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# ANTIOXIDANT POTENTIAL AND NUTRIENT CONTENT OF SELECTED SMALL INDIGENOUS SPECIES OF FISH

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## Abstract

The antioxidant level and nutrients in sixteen small indigenous fish species (SIS) of Bangladesh; eight marine and eight freshwater were measured by Thin Layer Chromatography (TLC) and 2, 2-Diphenyl 1-picrylhydrazil, 95% (DPPH) free radical scavenging method. The $IC_{50}$ determined by DPPH varied from 327.04±0.06 µg/ml to 1888.21±0.10 µg/ml of wet weight. The highest antioxidant activity was observed in *Heteropneustes fossilis* followed by *Mystus gulio*, *Hemirhamphus gaimardi*, *Mystus vittatus, Megalaspis cordyla*, *Silonia silondia*, *Colisa fasciatus*, *Amblypharyngodon mola*, *Oxygaster phulo*, *Gobioides anguillaris*, *Chela laubuca*, *Plotosus canius*, *Channa orientalis*, *Mugil cephalus*, *Coilia dussumieri* and *Tetraodon cutcutia*. The protein and lipid contents of the selected SIS ranged between 21.43 to 8.59%, and 7.22 to 1.75%, respectively. The study suggests the presence of potent antioxidant and appreciable amount of nutrients in selected fish samples. The top four potential SIS species consist of appreciable amount of protein and antioxidant are *Mystus gulio*, *Silonia silondia*, *Heteropneustes fossilis* and *Colisa fasciatus*.

Key words: antioxidant activity, proximate composition, DPPH frees radical scavenging, SIS

## Introduction

Antioxidants are one of the most important dietary functional components because they inhibit pathogenesis of various diseases such as cardiovascular disorders, diabetes, cancer, inflammation, aging and brain dysfunction. Reportedly, reactive oxygen species (ROS) have been linked to over 100 disorders [1]. Therefore, for maintaining a healthy biological system, it is critical to have the balance between oxidation and antioxidation. A variety of polyphenols, flavonoids, anthocyanins, vitamins have been reported as showing antioxidant [2, 3, 4].

Various epidemiological studies have suggested that consumption of fruits and vegetables is associated with reduced risk of cardiovascular diseases and cancer [ 5, 6, 7 ], neurodegenerative diseases such as Parkinson's and Alzheimer's diseases [8], as well as with inflammation and aging [9]. Both the hydrophilic and lipophilic antioxidants are essential to keep up physiological health of hydrophilic and lipophilic organs respectively. Besides, antioxidants are used in food industry and pharmaceuticals as additives. Widely used synthetic antioxidants are now under question due to their side effects like carcinogenicity [10]. Therefore, in response to the growing consumer concern about importance of dietary intake of antioxidants, and search for antioxidants and/or antioxidant principles from natural source have gained interest [ 10, 11, 12 ].

As a result, there is a worldwide call to develop a national food composition database. However, in Bangladesh most of the food composition tables currently used is many years back and some are taken from other country, people of which have different culture, food habit, weather etc. Moreover, information about the composition of food is necessary for nutritional education, training and research [13]. Knowing the nutritional status of fish species usually eaten by the people could contribute to remedying problems of malnourishment and obesity. Although the potential antioxidant activity of some plants and fruits [11, 14, 15, 16, 17] have been reported recently, it remains unknown in fish, one of the major sources of dietary functional components.

Fish is an essential and irreplaceable food in the rural Bangladeshi diet. The importance of fish in the diet is mainly considered for the presence of highly polyunsaturated fatty acids, especially n-3 fatty acids [18, 19, 20]. Small Indigenous fish Species (SIS), which are defined as species attaining a maximum length of 25 cm [21, 22]. In many parts of the South and Southeast Asia, including Bangladesh, a large diversity of found in open waters and closed water bodies contribute considerably to total fish intake. One of the noted advantages of SIS is that people, even the poor, can buy them in quantities they can afford. SIS is important as a source of protein and micro-nutrients such as calcium, zinc, iron and fatty acids [22] to the rural poor and also an opportunity of livelihood to a large number of fishers. The bioavailability of calcium from these small indigenous freshwater fish species is at par with that derived from milk [22].

Indigenous knowledge about these species and about their health benefits is high among rural population. For example, such species are often considered an essential part of the diet of pregnant women and lactating mothers. Such knowledge is, however, poorly documented and the antioxidant potential is not documented yet. Moreover, the knowledge of nutrient content of fishes is useful for quality control purposes in the processing industries. Therefore, this study is principally sought to assess antioxidant activity of the species concerned, and proximate composition, which indicate the desirability of the species from nutritional perspectives. Foods that are rich in protein and antioxidants are an essential component to a healthy well balanced diet.

## Materials and Methods

### Sample collection and preparation

Eight marine SIS species namely *Mystus gulio*, *Hemirhamphus gaimardi*, *Silonia silondia*, *Gobioides anguillaris*, *Coilia dussumieri*, *Mugil cephalus*, *Megalaspis cordyla*, *Chela laubuca* and eight freshwater SIS species namely *Heteropneustes fossilis*, *Amblypharyngodon mola*, *Oxygaster phulo*, *Channa orientalis*, *Mystus vittatus*, *Tetraodon cutcutia*, *Plotosus canius*, *Colisa fasciatus* were collected from different fish markets in Khulna, Bangladesh. After washing with distilled water, the muscle from collected fishes was separated from fish body by a sharp blade except *A. mola*.

Because of smaller size whole *A. mola* was used in sample preparation. Then 100-150 gram sample was homogenized in ethanol and filtered it. The filtrate was kept in the shaking water bath at 40ºc for drying. The fish extract was taken in a small vial and serial dilutions (0, 1, 50, 100, 200, 300, 400 and 500µg/ml) of fish extracts were prepared in ethanol. In this study, ascorbic acid (1, 5, 10, 50, 100 and 500µg/ml)

was used as a reference standard antioxidant. The samples were analyzed for antioxidants, moisture, protein, lipid and ash in triplicate.

**Estimation of antioxidant activity**

Initially antioxidant activity was determined by TLC method. After applying DPPH on the TLC plates, yellow or whitish color on purple background was observed in the ethanol extracts of fishes. Yellow or pale yellow color on indicated the presence of antioxidant components in the sample (Figure 1). Then, radical scavenging activity of fish extracts against stable 2,2 diphenyl 2 picryl hydrazyl hydrate (DPPH) was determined by the slightly modified method [23]. Freshly prepared DPPH solution (0.004% w/v) was taken in the test tubes, then extracts (stock solution) were added to the tubes and shaken vigorously so that the final volume was 3 ml. In the dark condition the tubes were allowed to stand for 30 min for the reaction to occur. The absorbance was determined at 517 nm using a spectrophotometer (HITACHI U-2910). First, the % inhibitions of DPPH free radical was measured [24], then % inhibitions were plotted against concentration and $IC_{50}$ was measured.

**Analysis of proximate composition**

Standard methods [25] were followed for the analysis of proximate composition.

**Statistical methods**

The results were expressed as Mean±SD. T-test was used to examine the difference between antioxidant level of freshwater and marine water species.

## Results and discussion

Antioxidants are important to protect tissues from oxidative damage caused by reactive oxygen species. Because of preference of fish in everyday diet of Bangladeshi, we have analyzed antioxidant activity and nutrients of 8 freshwater and 8 marine SIS (Table 1). In this experiment, the antioxidant level was measured *in vitro* by TLC method and DPPH free radical scavenging method. Ascorbic acid, a well-known antioxidant (Avocado Research Chemicals Ltd, Shore Road, Heysham, Lancs) was used as positive control. The antioxidant activity is expressed in terms of $IC_{50}$. The lowest $IC_{50}$ value showed highest antioxidant activity and the % inhibition increase with the increase of concentration. The IC50 of Heteropneustes fossilis and *Mystus gulio* were the lowest among freshwater and marine species of SIS respectively followed in ascending order as *Mystus vittatus*, *Colisa fasciatus*, *Amblypharyngodon mola*, *Oxygaster phulo*, *Plotosus canius*, *Channa orientalis*, *Tetraodon cutcutia* among freshwater species and *Hemirhamphus gaimardi*, *Megalaspis cordyla*, *Silonia silondia*, *Gobioides anguillaris*, *Chela laubuca*, *Mugil cephalus*, *Coilia dussumieri* among marine water species. However, no significant difference of antioxidant between fresh water and marine water species was observed ($P>0.05$). The result is contrast to previous study conducted by [26], which reported that antioxidant capacities in the seawater-adapted trout are higher than freshwater trout. This could be attributed due to highly diversification of fish. Of the 8 freshwater SIS *Heteropneustes fossilis*, *Mystus vittatus* and *Colisa fasciatus* showed higher antioxidant activity and their $IC_{50}$ value were 327.04 µg/ml, 405.02 µg/ml and 579.23 µg/ml respectively. The $IC_{50}$ values of 4 marine SIS species showing higher antioxidant activity were *M. gulio*, 364.18 µg/ml; *H. gaimardi*, 398.41 µg/ml; *M. cordyla*, 481.04 µg/ml and *S. silondia*, 489.20 µg/ml. This radical scavenging activity is lower than plants, fruits and essential oils [11, 14, 17, 27, 28, 29 ]. In the present study, we used only muscle of fish except *A. mola*. However, antioxidant activity varies in different edible parts of fishes such as muscle, liver and skin [30]. Nonetheless, this is the first ever study for probing such species for presence of antioxidants on SIS.

The species employed in the study were also tested for their proximate composition, e.g. moisture, protein, lipid and ash. The proximate composition of the species is within the acceptable limit for fish. Fish muscle contains 6-28% protein, 0.1-67% lipid and 28-96% water [31]. The moisture content of the analyzed SIS ranged 66.52% to 82.28%. The highest and lowest moisture content were recorded in *O. phulo* and *M. gulio* respectively. In this study, protein, the important constituent of fish was found to vary from 21.43% (*M. gulio*) to 8.59% (*O. phulo*). Other SIS species showing higher protein were *Silonia silondia* (19.20%), *Colisa fasciatus* (18.91%), *Mugil cephalus* (18.38%), *Plotosus canius* (18.18%) and *Tetraodon cutcutia* (17.21%). The analyzed protein content is quite higher than ripe tropical fruits although tropical fruits are reported to content high level of antioxidant. In general, protein content of different fruits is not greater than 3.5% [13, 32].

The carbohydrate present in the analyzed fish ranges between 1.75 % (P. canius) to 7.22 % ( M.

*cordyla*). Carbohydrate of fish is less concentrated than cereals because of their high water and protein content. In ripe Bangladeshi fruits carbohydrate ranges between 3.23 % to 11.94 % [13]. The ash content ranged from 2.14% to 6.67%, the maximum amounts were observed in *C. fasciatus* while minimum in *H. gaimardi*. The highest and lowest lipid content was analyzed in *M. cordyla*, 7.22% and *P. canius,* 1.75% respectively. In fishes from Indian waters, 30-50% of the total fatty acids are PUFAs [33] which could be a reason of reduced mortality of people consuming fish from coronary heart diseases [6, 34, 35].

Considering the nutritional roles of antioxidants and nutrients especially protein in disease protection and remedying malnourishment, *Mystus gulio*, *Silonia silondia* and *Heteropneustes fossilis*, *Colisa fasciatus* were the top SIS species among analyzed marine water and freshwater species respectively. Since antioxidants are believed to inhibit the formation of free radicals by arresting them, thereby protecting the cells from being damaged and the species employed being the high protein content, easily available and low cost ones; they obviously occupy an important place in nutrition supplement to the diets of the average people of the country.

The top four potential SIS species consist of appreciable amount of protein and antioxidant are *Mystus gulio*, *Silonia silondia*, *Heteropneustes fossilis* and *Colisa fasciatus*. Therefore, this low cost available SIS of Bangladesh could be an excellent source of nutrient and antioxidant thus provides us medicinal values. Studies on the specific type of antioxidants in the fish species concerned can be done in future.

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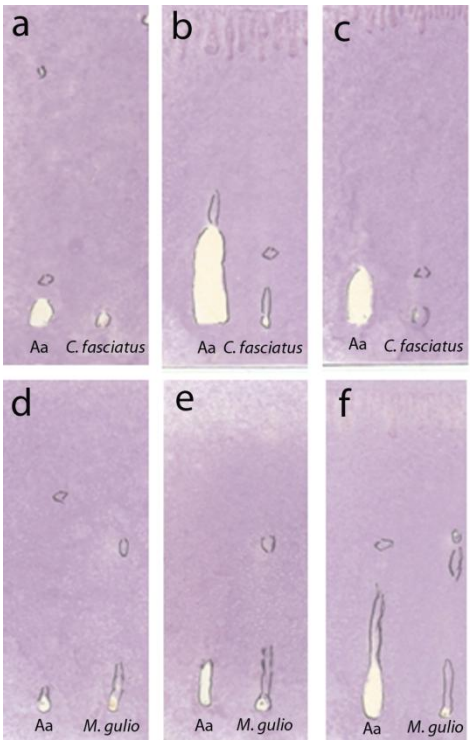


**Figure 1:** TLC plates of freshwater, *Colisa fasciatus* (a-c) and marine water, *Mystus gulio* (d-f) fishes in polar and non-polar solution. a, d: non polar solution (n-Hexane: Acetone=2:1); b, e: medium polar solution ($CHCl_3$:$CH_2OH$=5:1); c, f: polar solution ($CHCl_3$:$CH_2OH$:$H_20$ =40:10:1); Aa: Ascorbic acid.

| Scientific name | Local name | Common name | Moisture (%) | Protein (%) | Lipid (%) | Ash (%) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|---|---|
| **Freshwater species** | | | | | | | |
| ***Amblypharyngodon mola*** | Mola | Mola carplet | 77.51±0.59 | 12.77±0.46 | 4.40±0.49 | 3.08±0.11 | 609.40±0.01 |
| ***Channa orientalis*** | Cheng | Walking snakehead | 72.09±0.57 | 16.86±0.58 | 3.91±0.58 | 5.09±0.57 | 1106.50±0.07 |
| ***Colisa fasciatus*** | Khalisa | Banded gourami | 68.56±0.59 | 18.91±0.68 | 4.03±0.33 | 6.67±0.48 | 579.23±0.23 |
| ***Heteropneustes fossilis*** | Shing | Stinging catfish | 72.53±0.66 | 16.33±0.66 | 5.03±0.12 | 4.18±0.30 | 327.04±0.06 |
| ***Mystus vittatus*** | Ayre | Long-whiskered catfish | 75.61±0.54 | 13.59±0.54 | 3.88±0.38 | 4.51±0.57 | 405.02±0.09 |
| ***Oxygaster phulo*** | Chela | Finescale razorbelly minnow | 82.28±0.59 | 8.59±0.55 | 2.30±0.59 | 3.64±0.22 | 750.19±0.17 |
| ***Plotosus canius*** | Kain magur | Gray eel-catfish | 76.35±0.53 | 18.18±0.58 | 1.75±0.59 | 2.91±0.43 | 1029.17±0.21 |
| ***Tetraodon cutcutia*** | Potka | Ocellated pufferfish | 73.78±0.58 | 17.21±0.22 | 4.07±0.17 | 3.51±0.46 | 1888.21±0.10 |
| **Marine species** | | | | | | | |
| ***Chela laubuca*** | Chela | Finescale razorbelly minnow | 74.65±0.48 | 16.43±0.92 | 3.01±0.03 | 3.04±0.13 | 930.47±0.02 |
| ***Coilia dussumieri*** | Amadi | Goldspotted anchovy | 75.13±0.25 | 11.97±0.09 | 3.06±0.25 | 6.09±0.19 | 1161.00.±0.17 |
| ***Gobioides anguillaris*** | Shagor bele | Eel worm goby | 74.26±0.25 | 15.10±0.98 | 1.87±0.02 | 3.86±0.05 | 786.20±0.15 |
| ***Hemirhamphus gaimardi*** | Ekthuta | Quoy's garfish | 75.09±0.24 | 17.16±0.27 | 2.14±0.05 | 2.14±0.17 | 398.41±0.08 |
| ***Megalaspis cordyla*** | Kawa | Torpedo scad | 68.96±0.81 | 17.91±0.96 | 7.22±0.26 | 3.18±0.44 | 481.04±0.07 |
| ***Mugil cephalus*** | Parshe | Flathead grey mullet | 69.38±0.74 | 18.38±0.78 | 4.20±0.42 | 6.31±0.51 | 1152.15±0.07 |
| ***Mystus gulio*** | Nuna tengra | Long whiskers catfish | 64.52±0.98 | 21.43±0.64 | 5.62±0.68 | 4.42±0.39 | 364.18±0.05 |
| ***Silonia silondia*** | Shilong | Silond catfish | 68.06±0.37 | 19.20±0.50 | 6.18±0.43 | 4.32±0.56 | 489.20±0.11 |
| **Ascorbic acid (control)** | | | | | | | 22.98±0.03 |

**Table 1:** Antioxidant activity and proximate composition of freshwater and marine SIS.