Antibiotic efficacy and biochemical composition of ascidian, *Phallusia nigra* (Savigny, 1816) from the Palk Bay, Southeast coast of India

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Received 10 March 2015; revised 02 July 2015

An investigation was held out to examine the biochemical constituents present in the simple ascidian *Phallusia nigra* (*P. nigra*), such as protein, carbohydrate, lipids, moisture, acidity and ash content. Extractions of *P. nigra* (visceral) tissue sample in several solvents were assayed for antibacterial activity against foodborne pathogens. Protein content was found to be higher during the summer season in *P. nigra*, while the minimum quantity of lipid was recorded during monsoon season. Benzene and acetonitrile crude extracts inhibited the growth of foodborne pathogens. Chloroform extract didn't inhibit any strains.

[Keywords: *P. nigra*, proximate composition, mantle extraction, foodborne pathogens, Palk Bay Ascidian]

Introduction

Over the 50 years, a number of ascidian species have spread across a good deal of the globe. A great balance of natural compounds has been pulled up from marine invertebrates. Ascidians are conspicuous members of marine fouling and benthic communities. Ascidians accumulated high levels of vanadium in their tissues which is obviously non-toxic. Ascidians are capable of producing an unfavorable or toxic condition immediately above the surface as a chemical defense. Their soft-bodied morphology provides ascidians with little obvious structural defense from predation. Ascidians have been considered to rely on chemical defenses against predations. It is regarded as highly nutritious seafood. Consumption just after harvest is favored for the flavor as a metallic taste may become evident as the product becomes less fresh. Ascidians have increasingly come to be known as excellent model organisms in neurobiology and endocrinology, as good as in developmental biology and evolutionary biology.

Spicules, originally indented to provide more or less anti-predatory defenses for the marine invertebrates that secrete them in their tissues. Ascidians are characterized by a tough outer tunic made of the polysaccharide tunicin. Large populations of ascidians can also have a negative effect on coastal aquaculture systems through phytoplankton consumption, nutrient cycling and biodeposition. Temperate coastal areas ascidian is characterized by great seasonal fluctuations in environmental variables such as temperature and nutritional condition. Temperature and salinity are two of the most important factors affecting the distribution of marine invertebrates, thus determining invasion success non-native ascidian species.

Reproductive and developmental processes in ascidian are known to be affected by many different types of environmental factors, including salinity, temperature, food quality, photo period, and ocean hydrodynamics. High acidity due to sequestered sulfuric acid in outer tunic tissues, or high tissue concentrations of the heavy metal vanadium, currently, there are insufficient data to characterize environmental tolerances of sedans in India. Ascidians are rich sources of compounds with cytotoxic proprieties; these marine natural products can be used for the discovery and development of novel chemotherapeutic agents. In this paper, we detail the seasonal variation in physiological energetics and gross biochemical composition of ascidian *P. nigra* collected from Palk Bay, southeast coast of India. Present study aims at unraveling the biochemical composition of *P. nigra* and its antibiotic potentials.
Materials and Methods

Ascidian collection and extraction

Ascidian, *Phallusia nigra* (*P. nigra*) was collected regularly during four seasons in (1–2 m depth) Palk Bay region, Southeast coast of India, from February 2013 to January 2014. Collected samples were placed in zip lock bags in coolers on the ice and transported to the laboratory for further studies. Species were identified through as per the standard protocol\(^\text{21, 22}\) (Kott 1985 and 1990). Each individual was cleaned with the sea and sterile water by the hand of any associated plant or animal material. Ascidians tissue samples were air dried for a few days after drying made into powder using a mortar and pestle for biochemical analysis such as protein, carbohydrate and lipids.

Biochemical analysis

Ascidian (visceral) tissue extractions were based on methods given in McClintock *et al.*\(^\text{23}\) and in Koplovitz *et al.*\(^\text{24}\). Frozen tissues were transferred to at −80 °C freezer for several hours, then lyophilized (Freeze drier: Lark, Penguin) and re-weighed. The lyophilized tissues were then extracted with different solvent such as benzene, chloroform, acetonitrile thrice for 24 h to produce a lipophilic extract. Extracts were filtered through coarse filter paper and the solvent was removed using rotary evaporation to yield a dry lipophilic extract. Natural tissue level concentrations of extracts used for the biological activity.

The acidity of the outer surfaces of the ascidian tunics was measured using standard analytical pH strips. Tunic surface pH measurements were first conducted by gently laying a pH strip with a broad sensitivity range of 0 to14 whole pH units onto the outer tunic surface. Subsequently, higher resolution was obtained using pH strips capable of resolving pH to an accuracy of 0.3 pH units. Following measurements of the tunic surface, the tunic was sliced with a razor blade and the pH measured once again by inserting pH strips into the slice. This allowed a measurement of the internal pH of the tunic. Because the pH test was based on color visual discrimination, we considered this a qualitative measurement and chose to round each pH measurement off at 0.5 unit increments so as to be conservative.

To estimate the moisture content, 1g of fresh tissue was dried through a hot air oven at a constant temperature of 105°C for 24 hours\(^\text{21}\). The loss of weight was taken as moisture content.

Total protein was estimated using the Biuret method of Raymont *et al.*\(^\text{24}\). The total carbohydrate in a dried sample was estimated spectrophotometrically following the phenol-sulfuric acid method of Dubois *et al.*\(^\text{25}\).

The lipid in the dried sample tissue was gravimetrically estimated following the chloroform- methanol mixture method of Folch *et al.*\(^\text{26}\).

Ash content was determined gravimetrically by incinerating 1g dried sample in a muffle furnace at about 55°C for 6 hours\(^\text{27}\) and results are expressed in percentage.

Antibacterial assay

Antibacterial activity was determined against cultures of *Bacillus cereus* (MTCC 6629), *Salmonella enterica* (MTCC 3244), *Vibrio vulnificus* (MTCC 11) using the agar diffusion assay method.\(^\text{28}\) Three plates containing three wells were tested against ascidian sample at 1ml/well. Following incubation at 37°C for 24 hours, the diameters (mm) of the growth inhibition halos were determined using a perimeter.

Results

Biochemical analysis

A high level of acidity (pH 6.5%) was measured on the outer surface of the tunic of the ascidian, *P. nigra* during the summer season. While a low-level acidity (pH 2.3%) that ranged during monsoon season.

<table>
<thead>
<tr>
<th>Table 1. Biochemical composition of ascidian, <em>P. nigra</em></th>
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<tr>
<td><strong>Seasons</strong></td>
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<tr>
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<tr>
<td>Pre monsoon</td>
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<tr>
<td>Monsoon</td>
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<td>Post monsoon</td>
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<tr>
<td>Summer</td>
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Variations of moisture content in species of the ascidian, *P. nigra* were ranged from 68.3% to 97.8%. The minimum (68.3%) content was noticed in during the summer season while maximum (97.8%) content was recorded in during monsoon season.

The protein of ascidian varied from 7.1% to 15.40%. Maximum (15.40%) content of protein was observed in *P. nigra* during summer and the lowest content of protein was (7.1%) recorded on during monsoon season.

The carbohydrate content of ascidian, *P. nigra* was ranged from 2.3% to 8.9%. Minimum (2.3%) was observed during monsoon season and the maximum 8.29% was observed in during the summer season.

Fig.1. Biochemical composition of ascidian, *P. nigra* in seasonal wise.

The lipid content of ascidian, *P. nigra* was ranged from 1.03 to 3.95%. The minimum (1.03%) was recorded during monsoon season while the maximum content was observed in summer season.

Ash content in species of the ascidian, *P. nigra* was ranged from 0.14 % to 1.56%. The minimum (0.14%) was recorded in during monsoon season. While the maximum (1.59%) was recorded in during summer seasons.

Antibacterial assay

Benzene, chloroform and acetonitrile extract of ascidian tissue (visceral) studied in this work showed antimicrobial activity against some of the foodborne pathogens. The benzene extract showed antimicrobial activity against *B. cereus* MTCC 6629(10.25mm), *Salmonella enterica* MTCC 3224(12.55mm) and *V. vulnificus* MTCC 1145(11.12mm). Present results are given in Table 1. These findings suggested that *P. nigra* tissue (visceral) has an antimicrobial potential against foodborne pathogens.

Table 2. Antibacterial activity of ascidian, *P. nigra* against foodborne pathogens

<table>
<thead>
<tr>
<th>Food pathogens</th>
<th>Zone of Inhibition (mm)</th>
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<tbody>
<tr>
<td></td>
<td>Benzene</td>
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<tr>
<td><em>B. cereus</em> MTCC 6629</td>
<td>10.25</td>
</tr>
<tr>
<td><em>Salmonella enterica</em> MTCC 3224</td>
<td>12.55</td>
</tr>
<tr>
<td><em>V. vulnificus</em> MTCC 1145</td>
<td>11.12</td>
</tr>
</tbody>
</table>

Discussion

In this study estimate the tunic acidity, protein, carbohydrate, lipid, moisture and ash contents and antibacterial assay in species of the ascidian, *P. nigra*. The protein (15.40%) and carbohydrate (8.29%) was highly present in *P. nigra* while low acidity (2.3%), during pre-monsoon and protein content (7.1%) were recorded during monsoon. The water content of muscle obviously rises and this makes the flesh weak. This is the reason for the poor eating quality of spawning ascidians. Lipid content has been very low in the ascidians as compared to protein and carbohydrate. Epelbaum *et al.* reported ascidians environmental tolerances and preferences such as temperature and salinity, generally, *Botryllus schlosseri* survived environmental conditions of 10-25°C and 14-38‰, positive growth at 10-25 °C and 20-38‰ and *Botryllodes violaceus* tolerated environmental conditions between 5-25 °C and 20-38‰, positive growth at 15-25°C and 26-38‰. Low content lipids of ascidians have been already reported by Park *et al.*. The protein content of ascidians showed variation, these values are comparable with those previous reported. Defense in ascidians have also been evaluated in the context of food value with some investigators suggesting ascidian tissues are low in nutrients and energy. However, a number of studies have demonstrated that the nutritional value of ascidians is at or above that of other sessile marine benthic invertebrates such as sponges commonly consumed by predators. These studies indicate that ascidians are generally high in protein and carbohydrate and have tissue energy values ranging from 6.0 to 22.4 kJ per gram dry weight. In this study, the protein content was
presented to be the major biochemical composition in the (muscle) *P. nigra*. Similarly, high levels of protein occur in body components of the Antarctic solitary ascidians *Cnemidocarpa verrucosa* and likely reflect the contribution of insoluble protein to structural material including connective tissue. The protein was optimistically correlated by the salinity of the water. Ananthan et al previously estimated the protein, carbohydrate, lipid, moisture and ash contents of 10 ascidian species in Indian waters.

Paul et al reported that the tunicates have the potential to yield novel compounds with ecological, chemical and biomedical interest. Many studies have been conducted to examine the antimicrobial activity of ascidians against bacteria fungi even tumour cells. A chemical antibacterial defence has been suggested as one of an array of defence’s potentially available to sessile invertebrates. The extracts of *P. nigra* show promising results against food borne pathogens. Our findings are consistent with previous studies on ascidian. Abdul Jaffar Ali et al reported the maximum antibacterial activity of the crude methanol extracts of the test and mantle bodies *P. nigra* against the gram positive bacteria.

The results our studies the extracts of benzene and acetonitrile showed activity against foodborne pathogens. While no activity the extracts of chloroform. The maximum activity showed benzene extract against *salmonella enterica* MTCC 3225 and minimum activity was recorded in acetonitrile against *V. Vulnificus* MTCC 1445. Therefore, the current studies exposed the presence of potent antimicrobial compound against food borne pathogens and biochemical content. Hence further studies purified and characterized the bioactive compounds.

Acknowledgement

Authors thank to Dean and Director of CAS in Marine Biology and authorities of Annamalai University for providing the facilities and gratefully acknowledge the financial assistance of major research project (F.No.42-603/2013 (SR) dt.22.03.2013) by the University Grants Commission, New Delhi, India.

References


