

First Report of Seaweeds from Kuakata Coastline of Bangladesh with Morphology, Chlorophyll Contents and Biochemical Analysis

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Abstract: Seaweeds are very common marine food sources in many Asian countries for consuming and trading due to their nutritional values. Bangladesh has almost 710 km long coastline, although seaweeds exploration from this area is not completed yet. Kuakata coastline is located in southeastern part of this country with great economic importance; however, there have not been found any previous report on seaweeds. This study identified two intertidal seaweeds from this area and performed analysis in their morphological and chemical aspects. The seaweed *Enteromorpha compressa* has morphologically larger, and multicellular unbranched thallus of 4 cm long. It has comparatively lower amounts of total chlorophyll (1.80 mg/g), and higher level of carbohydrates (25.56 %) and moisture contents (9.46 %). Another one, *Ulothrix flacca* is unbranched filamentous algae with highest amounts of total chlorophyll (2.189mg/g), proteins (11.76 %) and lipids (5.52 %). This is the first report on seaweeds of Kuakata coastline, and first report on *Ulothrix flacca* from Bangladesh. The results will be helpful in food science, ecology and evolutionary biology of seaweeds.

Keywords: Macroalgae, Proline extraction, Carbohydrate contents, *Ulothrix flacca*, Intertidal algae.

Marine macroalgae, also called seaweeds, grow normally in the estuaries and shallow waters of sea. These organisms have been support other marine organisms as primary food and oxygen providers. For example, from ancient times they have been used as fodder for animal, fertilizer for farmland, cosmetics and source of drugs for human being. Certain species of macroalgae have been widely consumed as essential human foods in many Asian countries, because they contain considerable amounts of metabolites with nutrients value (1-3). Besides, algae

based lipids are the major dietary components for primary consumers and many marine invertebrates rely on these photosynthetic entity for maintaining their growth, survival and reproductive success (4). Thus they play very vital roles as fundamental constituents of marine ecosystems occupying the base of food chain in ocean.

Chlorophyll a and chlorophyll b, both are considered as major components of photosystems, and regarded as an extremely important biomolecule in photosynthetic organisms. They imply significant roles in the physiology, metabolism, productivity and overall economy of any photosynthetic organisms. Like other plants, its quantity is a good indicator of photosynthetic capacity with health benefits of seaweeds (5). However, seaweeds and other photosynthetic micro-algae are facing various climatic situations which may alter their productivity, community composition and morphological changes according to recent reports (6,7). In that case, study of an organic acid proline (Pro) would be a good signal indicator of stress adaptive responses in seaweeds, like other plants (8). Pro accumulation in plants already has been reported. Researchers emphasized that due to different abiotic and biotic stresses, plants produced proline in their cells (9-11). Moreover, seaweeds nutrient contents can vary with species to species, geography and seasonal variations, variations in water physicochemical properties, and these were evaluated by chemical compositions analysis (12-14). Even, many seaweeds show great variations in their morphological aspects in different period of life cycle, which makes very tough to distinguish between them in general (15,16).

Bangladesh has a coastline of about 710 km, including Kuakata (23.56 km), located in southeastern part with the second largest sea beach after Cox's Bazar. This coastline belongs to Barishal Division and stand as a part of Bay of Bengal, which supports a large number of marine flora and fauna, thus makes it as an integral part of



the marine biodiversity. Moreover, it is reported that 221 species of seaweeds are commercially being utilized globally and there are 42 countries who directly exploit seaweeds for economic purposes (17). Unfortunately, Bangladesh is not listed among the countries, although it has a huge opportunities. However, research information on algae from this area, even from Barishal Division is lack, except few earlier reports on microalgae (18-20).

Despite the scopes and areas of seaweed utilization is increasing globally, there is still lack of research reports concerning the distribution, total number of seaweed species, either native or invasive, along with their commercial importance in Bangladesh (21,22). Very few reports found on seaweed utilization in this country by Mog and Rakhyine tribal community as well as some people of St. Martin's Island (21). Therefore, the present study aimed to reveal the two intertidal seaweeds species from Kuakata coastline of Bangladesh along with their current morphology, chlorophyll contents and chemical composition.

MATERIALS AND METHODS

Plant Materials

Two intertidal seaweeds *Enteromorpha compressa* (L.) Greville and *Ulothrix flacca* (Dillwyn) Thuret were collected from several rocky shores of Kuakata coastline of Bangladesh during October 2020, and identified following authentic literature (23-25). Collected samples were rinsed with fresh water before moving to the laboratory and analyzed within few hours after each collection.

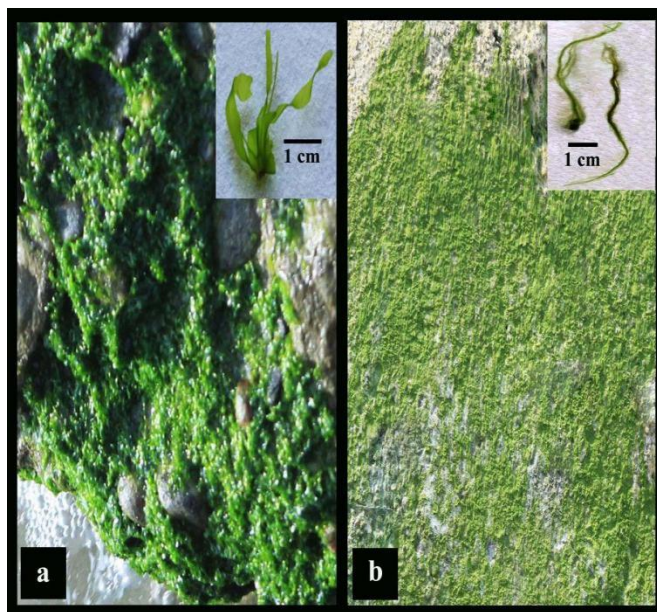


Fig.1. Two naturally growing seaweeds of Kuakata coasts with their habitats and close view of smaller

portion (Inset). (a) *Enteromorpha compressa*, (b) *Ulothrix flacca*.

Growth and Cell Morphology Study

Habitats and habits were examined by naked eyes and magnifying glass. Thallus, cell structure, size and shape was observed under light microscope (Olympus, CH30). Pictures were recorded with CMEX-18PRO (Euromex, Holland) using the ImageFocus software program. For confirmation of the obtained results more than 50 times repetitions were done.

Extraction Procedures

Chlorophylls, proline, lipid, protein, carbohydrate and moisture contents were extracted for analysis. Chlorophyll contents were extracted by 80% acetone and measured following standard methods (26,27). Proline (Pro) were extracted by the reagent 3% aqueous sulfosalicylic acid, and estimated according to Bates et al. (28). Then, chloroform and methanol as a mixture (1:2) was used for lipid extraction and measurement. Proteins were quantified according to Kruger protocols where bovine serum albumin was used as standard (29). Before estimating carbohydrates, collected samples were make oven dried (at 60 °C) till a constant weight, and then converted the dried materials into fine powder, and determined by modified phenol-sulfuric acid colorimetric method (30). Finally, 2 gm of each sample was heated up (at 105 °C) indirectly, until the moisture content was totally removed, and calculated in a moisture analyzer. The units of chlorophyll contents and proline contents were presented as mg/g of fresh sample. Units of lipids, proteins, carbohydrates and moisture contents were presented as % of dry weight (DW).

Data Analysis

All extractions were performed in triplicates and the obtained data were processed by MS Excel 2010 software program. All statistical works were done by JMP software program.

RESULTS AND DISCUSSION

Morphology Studies

In case of *Enteromorpha compressa*, this research noted the upper region of intertidal zone as its common habitat in the studied area, and attachment was mostly in rocks using disk-like holdfast. The observed holdfast of the species was made up of several rhizoids. The thallus was flattened, unbranched and yellowish-green colored. Generally, the thallus was narrower in the base, and gradually widens towards the center. From each holdfast, up to 4.5 cm thalli were emerged which were soft in nature and easy to break from attached habitats.

Sometimes they grew spirally along the main axes and less to moderate dense upon the habitats. Each thallus was usually shorter and looking like a simple leaf of 4 cm long and 2.5 mm breadth ((Fig. 1a, 2a). Cells arrangements were looking single layered, and irregular in shape. For example, rectangular, oval to oblongular were observed under microscope. Cell size was also variable, 10 to 20 μm in length and 3 to 10 μm breadth (Fig. 2b). Inter cellular space was almost 5 to 10 μm . The microscopic view revealed that, thallus cells were arranged in rows; however, the arrangement was almost circular in center area. Each cell had green protoplast consist of single to two pyrenoids. Normally pyrenoids were located in the marginal area of the protoplast, and clearly visible by orcein staining technique (Fig. 2c).

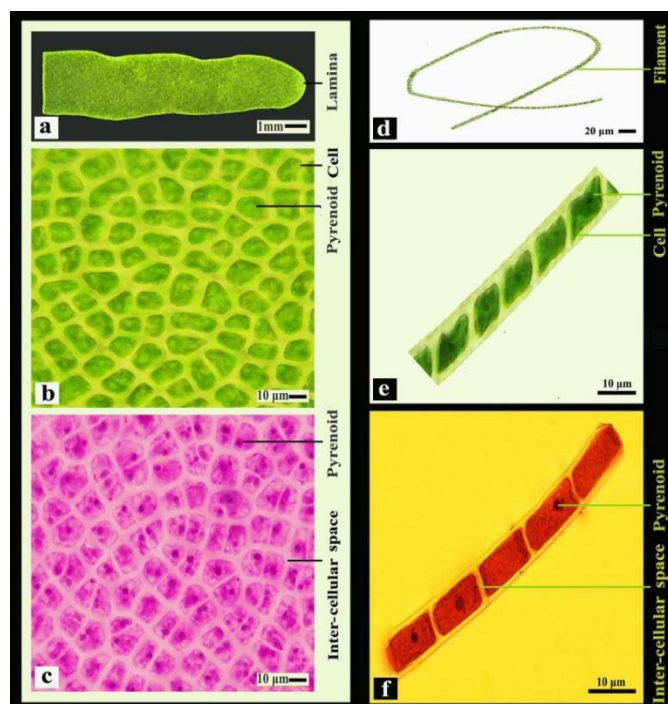


Fig.2. Microscopic view (4X and 40X) of *Enteromorpha compressa* (a, b, c), and *Ulothrix flacca* (d, e, f). (a) thallus, (b) cells with glycerin mounted, (c) cells with orcein stained, (d) Single filament, (e) mature cells with glycerin, (f) cells with orcein stained.

The other species, *Ulothrix flacca* was non-branched, filamentous and hair likes bright to dark-green colored, and found in the upper region of intertidal zone of the studied area. They grew in nearby habitats of *E. compressa* in every examination. Rocks, plastic ropes or other substrates were the common habitats of the alga, and they grew densely upon the habitats (Fig. 1b). This alga was considerably hard in nature and tightly attached with the habitats. Thallus length was variable with undefined length, and special type of holdfast cells were

seen at the base of each filament. Average length and breadth of thallus was measured up to 10 cm and 5 μm , respectively (Fig. 2d). They were surrounded by a mucilaginous sheath with variable width. The cells were cylindrical and mostly ellipsoidal with dimensions of 8–12 μm length and 3–8 μm breadth. Cells were scarcely adherent to each other according to a linear series. Young cells were cylindrical with single pyrenoid and mature cells were barrel-shaped with several pyrenoids (Fig. 2e). The mature cells protoplast was looked like concentrated to two small portions, but separated from each other. Pyrenoids in young filament were clearly visible in orcein stains, although rarely few cells found without pyrenoid (Fig. 2f).

Chlorophyll Contents and Biochemical Analysis

Since we know chlorophylls are the only places where photosynthesis occurs, the amounts of chlorophylls refer the productivity or C-sequestration capacity of any photosynthetic organisms. This experiment found *U. flacca* with higher amounts of chlorophylls than *E. compressa* in respects of chl-a, chl-b and total chlorophylls (Fig. 3). The results meant *U. flacca* was more productive with higher CO_2 capturing capacities. In case of proline (Pro) accumulation, one special type of organic acid that plants accumulate during their developments in stressful conditions, *E. compressa* showed higher amount (2.64 mg/g) than *U. flacca* (2.24 mg/g) (Fig. 3). Since Pro is regarded as a stress tolerance signaling molecule in photosynthetic organisms, the obtained result meant that *E. compressa* exposed to more stress conditions in Kuakata coastline area due to changes in salinity, draught or temperature range according to the previous scientific evidences (31,32).

In terms of carbohydrate synthesis, *E. compressa* produced significantly higher amount (25.56 % of DW) which was more than double of *U. flacca* (10.42 % of DW).

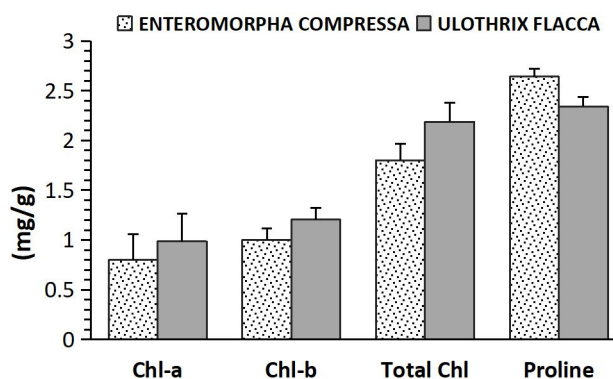


Fig.3. Chlorophyll and accumulated proline contents of the two studied seaweeds. Bar represents standard deviation.

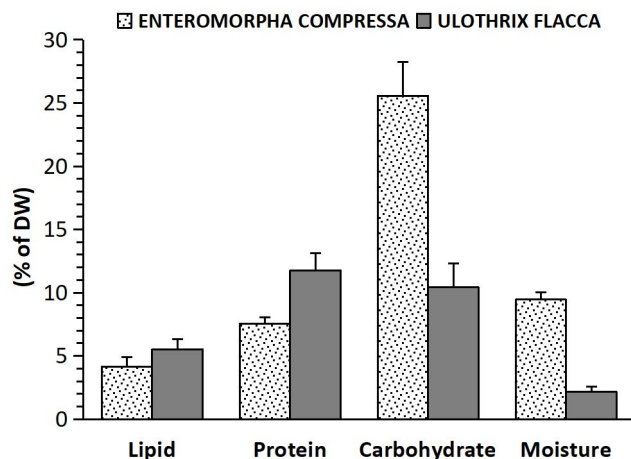


Fig.4. Lipid, protein, carbohydrate and moisture contents of studied seaweeds. Units were presented as percentage of dry weight with standard deviation.

As carbohydrates work as immediate energy source for almost all animals, this alga could be used as a food or fodder mainly due to its higher carbohydrate contents (33). Moreover, it was reported that comparatively higher amount of carbohydrate was found in *Ulva sp.*, but this research emphasized *E. compressa* also has high amount of carbohydrates in their body (34). On the other hand, *U. flacca* showed comparatively higher amounts in lipid and protein contents than *E. compressa* (Fig. 4). Normally, seaweeds possess approximately 16-30 % of proteins in their body; however, this study revealed the two seaweeds had lower amounts of proteins (7.55 % DW and 11.76 % DW, respectively) than other available seaweeds. Lipid contents in *U. flacca* was almost 6% in our study which was higher than the other one, although it was reported that total lipid contents of seaweeds are always less than 4% of DW (35). The result meant that this alga produced lipids more than the production by other seaweeds. In terms of moisture content, highest amounts was determined in *E. compressa* (9.46%) comparatively than *U. flacca* (2.15%). Furthermore, moisture content has significant roles in flavor, texture and overall food quality, and can affect the physical and chemical properties of an organism. Consequently, *E. compressa* would be easily degradable, while *U. flacca* would be more durable.

The statistical analysis showed that there was a significant differences (at $P < 0.05$) between the two seaweeds concerning their chlorophyll contents along with other calculated chemical compositions. That means the two seaweeds have different nutrient compositions. For the best knowledge of the authors, this is the first report of seaweeds from Kuakata coastline of Bangladesh with their morphological studies, chlorophylls estimation and biochemical analysis, because there was not found

any report on seaweeds in the region. However, the report will contribute to the country's food and nutrition sciences research as well as will contribute to the field of ecology and evolutionary biology.

CONCLUSION

This investigation found only two intertidal seaweed species, namely *E. compressa* and *U. flacca* in the coast of Kuakata of Bangladesh. As there was not found any previous reports on seaweeds from this coast, this is the first report of seaweeds from this area. Moreover, *E. compressa* is morphologically larger and consisted with higher amounts carbohydrate and moistures contents, while *U. flacca* has significant level of proteins and lipids.

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CONFLICT OF INTEREST

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RH have planned, designed, worked out and written the manuscript. The other authors have equally contributed to the extraction procedures and data analysis.

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