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# Antioxidant Potential and Nutrient Content in Selected Fish Species of Different Feeding Habits in Bangladesh

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## ABSTRACT

This study reports the antioxidant potential and chemical composition of muscle from 30 fish species of different feeding habits namely carnivore, herbivore and omnivore. Different *in vitro* assays used for determining antioxidant potential of extracts of fish species were: thin layer chromatography (TLC) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity. No significant difference of antioxidant activity or nutrient content was observed among carnivorous, herbivorous and omnivorous fishes. The $IC_{50}$ measured by DPPH method varied from 154 to 2139 µg/ml of wet weight. The lowest $IC_{50}$ value was observed in *C. idella*followed by *P.ticto, C. striatus, C. punctatus, L. rohita, A. testudineus, M. cordyla, O. mossambicus and G. giuris.* Themoisture content of samples ranged from 62 - 85%. The protein content washigh in *C. idella* (24.3%) and low in *L. calbasu* (10.57 %). The lipid and ash content analyzed in the selected fish species ranged from 1.17-7.94% and 1.31-4.80%, respectively. Overall, the results suggestthat fish of different feeding habit can be exploited for their antioxidant and nutrients components and used for consumption as well as value addition in food formulations.

**Keywords:** Antioxidant activity, DPPH free radical scavenging, proximate composition, herbivore, carnivore, omnivore

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## INTRODUCTION

Antioxidant means 'against oxidation'. An antioxidant is any substance that retards or prevents deterioration, damage or destruction by oxidation[1].Antioxidant also possesses the ability to protect the body from damages caused by free radicals[2]. Several studies have been established that antioxidants inhibit diseases such as cerebrovascular disease, cancer, arteriosclerosis, heart disease, senility, aging, behcet's disease, crohn's disease, cataracts, sunburn, ulcers, osteoporosis, rheumatoid arthritis, diabetes mellitus, emphysema, stroke, rheumatoid arthritis, hemorrhagic shock, cardiovascular disorders, cystic fibrosis, neurodegenerative diseases (e.g. parkinsonism, Alzheimer's disease), gastrointestinal ulcerogenesis, AIDS and even early senescence[3]. Natural products with antioxidant activity may be used to reduce oxidative damage in human body[4]. Nutrients that have antioxidant and anti-inflammatory activity have been shown to reduce the risk of several forms of cancer. Some of such antioxidants including glutathione, ubiquinol, and uric acid are produced during normal metabolism in the body[5]. Other lighter antioxidants are found in the diet. Although there are several enzymatic systems within the body that scavenge free radicals, body cannot manufacture some micronutrients that must be supplied in the diet. Of them, the principle micronutrient antioxidants are vitamin E (α-tocopherol), vitamin C (ascorbic acid), and β-carotene[6]. As a result, attempts have been made to study the antioxidant potential of a wide variety of vegetables and fruits in abroad and Bangladesh[7-13]. Strong antioxidant activities have been found in berries, cherries, citrus, prunes, pomelo, and olives. Unlike vegetables and plants, the study of antioxidant on fish is scanty. Previously few studies on the antioxidant activity have been reported in fish species[14, 15]. In addition, lipophilic antioxidants potential has been reported particularly in the marine fish species[16, 17]. However, fish is important in diet because of a source of protein and polyunsaturated fatty acids. Omega-3 fatty acids and natural triglycerides from fatty fish have anti-inflammatory and antioxidant activity[18]. Since fish of different feeding habit is an irreplaceable food item in Bangladesh have been cultured, experimental strategies have been designed for the screening and identification of antioxidative compounds from fish species of different feeding habit in Bangladesh. In the present study, antioxidant and proximate composition were identified from the muscle of 30 fish species including each 10 species from carnivore, herbivore and omnivore in a sense that the antioxidative compounds and proximate composition of fishfrom different feeding habit could be different. Herbivores are those animals that feed principally on vegetable matter, like leaves, fruit, roots, tubers, flower, nectar, etc[19]. Carnivores are those animals that feed principally on the meat of other animals, like the muscles, bones, or

internal organs. And, omnivores feed on both vegetation and meat, which includes all kinds of plants, fruits, animal or insect they can find and catch.*C. idella*, *P.ticto*, C.*striatus*, C.*punctatus*, *L.rohita* and *A. testudineus*showed lower $IC_{50}$ among all species.

## MATERIALS AND METHODS

### Sample collection and preparation

On the basis of different habits, 30 fishes classified into three groups as herbivore, carnivore and omnivore (Table 1) were purchased 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 g fish sample was homogenized in ethanol and filtered it. The filtrate was kept in the shaking water bath at 40 $^{o}$c for drying. The fish extract and ascorbic were taken in a small vial and serial dilutions (0, 1, 50, 100, 200, 300, 400 and 500 µg/ml)were prepared in ethanoland 0.004% DPPH were added to measure free radical scavenging activity. In this study, ascorbic acid 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 (Figure 1). Yellow or pale yellow color indicated the presence of antioxidant components in the sample. Then, radical scavenging activity of fish extracts against stable DPPH was determined by the slightly modified method explained by Brand-Willliamset al.[20]. 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 would be 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[21], then % inhibitions were plotted against concentration and the inhibitory conc. 50% ($IC_{50}$)was measured.

### Analysis of proximate composition

Moisture:Moisture was determined by complete drying of the sample at $105^{0}$ C in an oven[22]. The percentage of the moisture content was calculated by the following equation.

% Moisture= (Weight of the sample – Weight of the dried sample) × 100 / Weight of the sample.

Protein:Protein content of fish samples was determined by micro kjeldahl method. After

competition of digestion, distillation and titrationof the samples finally the percentage of gross portentous nitrogen was calculated out with the following formula[23].

% N= Volume of HCL ×Normality of HCL× 0.014 / Weight of Sample (gm).

Percentage of protein= % N ×6.25 (Conversion factor)

Lipid:Most methods of measuring lipid content depend on extracting the lipid by dissolving it in a suitable solvent. Lipid content of samples was determined by following Bligh & Dryer method[24].The percentage of the lipid content was calculated by equation.

% Lipid= (Weight of lipid / Weight of sample) × 100

Ash:The ash content of a sample is the inorganic residue left after complete removal of the organic residue by muffling at about 550 $^0$C to 600 $^0$C in a muffle furnace for 6-8 hours till the residue become white. Finally the percentage of ash content was calculated by the following formula.

% Ash = (Weight of ash / Weight of the sample) × 100

**Statistical methods**

The results were expressed as mean±SD. T-test was used to examine the difference between antioxidant levels and proximate composition of fish samples.

## RESULTS AND DISCUSSION

Human being requires a diet which is balanced in nutrients e.g. polyunsaturated fatty acids (PUFAs), protein and minerals, and high in antioxidants. Fish is well known as a source of protein and PUFA. Since fish are preferred in diet of Bangladeshi and different types of fish are available, 30 fish species of different feeding habits, namely herbivore, carnivore and omnivore were opted for the analysis of antioxidants and proximate composition (Table 1).

**Table 1: Antioxidant activity and proximate composition of herbivore, carnivore and omnivore fish species**

| Scientific name | Local name | Common name | Moisture (%) | Protein (%) | Lipid (%) | Ash (%) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|---|---|
| **Herbivore** | | | | | | | |
| *Ctenopharyhngodonidella* | Grass carp | Grass carp | 62.07±0.70 | 24.30±0.41 | 7.94±0.18 | 4.80±0.03 | 154±0.02 |
| *Hypophthalmichthysmolitrix* | Silver carp | Silver carp | 78.01±0.11 | 16.01±0.30 | 2.83±0.61 | 2.56±0.11 | 963±0.24 |
| *Labeobata* | Bata | Bata | 74.51±0.39 | 21.24±0.62 | 2.06±0.03 | 2.31±0.06 | 966±0.34 |
| *Puntiusgonionotus* | Thai Shorputi | Silver barb | 73.05±0.37 | 20.21±0.46 | 4.25±0.37 | 2.73±0.14 | 1679±0.18 |
| *Puntiusticto* | Titputi | Ticto barb | 79.2±0.20 | 17.41±0.01 | 1.49±0.07 | 1.37±0.13 | 268±0.28 |
| *Labeocalbasu* | Kalbasu | Orangefinlabeo | 80.05±0.19 | 10.57±0.06 | 3.59±0.06 | 3.80±0.49 | 982±0.07 |
| *Labeorohita* | Rui | Rohulabeo | 73.5±0.07 | 17.85±0.02 | 3.56±0.08 | 2.80±0.26 | 397±0.02 |
| *Tenualosailisha* | Ilish | Hilsa shad | 81.26±0.23 | 12.42±0.27 | 1.17±0.06 | 3.78±0.82 | 1011±0.05 |
| *Pomadasys hasta* | Sadadatina | Silver grunt | 76.09±0.49 | 18.45±0.31 | 3.54±0.17 | 1.87±0.47 | 588±0.06 |
| *Amblypharyngodonmola* | Mola | Molacarplet | 68.45±0.21 | 18.90±0.31 | 7.50±0.10 | 3.69±0.09 | 520±0.09 |
| **Carnivore** | | | | | | | |
| *Channapunctatus* | Taki | Spotted snakehead | 74.78±0.09 | 18.43±0.51 | 4.57±0.41 | 1.59±0.61 | 346±0.04 |
| *Channastriatus* | Shol | Striped snakehead | 69.71±0.32 | 21.30±0.24 | 3.47±0.13 | 2.20±0.17 | 328±0.01 |
| *Mystusaor* | Ayre | Long- whiskered catfish | 72.45±0.25 | 20.12±0.08 | 5.21±0.05 | 1.98±0.58 | 1170±0.12 |
| *Notopteruschitala* | Chital | Clown knifefish | 74.69±0.41 | 15.42±0.23 | 5.68±0.07 | 3.27±0.39 | 2139±0.21 |
| *Notopterusnotopterus* | Foli | Bronze featherback | 78.07±0.01 | 18.32±0.70 | 2.66±0.18 | 1.31±0.04 | 1757±0.15 |
| *Harpadonnehereus* | Loyta | Bombay-duck | 79.76±0.22 | 13.76±0.42 | 4.56±0.13 | 2.03±0.08 | 829±0.09 |
| *Latescalcarifer* | Vetki | Brramundi | 85.43±0.04 | 11.64±0.03 | 1.32±0.10 | 1.52±0.14 | 1482±0.05 |
| *Megalaspiscordyla* | Kawa | Torpedo scad | 74.87±0.50 | 17.54±0.13 | 5.39±0.08 | 2.19±0.51 | 408±0.03 |
| *Polynemusparadiseus* | Taposhi | Paradise threadfin | 73.97±0.40 | 15.54±0.32 | 5.43±0.19 | 3.23±0.52 | 1111±0.07 |
| *Trichiurushaumela* | Churi | Largeheadhairtail | 68.21±0.12 | 23.26±0.33 | 5.87±0.19 | 2.39±0.44 | 949±0.02 |
| **Omnivore** | | | | | | | |
| *Anabas testudineus* | Koi | Climbing perch | 76.59±0.10 | 18.25±0.19 | 1.30±0.06 | 2.32±0.07 | 398±0.11 |
| *Mackerel sp* | Konkon | Konkon | 77.24±0.41 | 13.70±0.52 | 2.69±0.43 | 4.01±0.05 | 531±0.06 |
| *Pampuschinensis* | Rupchanda | Chinese silver pomfret | 77.15±0.45 | 14.30±0.04 | 3.45±0.28 | 3.10±0.02 | 600±0.08 |
| *Rhinomugilcorsula* | Khorsula | Khorsula | 75.98±0.31 | 13.67±0.25 | 6.87±0.43 | 3.12±0.12 | 1266±0.09 |
| *Scatophagusurgus* | Bishtara | Spotted scat | 78.34±0.51 | 17.98±0.42 | 2.17±0.27 | 1.90±0.47 | 872±0.02 |
| *Catlacatla* | Catla | Catla | 76.8±0.42 | 12.87±0.25 | 6.87±0.03 | 2.79±0.43 | 538±0.03 |
| *Cyprinuscarpio* | Common carp | Common carp | 68.6±0.17 | 22.34±0.18 | 4.06±0.51 | 4.23±0.43 | 824±0.17 |
| *Glossogobiusgiuris* | Balia | Tank goby | 70.2±0.23 | 21.37±0.05 | 5.91±0.17 | 1.30±0.57 | 492±0.19 |
| *Oreochromismossambicus* | Tilapia | Mozambique tilapia | 71.95±0.16 | 15.85±0.05 | 6.94±0.23 | 4.26±0.7 | 411±0.07 |
| *Oreochromisniloticus* | Nilotica | Nile tilapia | 79.12±0.14 | 11.45±0.37 | 5.32±0.17 | 2.52±0.28 | 1028±0.05 |

Because of varying antioxidant level in different edible parts of fishes such as muscle, liver and skin[15, 25], we used only muscle of fish except *A. mola*. Antioxidant activity was determined initially by observing yellow or pale yellow color on TLC plate (Figure 1), and then measured by DPPH assay[26]. The antioxidant level was measured by $LC_{50}$ and ascorbic acid was used as positive control. Antioxidants of the recorded fish samples measured by $IC_{50}$ varied from 154 to 2139 µg/ml of wet weight.

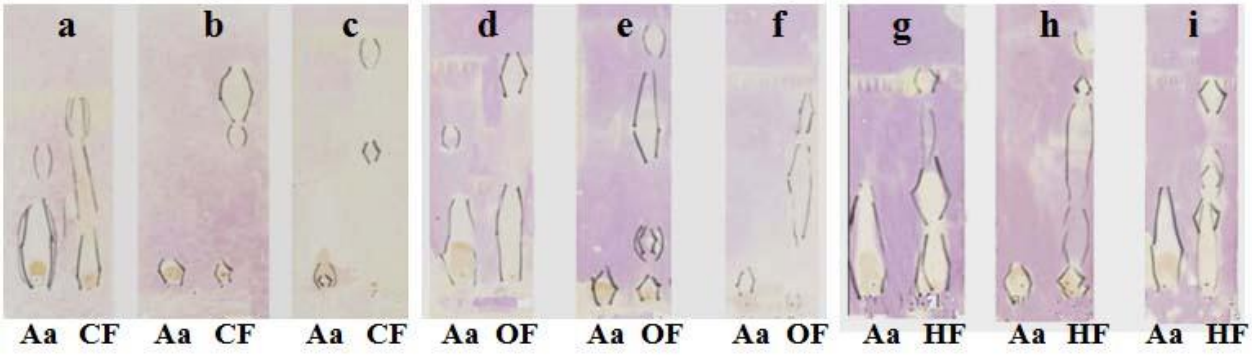


**Figure 1: TLC plates of Carnivore, *Channastriatus* (a-c), omnivore, *Anabas testudineus* (d-f) and Herbivore, *Ctenopharyhngodonidella*(g-i) fishes in polar and non-polar solution. A, d, g: non polar solution (n-Hexane: Acetone=2:1); b, e, h: medium polar solution ($CHCl_3$:$CH_2OH$=5:1); c, f, i: polar solution ($CHCl_3$:$CH_2OH$:$H_20$ =40:10:1); Aa: ascorbic acid; CF: carnivorous fish; OF: omnivorous fish and HF: herbivorous fish.**

The lowest value was observed in *C. idella* followed by *P.ticto, C. striatus, C. punctatus, L. rohita, A. testudineus, M. cordyla, O. mossambicus, G. giuris, A. mola, M. sp, C. catla, P. hasta, P. chinensis, C. carpio, H. nehereus, S. urgus, T. haumela, H. molitrix, L. bata, L. calbasu, T. ilisha, O. niloticus, P. paradiseus, M. aor, R. corsula, L. calcarifer, P.gonionotus, N. notopterus* and *N. chitala*. Although herbivore fishes are supposed to contain high antioxidant component because of their feeding habit as they consume mainly plants, no significant difference of antioxidant level was found among the fish species of different feeding habit. This could be attributed due to highly diversification of feed ingredients. There are differences in total antioxidants in plankton[27] and dietary plants[28]. $IC_{50}$ value of herbivore, carnivore and omnivore fishes ranged 154–1679, 328–2139 and 398–1266 µg/ml, respectively. Considerably higher antioxidant activity (low $IC_{50}$ level) was observed in three herbivores, two carnivores and only one omnivore fishes. e.g. *C. idella (*154µg/ml), *P.ticto* (268 µg/ml), C.*striatus*(328 µg/ml), C.*punctatus* (346 µg/ml), *L.rohita* (397 µg/ml) and *A. testudineus* (398 µg/ml), while the $IC_{50}$ of ascorbic acid used as a positive control was estimated as 16 µg/ml (Figure 2 and 3).

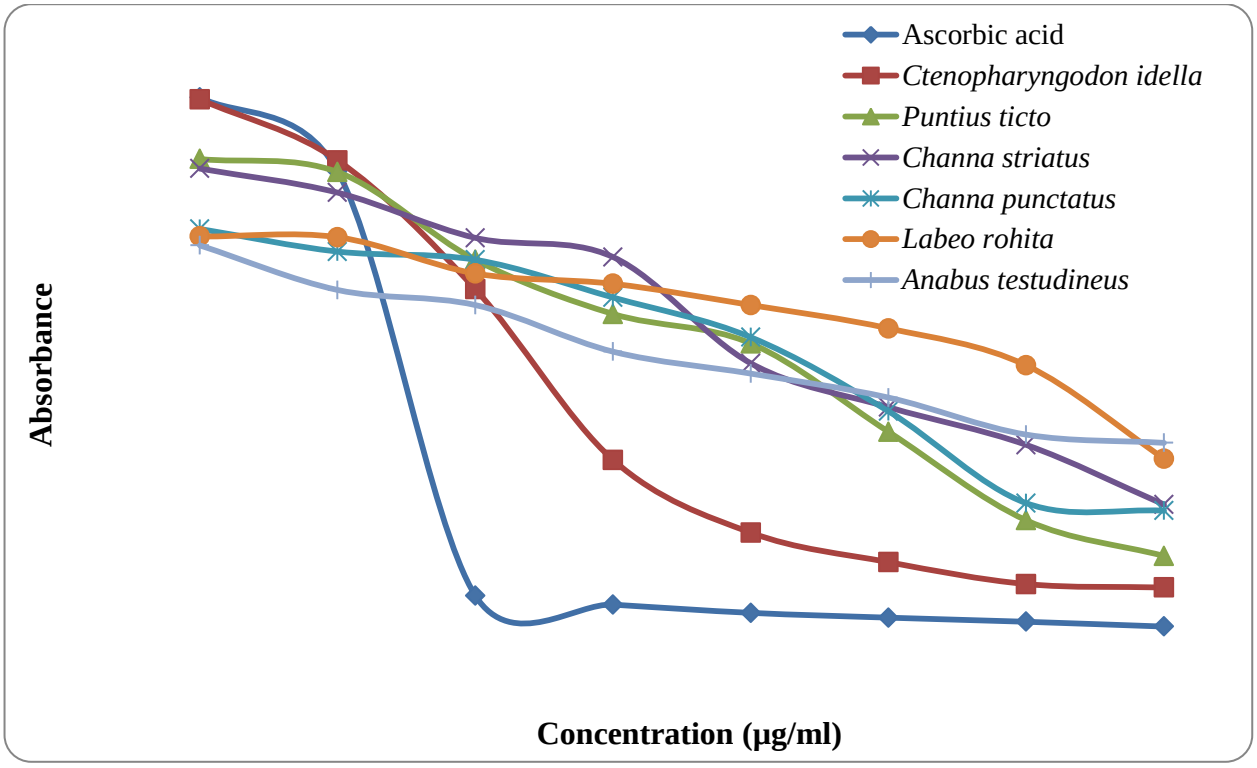


**Figure 2: DPPH radical scavenging activity of fish extracts from carnivore, herbivore and omnivore. Different concentration of 0-500 µg/ml was used for the assay. Ethanol extracts and they are compared with standard ascorbic acid.**

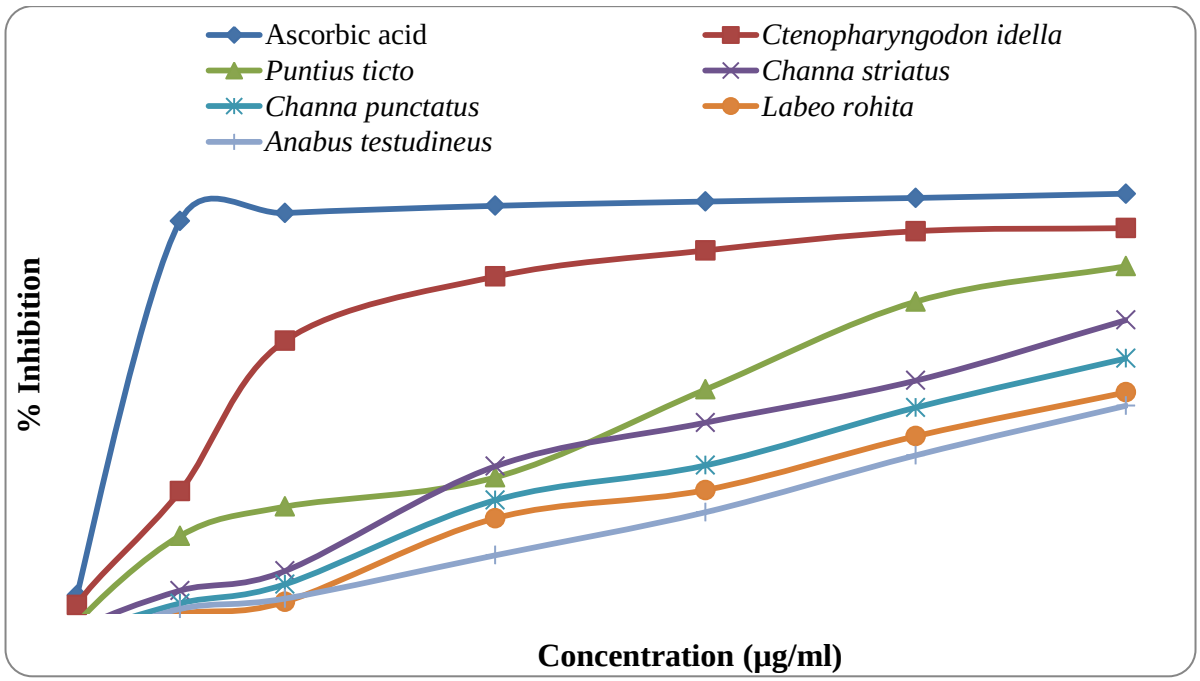


**Figure 3: Comparison of % inhibition for DPPH scavenging activity between ethanol extracts of different feeding habit fish and ascorbic acid. Different concentration of 0-500 µg/ml was used for the assay.**

These findings compose *C. idella*, *P.ticto*, C.*striatus*, C.*punctatus*, *L.rohita* and *A. testudineus* to be a good source of antioxidant. *L. rohita* and *A. testudineus* were also identified as two of the three potential Malaysian freshwater fish species with high antioxidant activities[15].

The fish species analyzed in the study were also tested for their proximate composition with a view to measure moisture, protein, lipid, ash content and find out the top fish species for diet, which could be suggested for culture. The highest and lowest moisture content were observed in *L.calcarifer* (85.43%) and *C.striatus* (69.71%) respectively. The protein, lipid and ash content of the potential six species having higher antioxidants varied from 17.41 to 24.30%, 1.30 to 7.94% and 1.37 to 4.80% respectively. The overall protein, lipid and ash contents ranged 10.57-24.30%, 1.17-7.94% and 1.30-4.80% respectively, which are within the acceptable limit. Fish muscle contains 6-28% protein, 0.1-67% lipid and 28-96% water[29]. The analyzed protein content is quite higher than ripe tropical fruits although tropical fruits are reported to content high level of antioxidant[30, 31]. And, carbohydrate of fish is less concentrated than cereals because of their high water and protein content. In ripe Bangladeshi fruits carbohydrate ranges from 3.23% to 11.94%[30]. In addition, 27-50% of the total fatty acids from fishes of Indian waters contain are PUFAs[32-34], which could be a reason of reduced mortality of people consuming fish from coronary heart diseases[35-35]. The species showing higher protein content were *C.idella*(24.30%), *T.haumela* (23.26%), *C.carpio*(22.34%), *G.giuris*(21.37%), *C.striatus*(21.30%), *L.bata*(21.24%), *P. gonionotus*(20.21%) and *M. aor*(20.12%). Among these species, *C. idella* contains the highest level of lipid and ash content. Another species, *C. striatu*s contains 3.47% lipid and 2.20% ash. Considering the proximate composition and antioxidant level, the top fish species for diet is*C. idella* and *C. striatus* which contains considerable amount of protein, lipid ash and antioxidant level.

## CONCLUSION

This study focused on the fish antioxidant activity and proximate composition of herbivore, carnivore and omnivore fish species. The top six potential Bangladeshi fish species having outstanding antioxidant level are *C. idella*, *P.ticto*, C.*striatus*, C.*punctatus*, *L.rohita* and *A. testudineus.* These fish species can be recommended as part of daily diet. *C. idella*and *C. striatus*has the higher nutrient profile as well as considerable amount of antioxidant. The fish extracts can also be used as an alternative source of natural antioxidants to replace synthetic antioxidants as they may be able to protect the human body from free radicals and retard the progress of many chronic diseases. Studies on the specific type of antioxidants in the specific part

of fish concerned can be done in future.

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