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PROXIMATE COMPOSITION AND PRELIMINARY SCREENING OF SECONDARY METABOLITES FROM VARIOUS TISSUES OF PUFFER FISH *AROTHRON STELLATUS* FROM THOOTHUKUDI COAST

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ABSTRACT

This study is to report the proximate composition of different parts, liver, skin,muscle and intestine of puffer fish Arothron stellatus as well as the screening of secondary metabolites namely alkaloids, steroids, alcoholic compounds, glycosides and amide group containing compounds from extracts of liver, skin, muscle and intestine of A.stellatus. All tissues reported in this study showed high amounts of protein in skin (21.92±0.52 mg/g), muscle (18.23±0.11 mg/g) and intestine (9.8±0.52 mg/g). At the same time liver (60.56±0.8 mg/g) contained high amount of lipid, while intestine (10.53±0.33 mg/g), muscle (1.2± 0.21 mg/g) and skin (0.51±0.07mg/g) contained lower proportion of lipid. The moisture contents were ranging between 28.91 to 78.73 mg/g. The analyzed tissue samples contained only the least amount of carbohydrates. The ash contents ranged between 0.62 to 4.05 mg/g. In secondary metabolite screening, the extracts of liver, skin, muscle and intestine showed positive test of alkaloids, alcohols and glycosides. Except the liver, all the tissues showed positive test for amide group. Steroids are completely absent in all tissues. Further purification of the extracts will provide new bioactive compounds with therapeutic potentials.

Keywords: *Arothron stellatus, Secondary metabolites, Proximate, Glycosides*

INTRODUCTION

Most of the marine organisms live in a hostile environment, having developed a well defence mechanism for their survival (Garson, 1989). The bio resources present in the marine ecosystem have potent bio molecules which includes many natural organic compounds. These organic compounds are reported to have biological activities like anti tumour, antimicrobial,analgesic etc. (Rajamanikandan et al., 2011). Marine fish are at high nutritive value. Proteins from fish have shown biological activities (Khara, 2013).

Arothron stellatus is widespread in the Indo Pacific and appears to be common. It is a giant among puffer fish reaching lengths in excess of one meter. It is common in the coast of India, where it is considered one of the three major species of puffer fish (Cole *et.al.,*2008). *A.stellatus,*also known as the stellate puffer, starry puffer or starry toad fish is a demersal marine fish belonging to the family Tetraodontidae. It secretes a violent poison, the

tetrodotoxin, which protects it from voracious predators. In order to ward off potential enemies, they can inflate their bodies by swallowing air or water. It posses toxic muscle, intestine, liver and gonads (Berry and Hassan, 1973). The liver and skin are toxic, the ovaries are extremely toxic (Nakabo, 2002). The degree of toxicity varies by species and also according to geographic area and season (Allen and Randall 1977 and Allen and Erdmann 2012).

Compared with the general fish, puffer fish meat had high protein content, low fat content and rich in essential amino acids and rich and minerals. In addition to meat, sexual gland also had high nutritional value. Testis was rich in protamine, DNA, Zn and Se and was praised as "beauty milk" in the history (Yuqui et al., 2014). *A. stellatus* is highly palatable if handled with care. It is consumed in Japan, where it retails under the name "Shoramifugu" (Yu, 2003). This species is of no economic value in Qatar (Al- Khayat and Al- Ansi, 2007). This species is not sold in the local markets, but it is dried, stretched and utilized by local fisherman (Stanley Pers- Conmm, 2014). In Thoothukudi, coast, puffers are easily found and classified as trash fish. They have no market value and not consumed by local people. But the fishermen have been exporting the properly cleaned and processed flesh of this fish to various parts of Tamil Nadu and Karnataka.

It is already reported that puffer fish might be consider as nutritious food due to its content of good amount of oil, protein, minerals etc.(Hasan, 1997). Proximate composition of banana puffe fish, *Lagocephalus lunaris* (Nurjanah *et al*., 2015), *Takifugu obscurus* (Yuqi *et al.,* 2014), and yellow puffer fish *Xenopterus naritus* (Azman *et al.,* 2015) has been reported. Uddin *et al* (2013) reported the presence of secondary metabolites in tissues of puffer fish *Tetraodon cutcutia* available in Bangaladesh. Currently, the study of the nutritional value of the puffer fish tissues has not yet completed. Due to little published information, the study was attempted to determine the proximate composition and also screen potential bioactive compounds in the form of secondary metabolites from various tissues of puffer fish *Arothron stellatus* collected from Thoothukudi Coast.

MATERIAL AND METHODS

Specimens of puffer fish *A. stellatus* were collected from fishing harbor, Thoothukudi coastal area. They were kept in cold iced box and transported to the laboratory

Proximate analysis

The specimens were dissected to remove the liver, skin, intestine and muscle. The tissue samples were dried in an electric oven between 70- 80^{o}C until the samples get constant weight and used for the estimation of protein (Lowery *et al.,* 1959), carbohydrates (Dubois *et al*.,1956), and lipids (Folch *et al*., 1957)

Screening of secondary metabolites

The tissue samples of liver, skin, muscle and intestine were cut into small pieces. 10 grams of each tissue was homogenized with 50 ml of 0.1% of acetic acid and were kept in water bath around 45^{o}C for 10 minutes, cooled and centrifuged off. Then it was stored at the deep freezer at – 20^{o}C for further analysis (Kawabata,1979). Preliminary qualitative secondary metabolites screening were carried out with the following methods

Steroids

100 mg of the extracts were dissolved in 5ml of chloroform and a few drops of concentrated sulphuric acid are added to it followed by addition of 2- 3 drops of acetic anhydride. Development of a little green colour indicates the presence of the steroid (Feigl, 1997).

Alkaloids

About 100 mg of the extracts were taken on a watch glass, then 2 ml of HCl is added and the mixture is stirred with a glass rod. Half drop of Mayer's reagent is added to the solution and formation of a brick red precipitate on the watch glass indicates the presence of alkaloid (Pal and Chakraborty, 1987).

Alcohols

100 mg of the extracts were dissolved in 0.5 ml of ceric ammonium nitrate reagent, and shaken well. Development of a yellow to red colour indicates the presence of alcoholic hydroxyl group (Vogel, 1967)

Cardiac glycosides

About 100 mg fish extract was dissolved in 1 ml glacial acetic acid containing one drop of ferric chloride solution, and this was then under layer with 1 ml of conc. H_2SO_4 .A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardenolides (Burger, 1960).

Amide Test

100 mg of the extracts were dissolved in 3 ml of 20% NaOH and the solution is boiled for 15- 20 minutes. Then the liberation of ammonia gas indicates the presence of amide group. The ammonia gas is identified by its pungent odor and also the gas turns red litmus to blue (Meyer *et al.,* 1982).

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of muscle, liver, skin and intestine of *A.stellatus* including moisture, protein, carbohydrates and ash contents are presented in Table 1.The moisture, protein, carbohydrates and ash contents of liver were 28.91mg/g, 4.1mg/g, 0.062 mg/g and 0.62 mg/g respectively. They were lower than that of muscle, skin and intestine but the lipid content in the liver was 60.56 mg/g much higher than others.The liver serve to store fat. Lipid is an energy source that is most effective when compared with carbohydrate and protein, since 1 gram of fat can produce 9 kcal. The value is higher compared to the energy produced by 1 gram of protein and carbohydrates which is 4 kcal. Fat can also be used as a source of essential fatty acids and vitamins (A, D, E , K) (Winarno, 2008). The lipid content in the muscle of *A. stellatus* was 1.21± 0.21 mg/ g higher than that of banana puffer fish (0.15± 0.03%) and *Takifugu rubripes* (0.07 %) based on the research result by Nurjanah *et al*., (2015) and Saito and Kunisaki (1998) respectively. According to Jacob *et al*., (2008), the lipid content in fish is influenced not only by the type of fish but by feeding habit, type of food, age, environment, seasons and nutrition sufficiency level.

Protein is a substance that is very important for the body. In addition to functioning as an energy source, it also serves as regulating the building substances. Protein is a source of amino acids containing the elements of C, H, O and N which are not owned by fats and carbohydrates (Winarno, 2008). The analysis results of protein showed that the highest value in the skin (21.92± 0.52 mg/g) followed by muscle (18.33± 0.11 mg/g), intestine (9.87± 0.52 mg/g) and the lowest in the liver (4.1± 0.13 mg/g). Tetradotoxin exists in puffer as a complex which joints to protein (Qin *et al.,* 2008), it results in lower protein content in the liver.

The moisture content had the highest value in muscle, skin, and intestine of *A.stellatus*. The highest moisture was in the muscle (78.73±0.52 mg/g) followed by skin (73.10±0.65 mg/g) and the lowest was liver (28.91±0.22 mg/g). The water content in food greatly affects the quality and shelf life of the food material. The higher the water content in the material, the more quickly the material quality will degenerate, which may become a source of living microorganism to grow. According to Ayas and Ozugul(2011), the differences in the moisture contents could be caused by the types, biota ages, differences in environmental conditions and the levels of the organism freshness.

Carbohydrate content showed that *A.stellatus* intestine sample had the highest value 3.98±2.10 mg/g. Maeanwhile the low carbohydrate was found in the liver (0.062±0.0025 mg/g). The muscle had carbohydrates 0.94±0.75 mg/g whereas the skin contains 0.62±0.72 mg/g. The carbohydrate in the fish meat was polysaccharide,that is glycogen. Glycogen contained in the fishery products was approximately 1% carbohydrate content in fish meat ranged from 0.05 to 0.85% glycogen, 0.038% glucose and 0.006 to 0.43% lactic acid (Adawyah, 2007).

Secondary metabolites Screening

Secondary metabolites are produced in nature and serve survival function for the organisms producing them (Demain, 2000). Large number of marine organisms produce toxins or poisons for defence mechanism or as secondary metabolites which have shown effectiveness in treating many diseases. The secondary metabolite screening and qualitative estimation of *A.stellatus* liver, skin, muscle and intestine tissue extract showed that liver extract exhibited characteristics test of alkaloids, alcohols, amides, cardiac glycosides and negative tests for steroids. Meanwhile skin, muscle and intestine extracts were assigned with positive test for alcohols, alkaloids, amides and glycosides and negative for steroids as shown in Table 2. The metabolites in the liver extract of *A stellatus* coincides with the liver extract of puffer fish *Tetradon cutcutica* from Bangaladesh (Uddin *et al*., 2013).

CONCLUSION

The present finding reveals that *A.stellatus* is a good source of proteins and lipids also contains some important bioactive compounds in the form of secondary metabolites. Further studies on the characterization of bioactive compounds from various parts of this puffer fish may provide new chemical compounds with

pharmacological activities and also provide information regarding the safe consumption of puffer fish to the public.

Table 1 Proximate composition in the tissues of puffer fish *Arothron stellatus* (mg/g Mean±SD)

Tissue	Protein	Carbohydrate	Lipid	Moisture	Ash
Skin	21.92±0.52	0.62± 0.72	0.51± 0.07	73.10± 0.65	3.1± 0.32
Muscle	18.33± 0.11	0.94± 0.75	1.2± 0.21	78.73± 0.52	4.05±1.8
Liver	4.1± 0.03	0.062± 0.003	60.56± 0.08	28.91± 0.22	0.62±0.03
Intestine	9.8± 0.52	3.98± 2.10	10.53± 0.33	70.15± 0.35	2.9± 0.52

Table 2 Secondary metabolites of the different tissue extracts of puffer fish *Arothron stellatus*

Tissue extract	Steroids	Alkaloids	Alcohols	Cardiac glycosides	Amide
Liver	-	+	+	+	-
Muscle	-	+	+	+	+
Skin	-	+	+	+	+
Intestine	-	+	+	+	+

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