was taken and mixed well with 3 ml of sodium methylate solution and incubated at 60°C for 15 minutes. After incubation, 1 ml of sodium metaperiodate solution was added immediately and then 1 ml of acetyl acetone reagent was mixed and it was heated at 60°C for another 10 minutes. It was allowed to cool at room temperature. 0.1% of triglyceride solution in isopropanol (standard) and distilled water (blank) were simultaneously treated as above. The tubes were centrifuged and the absorbance was measured at 410 nm.

The triglyceride concentration (in mg/dl) = \( \frac{\text{OD of test}}{\text{OD of standard}} \times 100 \)

AMINO ACID ANALYSIS OF S. variolaris

The analysis of the amino acid composition of the S. variolaris was carried out in an amino acid analyzer (HPLC specially designed for amino acid analysis; Shimadzu LC – 10 As, Japan) using the method of Bidlingmeyer et al. (1984). Samples were hydrolyzed using 6 N HCl (1% w/v) phenol vapour at 110°C for 24 h under vacuum. Protein hydrolysate was filtered through Whatmann No- 42 filter paper quantitatively, flash evaporated to remove HCl and made up to 100 μl in 0.05 M HCl. The sample (100 μl) was injected through the sample loop in the amino acid analyzer and the amino acids were detected by spectrofluorometer. The chromatogram was recorded.
COMPUTER ANALYSIS OF THE AMINO ACID SEQUENCE

The obtained amino acid sequence was analyzed using BACTIBASE database (a data repository of bacteriocins developed by the Functional proteomics and Alimentary Bio-preservation Research at Institute of Applied Sciences Tunis (ISSBAT), Tunisia in collaboration with Neutraceuticals and Functional Foods Institute (INAF), Laval University, Canada). Similarity search was performed using the FASTA program and sequence alignment using CLUSTAL W of the database. The physicochemical profile of the protein was also predicted using the software.

ESTIMATION OF MINERAL CONTENT

PREPARATION OF SAMPLE SOLUTION FOR MINERAL ANALYSIS

The solutions of samples for mineral analysis were prepared by wet digestion method. Clean and dried samples of *S. variolaris* gonad and exoskeleton were taken in a volumetric flask and added 50 mL of double distilled water with the sample. These were boiled for about four hours and then evaporated the solvent. At room temperature 100 mL of HNO₃ and HClO₄ mixture (5:1 v/v) were added with the boiled sample. This was refluxed at 120-125°C (20 hours) until a clear solution appeared. The volume of the solution has been reduced to about 3-5 mL by condensation. At room temperature added few mL of distilled water and filtered through Whatman-40 filter paper into a 100 mL volumetric flask and made up to the mark with double distilled water. All the sample solution under the present investigation were prepared in similar manner and stored at room temperature for Spectroscopic measurement. A blank solution was also prepared for each group of sample by using all reagents except the sample.
ANALYTICAL TECHNIQUES

Standard stock and working solutions of potassium, sodium, phosphorous and magnesium were prepared using standard methods (Greenberg et al., 1992). Concentration of potassium and sodium were determined using flame photometer at 767 and 589 nm respectively. Concentration of magnesium was determined using atomic absorption spectrophotometer at 285.2 nm. Concentration of phosphorous was determined using the phospho-vanadomolybdate method for colour development and absorbance measured with spectrophotometer at 470 nm (Bassett et al., 1987 and HACH, 1992).

ESTIMATION OF PHYSICOCHEMICAL PARAMETERS

1. ESTIMATION OF MOISTURE CONTENT

Moisture content of the samples was determined according the AOAC (1990) method. Preweighed wet samples were dried in an oven at 105°C until a constant mass was obtained. The percentage of moisture content was calculated as follows:

\[
\text{Percentage of moisture} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100
\]

2. ESTIMATION OF ASH CONTENT

The dried samples were minced, weighed and ignited in the crucible. It was then transferred in the muffle furnace. The ash content was determined by ashing the samples at 560°C for 6-8 hours until the residue become white (AOAC, 1995). The residue was weighed and the percentage of ash was calculated.
Percentage of ash = \frac{\text{Dry weight of the sample}}{\text{Original weight of the sample taken}} \times 100

**STATISTICAL ANALYSIS**

Triplicate determinations were carried out for each biochemical, mineral and physicochemical analysis and the results were expressed as mean ± SD
RESULTS

The biochemical constituents of the body parts of *S. variolaris* were shown in table 1.1 and figure 1.1. Of the biomolecules analyzed protein, carbohydrate, total cholesterol and triglyceride were found to be maximum in gonad and minimum in exoskeleton. The gonad of *S. variolaris* contains the high amount of protein (149 ± 0.82 mg/g) whereas the exoskeleton contain low amount of protein (134.33 ± 1.25 mg/g). The carbohydrate content was almost same in gonad (4.63 ± 0.17 mg/g) and exoskeleton (4.37 ± 0.79 mg/g). The total cholesterol values of gonad and exoskeleton were 2.24 ± 0.07 mg/g and 1.71 ± 0.17 mg/g respectively. Likewise, the triglyceride level was generally high in gonad (3.63 ± 0.08 mg/g) and it was relatively low in exoskeleton (2.68 ± 0.15 mg/g).

Table 1.2 and 1.3 shows the amino acid profiles of *S. variolaris* gonad and exoskeleton. Totally nine amino acids were recorded in *S. variolaris* gonad (Figure 1.2). Among them four were essential amino acids *viz.*, methionine (0.0079 µg/mg), histidine (0.0062 µg/mg), valine (0.0058 µg/mg) and leucine (0.0008 µg/mg). Five aminoacids *viz.*, proline (0.0437 µg/mg), alanine (0.0125 µg/mg), glutamate (0.0115 µg/mg), tyrosine (0.01 µg/mg) and arginine (0.0002 µg/mg) were non-essential amino acids.

A total of ten amino acids were observed in *S. variolaris* exoskeleton (Figure 1.3). Among them five were essential amino acid and five were non-essential amino acids. The main essential amino acids found in exoskeleton were methionine (0.0139 µg/mg), valine (0.0119 µg/mg), histidine (0.0053 µg/mg), leucine (0.0019 µg/mg) and phenyl alanine (0.001 µg/mg). While the main non-essential amino
acids were proline (0.0669 µg/mg), alanine (0.025 µg/mg), tyrosine (0.0208 µg/mg), glutamate (0.0075 µg/mg) and arginine (0.0004 µg/mg). In both gonad and exoskeleton, leucine the essential amino acids and arginine the non-essential amino acids were present in very low quantities.

Mineral elements composition of gonad and exoskeleton of *S. variolaris* was presented in table 1.4 and figure 1.4. Among the estimated minerals, potassium and phosphorous were high in gonads. Potassium content of gonad and exoskeleton were estimated to be 3.23 ± 0.04 mg/g and 1.22 ± 0.03 mg/g respectively. The phosphorous content recorded in gonad was 1.21 ± 0.01 mg/g and it was 0.59 ± 0.03 mg/g in exoskeleton. It was also observed that sodium was maximum in exoskeleton and minimum in gonads. The sodium content was 32.12 ± 0.10 mg/g in gonad and 33.52 ± 0.03 mg/g in exoskeleton. The magnesium content was very low in both gonad and exoskeleton when compared to other minerals and it was 0.29 ± 0.01 mg/g in gonad and 0.64 ± 0.01 mg/g in exoskeleton.

Table 1.5 and figure 1.5 represent the physicochemical composition of *S. variolaris* gonad and exoskeleton. In the present study, the moisture content was maximum in gonad (75.96%) and minimum in exoskeleton (9.64%). Whereas, the exoskeleton recorded the highest ash percentage (81.62%) and the gonad recorded lowest ash percentage (5.58%).
<table>
<thead>
<tr>
<th>S. No</th>
<th>Biochemical constituents (mg/g)</th>
<th>Body parts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gonad</td>
</tr>
<tr>
<td>1.</td>
<td>Protein</td>
<td>149 ± 0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exoskeleton</td>
</tr>
<tr>
<td></td>
<td></td>
<td>134.33 ± 1.25</td>
</tr>
<tr>
<td>2.</td>
<td>Carbohydrate</td>
<td>4.63 ± 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exoskeleton</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.37 ± 0.79</td>
</tr>
<tr>
<td>3.</td>
<td>Total cholesterol</td>
<td>2.24 ± 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exoskeleton</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.71 ± 0.17</td>
</tr>
<tr>
<td>4.</td>
<td>Triglycerides</td>
<td>3.63 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exoskeleton</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.68 ± 0.15</td>
</tr>
<tr>
<td>S.No</td>
<td>Free amino acids</td>
<td>Time (min)</td>
</tr>
<tr>
<td>------</td>
<td>-----------------------</td>
<td>------------</td>
</tr>
<tr>
<td>1.</td>
<td>Essential amino acids</td>
<td>Methionine</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>Histidine</td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td>Valine</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>Leucine</td>
</tr>
<tr>
<td>5.</td>
<td>Non-essential amino acids</td>
<td>Proline</td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td>Alanine</td>
</tr>
<tr>
<td>7.</td>
<td></td>
<td>Glutamate</td>
</tr>
<tr>
<td>8.</td>
<td></td>
<td>Tyrosine</td>
</tr>
<tr>
<td>9.</td>
<td></td>
<td>Arginine</td>
</tr>
</tbody>
</table>
**TABLE 1.3**

**Amino acid composition of *S. variolaris* exoskeleton**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Free amino acids</th>
<th>Time (min)</th>
<th>Area</th>
<th>Concentration (µg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Essential amino acids</td>
<td>Methionine 31.383</td>
<td>23884</td>
<td>0.0139</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>Histidine 44.943</td>
<td>19755</td>
<td>0.0053</td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td>Valine 27.425</td>
<td>24905</td>
<td>0.0119</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>Leucine 33.543</td>
<td>3818</td>
<td>0.0019</td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td>Phenyl alanine 37.36</td>
<td>2083</td>
<td>0.001</td>
</tr>
<tr>
<td>6.</td>
<td>Non-essential amino acids</td>
<td>Proline 14.01</td>
<td>22968</td>
<td>0.0669</td>
</tr>
<tr>
<td>7.</td>
<td></td>
<td>Alanine 21.495</td>
<td>33689</td>
<td>0.025</td>
</tr>
<tr>
<td>8.</td>
<td></td>
<td>Glutamate 13.317</td>
<td>12669</td>
<td>0.0075</td>
</tr>
<tr>
<td>9.</td>
<td></td>
<td>Tyrosine 34.945</td>
<td>14644</td>
<td>0.0208</td>
</tr>
<tr>
<td>10.</td>
<td></td>
<td>Arginine 51.217</td>
<td>4244</td>
<td>0.0004</td>
</tr>
</tbody>
</table>
### TABLE 1.4

**Mineral composition of *S. variolaris* gonad and exoskeleton**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Mineral elements (mg/g)</th>
<th>Body parts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gonad</td>
</tr>
<tr>
<td>1.</td>
<td>Potassium</td>
<td>3.23 ± 0.04</td>
</tr>
<tr>
<td>2.</td>
<td>Sodium</td>
<td>32.12 ± 0.10</td>
</tr>
<tr>
<td>3.</td>
<td>Phosphorous</td>
<td>1.21 ± 0.01</td>
</tr>
<tr>
<td>4.</td>
<td>Magnesium</td>
<td>0.29 ± 0.01</td>
</tr>
</tbody>
</table>

### TABLE 1.5

**Physicochemical composition of *S. variolaris* gonad and exoskeleton**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Physicochemical parameters (%)</th>
<th>Body parts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gonad</td>
</tr>
<tr>
<td>1.</td>
<td>Moisture</td>
<td>75.96</td>
</tr>
<tr>
<td>2.</td>
<td>Ash</td>
<td>5.58</td>
</tr>
</tbody>
</table>
FIGURE 1.1

Biochemical composition of *S. variolaris* gonad and exoskeleton

![Biochemical composition of *S. variolaris* gonad and exoskeleton](image)
FIGURE 1.2

Aminoacid composition of *S. variolaris* gonad
FIGURE 1.3

Aminoacid composition of S. variolaris exoskeleton
FIGURE 1.4

Mineral composition of *S. variolaris* gonad and exoskeleton

FIGURE 1.5

Physico chemical composition of *S. variolaris* gonad and exoskeleton
PLATE 1.1

*Stomopneustes variolaris*

Gross View

Cross section of *S. variolaris*

Exoskeleton of *S. variolaris*

Gonad of *S. variolaris*
DISCUSSION

Pharmacognostic evaluation of crude drugs plays a very important role in identification, purity and quality of crude drugs. It is one of the simplest and cheapest method to establish the correct identity of the source material. Various kinds of marine organisms, from different tidal habitats form the vital and renewable source of food and pharmaceuticals. Sea urchin is one of the key marine animal screened for bioactive materials. The knowledge of biochemical composition of any edible organisms is extremely important, because the nutritive value is reflected in its biochemical contents (Nagabhushanam and Mane, 1978).

Protein is an important biochemical macronutrient and it plays a vital role in determining the texture and quality of animal muscle. It is used to make bone, skin, nails, hair, muscle, cells and play a role in improving brain function and aiding many aspects of health including blood pressure and cardiovascular problems. Protein was the most dominant biochemical constituent in *S. variolaris* and it was moderately high in gonad (149 ± 0.82 mg/g) than exoskeleton (134.33 ± 1.25 mg/g). Similar study carried out by Mol *et al.* (2008) showed that the sea urchin *P. lividus* contain 12.03 ± 1.26% of protein. Shikov *et al.* (2012) found that the sea urchin *S. droebachiensis* contain 39.7% protein. Bragadeeswaran *et al.* (2013) elucidated that the sea urchin *T. toreumaticus* contain 2.70 mg/ml protein. Compared with these studies the protein content in *S. variolaris* is very high. These differences are related with the omnivorous feeding habit and adequate food present in the surrounding. Mayne and Robinson (1998) reported the positive relationship between feeding habit and total body protein of organism.
Most underdeveloped countries in the world have been facing malnutrition problems, especially shortage of protein in human and animal feed. Human body needs a large amount of protein but unlike carbohydrate and fat, the body does not store protein. 100 grams of sea urchin gives 13.3 grams of protein that will meet 29% of daily protein requirement in women and 24% of daily requirement in men. *S. variolaris* gonad is rich in protein and a delicious food, sea urchin sushi is made from sea urchin gonads. Each ounce of sea urchin sushi contains 3.2 grams of protein (Willet, 2012). This protein-rich *S. variolaris* can overcome this malnutrition problem.

Carbohydrate is an essential component of living cells as it supplies the energy needed for physiological and metabolic processes. Unlike proteins, carbohydrate is almost the same in gonads (4.63 ± 0.17 mg/g) and exoskeleton (4.37 ± 0.79 mg/g). Sea urchins are poor in carbohydrate content, but it has a significant role in body coating and fertilization events (Ghazarian *et al.*, 2010). Mol *et al.* (2008) reported a concentration of 2.8% of carbohydrate in the roe of the sea urchin, *P. lividus*. Bragadeeswaran *et al.* (2013) recorded 2.15 mg/ml of carbohydrate in the sea urchin *T. toreumaticus* sampled in southeast coast of India, which represents a carbohydrate content lower than those found in the present study.

In the present study the carbohydrate content was less when compared with protein. This may be due to the limitation of carbohydrate and abundance of protein in the natural aquatic food web. Pushparajan *et al.* (2012) reported similar views while working with shrimp larval ingress in pichavaram mangroves, south east coast of India. Moreover, this low value supports the view that carbohydrate plays an insignificant role in energy reserve in aquatic animals (Love, 1970). High protein
and low carbohydrate diets are used to prevent or manage some chronic diseases and conditions including cardiovascular diseases, metabolic syndromes, high blood pressure and diabetes. Due to its high protein and low carbohydrate content sea urchin *S. variolaris* gonad can be used as an ideal substitute for commonly used food such as meat, fish and poultry along with non- starchy fruits and vegetables.

In the present study, cholesterol and triglycerides were found to be maximum in gonads and minimum in exoskeleton. This high amount of cholesterol and triglycerides in the gonad may be correlated with the maturation of gonadal follicles and time of spawning. Yan *et al.* (2009) observed an increase in the protein and lipid content in association with the gametogenesis in the gonads of the razor clam, *Sinonovacula constricta*. At normal levels, cholesterol is an important body constituent used in the structure of cell membranes, synthesis of bile acid and steroid hormones. Triglycerides make up about 95% of all dietary fats and it can be used for energy by cells. But if their level in the blood increases above the threshold level, it becomes a silent danger. In *S. variolaris* the cholesterol and triglyceride level is low when compared with other biochemical constituents such as protein and carbohydrate. Dieticians suggested that the high protein and low fat diets were effective for weight loss and healthy blood lipid profile. The coastal people believed that eating this sea urchin in raw condition can improve physical strength and health. This study proved this traditional belief.

Amino acids are organic compounds serving as building blocks of proteins and as intermediate in metabolism. Amino acid profile of *S. variolaris* gonad and exoskeleton revealed the presence of nine amino acids in the gonad and ten amino acids in the exoskeleton fractions. The essential amino acids methionine, histidine,
valine and leucine were present in both the body parts, whereas phenyl alanine was present only in exoskeleton. Supplementation of human diet with *S. variolaris* gonad may supply the much needed essential amino acid to humans, because these amino acids are not synthesized in the body by *de nova* mechanism.

Methionine and valine are used to treat liver diseases. Histidine is used for rheumatoid arthritis and allergic diseases. Leucine is necessary for nitrogen equilibrium in adults and it prevents breakdown of muscle protein after trauma on severe stress. Phenyl alanine is used for depression, chronic pain, osteoarthritis and vitiligo. However, these essential amino acids were present only in trace concentrations when compared with non-essential amino acids. Shikov *et al.* (2012) reported that glycine, alanine, leucine and valine were dominant, followed by methionine, isoleucine, lysine, serine, threonine, phenyalanine, proline, histidine, tyrosine and tryptophan in the green sea urchin *S. droebachiensis*. They also reported that essential amino acids were found in higher concentration than non-essential amino acids. Similarly, in the purple sea urchin *A. crassispina* glycine, valine, methionine, lysine and arginine content was high (Osako *et al.*, 2007).

Non-essential amino acids support tissue growth and repair, immune function, red blood cell formation and hormone synthesis. In the present study, the non-essential amino acids proline, alanine, glutamate and tyrosine were found to be dominant among the free amino acids in *S. variolaris*. Among the non-essential amino acids proline is an osmoprotectant and therefore it is used in many pharmaceutical applications. Alanine increases immunity and provides energy for muscle tissue, brain and the central nervous system. Glutamate is an important neurotransmitter which plays a key role in long term potentiation and it is also
important for learning and memory (Sapolsky, 2005). Tyrosine is used for stress, cold, fatigue and sleep deprivation. Arginine is used to treat heart and blood vessel conditions including congestive heart failure, chest pain, high blood pressure and coronary artery diseases. Human body cannot synthesize several non-essential amino acids in large quantities in accordance with the body’s requirements and it requires conditional amino acids such as proline, tyrosine during illness and stress. In the present study high amount of non-essential amino acids was noticed in S. variolaris and administration of S. variolaris gonad may be helpful in solving this problem.

The findings of the present study was in concordant with the findings of Kaneko et al. (2012), who reported the presence of higher levels of alanine and glycine in the black sea urchin D. setosum gonads. Present study also support the view of Mol et al. (2008), who observed that the glutamic acid, glycine, aspartic acid, lysine and arginine were dominant in the sea urchin P. lividus roe. Chen et al. (2013) found that the most dominant amino acid of sea urchin T. gratilla was glycine, whereas alanine, proline, arginine and glutamic acid were the predominant free amino acids.

Free amino acids mainly contribute to the flavor, taste and physiological functions of sea foods (Hall and Ahmad, 1992 and Huang et al., 2002). Unpleasant fishy tastes and other undesirable sensory factors can be responsible for the low consumption of sea food supplements (Mueller and Talbert, 1988 and Jones and LeCornu, 1994). Fuke and Ueda (1996) indicated that the main flavor giving amino acids in sea urchin gonad were glycine, glutamic acid, alanine, methionine, valine
and arginine. The present study revealed that these flavor enhancing amino acids were high in *S. variolaris*.

Mineral elements constitute important components of hormones, enzymes and enzyme activators in human nutrition. Mineral deficiencies can cause biochemical, structural and functional pathologies. Although mineral elements make a small proportion of total chemical composition and body weight of animal, but their physiological importance and pharmacological activities cannot be ignored. Gopakumar (1997) reported that the sea foods are excellent sources of Ca, P, I, P, Na, Fe and Zn. In the present study, potassium and phosphorous were maximum in gonad and minimum in exoskeleton. Normally higher potassium intakes may be beneficial in preventing kidney stone formation. On the other hand, phosphorous helps in kidney function, muscle contraction, normal heart beat and nerve signaling. Zhenglun (1994) reported that the pearl shells from South China Sea were rich in Ca, P and Zn contents. In humans, sodium is an essential nutrient that regulates blood volume, blood pressure, osmotic equilibrium and pH. Magnesium is used as an antacid for acid indigestion. In the present study these minerals were found to be maximum in exoskeleton and minimum in gonad. Rajagopal *et al.* (1998) reported the importance of Ca, Mg and K in the human nutrition.

The physicochemical parameters are mainly used in judging the purity and quality of the drug. According to Bassey *et al.* (2011), knowledge of the moisture content of food stuff serves as a useful index of keeping their qualities. In the current investigation, moisture content was maximum in gonad (75.96%) and minimum in exoskeleton (9.64%). Moisture is an inevitable component of crude drugs. Crude drugs with high moisture content can be easily deteriorated due to fungus
(Mukherjee, 1996). So during drug development it can be eliminated as far as practicable. This high level of moisture content in the S. variolaris gonad was supported by previous studies. Hiroshi et al. (1998) and Cruz-Garcia et al. (2000) reported that gonads of sea urchin species S. nudus and P. lividus respectively contain high moisture content.

Ashing is an important tool for detecting the adulteration in crude drugs. Ash is one of the least studied chemical constituents in marine animals. In the present study, ash content was uniformly high in exoskeleton (81.62 %). This high level ash content in the exoskeleton may be due to the high level of chitin strengthened by high level of calcium in the sea urchin shell. Similar result was observed by Mol et al. (2008) in P. lividus (2.25 ± 0.24%). Pais et al. (2011) reported the ash content in the sea urchin P. lividus gonad was 1.88 ± 0.003% to 2.35 ± 0.25%. Similar to this finding the present study also revealed the presence of low ash content (5.58%) in the gonad.

A newer species should be recommended for human consumption only after assessing the nutritional value of the species. Even though Indian sub-continent is endowed with numerous marine animals suitable for human consumption, our knowledge on its nutritional value is fragmentary. The nutritional values of this sea urchin do not bring the limelight so far, so consumption of these nutrient rich animals has not attracted attention. On the basis of the current findings it can be assumed that the sea urchin S. variolaris is a good source of some important nutrients and minerals. Moreover, pharmacognostical parameters analyzed in the current investigation serve as a standard data for quality control studies of pharmaceutical preparation from S. variolaris in future.