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Studies of Marine Seaweed *Sargassum flavicans*

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Abstract: The marine seaweed *Sargassum flavicans* belonging to the class Phaeophyceae grows naturally in Saint Martin's Island, Cox's Bazar, Bangladesh. *S. flavicans* was collected from the Island for fatty acid analysis and investigation of its biological properties. The dried seaweed (~210 g) was extracted with a mixture of dichloromethane and methanol (1:1). The crude extract (9.0 g) was suspended in water and was partitioned successively with hexane, dichloromethane, and finally with 2-butanol. Cytotoxicity assay of different extracts of the seaweed was carried out on the HeLa cell line, a human cervical carcinoma cell line, and none of the extracts was found to be cytotoxic. Antioxidant activity, antioxidant capacity, and phenolic content of extracts were also evaluated and the dichloromethane extract contained more phenolic content (264.67 ± 0.87 mg gallic acid equivalent per gram of dry extract) and antioxidant capacity (388.0 ± 9.55 mg ascorbic acid equivalent per gram of dry extract) compared to other extracts of *S. flavicans*. Analysis of fatty acid compositions in oil was also carried out by gas chromatograph equipped with flame ionization detector (GC-FID) and palmitic acid (46.75 %), stearic acid (18.45 %) as well as oleic acid (19.93 %) were the major acids in the oils.

Keywords: Sargassum flavicans, palmitic acid, myristic acid, antioxidant activity, cytotoxicity.

INTRODUCTION

The marine seaweed *Sargassum flavicans* belongs to the class Phaeophyceae. *Sargassum* is a genus of brown macroalgae in the order Fucales. It is normally brown to yellowish with a length up to 10 m. It is found along the coasts of the Indian Ocean to Bangladesh and Myanmar region. The Atlantic Ocean's Sargasso Sea was named after the algae, as it hosts a large amount of *Sargassum* (Davis et al., 2003). It is a potential bio-indicator of industrial nutrient enrichment (Alquezar et al., 2013) and the local people of Saint Martin's island use them as vegetables. The plant is widely used as food, feed, fertilizer, and medicine. Macroalgae mean several species of macroscopic, multicellular and marine algae (Smith et al., 1944)**.** At the beginning of 2011, 3 million tons of seaweed was produced by Indonesia and the production of seaweed reached almost 10 million tons by the end of 2011 **(**FAO, 2018)**.** Many species of *Sargassum* are distributed throughout the temperate and tropical oceans of the world and the genus is widely known for its planktonic (free-floating) species. Most species within the class Phaeophyceae are predominantly cold-water organisms that benefit from

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nutrients upwelling, but the genus *Sargassum* appears to be an exception (Hogan et al., 2011). *S. flavicans* grows naturally in Saint Martin's Island in Bangladesh and there is no reported antioxidant activity of the seaweed from Bangladesh to the best of our knowledge. Therefore, the objective of this study was to biological activity studies of marine seaweed *S. flavicans* grown in Saint Martin's Island.

MATERIAL AND METHODS

Collection of plants: The plant was collected from Saint Martin's Island, Cox's Bazar, Bangladesh and was identified by the Botanist, Department of Botany, University of Dhaka, Bangladesh with a preparation of a voucher specimen (SMI001). The leaves were first dried in room temperature and then in the oven at 40°C and finally dried materials were ground into power.

Extraction: The powdered seaweed (210 g) was extracted with mixture of dichloromethane and methanol (1:1). The crude extract (9.0 g) was suspended in water and was partitioned successively with hexane, dichloromethane and finally with 2-butanol. All the extracts were separately concentrated using rotary vacuum evaporator at 40°C under reduced pressure, and n-hexane (1.90 g), dichloromethane (4.10 g), and 2-butanol (1.30 g) extracts were obtained.

Total Phenolic Content: The total phenolic content (TPC) was determined by the modified folin-ciocalteu method (Wolfe et al., 2003). 0.5 mL of different extracts of *S. flavicans* was taken separately in different test-tube, then 5 mL of Folin-ciocalteu's reagent (1: 10 v/v distilled water) and 4 mL (75g/L) of sodium carbonate were added. The solution was then vortex for 15 seconds and allowed to stand for 30 minutes at 40°C. The absorbance was measured at maximum 765 nm against the blank in a double beam UV/Visible spectrophotometer (UV-1800). The total phenolic content was determined and expressed as mg gallic acid equivalents per gram of dry extract using the equation obtained from a standard gallic acid calibration curve, $y = 0.003x - 0.001$, $R^2=0.996$.

Total Antioxidant Capacity: The total antioxidant capacity of the sample extracts was evaluated by the phosphomolybdenum assay method (Prieto et al., 1999). The subsequent formation of a green phosphate-Mo (V) complex in acidic condition was visible. The 0.3 mL of each extract was allowed to mix with 3.0 mL of the reagent solution. This reaction mixture was incubated at 95°C for 90 minutes. The absorbance was measured at 695 nm using a spectrophotometer against a blank solution. The total antioxidant capacity was measured and expressed as mg ascorbic acid equivalents per gram of dry extract using the equation obtained from a standard ascorbic acid calibration curve, $y = 0.002x - 0.006$, $R^2= 0.991$.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity: The stable DPPH radical-scavenging activity was evaluated using the previous modified method (Gupta et al., 2003). Stock solution (1mg/mL) of the different solvent fractions of *S. flavicans* was prepared in respective solvent systems from which serial dilutions were carried out to obtain the concentrations of 5, 10, 20, 40, 50, 80, 100, 200, 250, 400 µg/mL. 2 mL of 0.1 mM DPPH solution was added to 2 mL of extract solution at different concentrations. The absorbance was measured at 517 nm against a blank with spectrophotometer.

Cytotoxicity assay on cancer cell line: Cytotoxicity assay was examined against HeLa cell line, a human cervical carcinoma cell line and was maintained in DMEM (Dulbecco's Modified Eagles medium, Sigma, Germany) containing 1% penicillin –streptomycin (1:1) and 0.2% gentamycin and

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10% fetal bovine serum (FBS). Cells (4×10^4/200 µL) were seeded onto 48-well plate and incubated at 37°C + 5% CO_2. Next day, 50 µL sample (filtered) was added each well. Cytotoxicity was examined under an inverted light microscope after 48 hour of incubation. n-Hexane, dichloromethane, and methanol extracts of *S. flavicans* were tested against HeLa cell line. Duplicate wells were used for each sample (Jae et al., 2018).

Analysis of fatty acid compositions: Fatty acid compositions of oil from *S. flavicans* were analyzed following the earlier described procedure (Shoeb et al., 2013).

RESULTS & DISCUSSION

Phenolic Content: Total phenolic content (TPC) is the process to measure the amount of phenolic compounds present in the plants. Phenolic compounds have redox properties that allow them to act as antioxidants (Taigang et al., 2010, Johari et al., 2019). The concentration of the gallic acid solution (05-100 ppm) was used for making a calibration curve (Fig. **1**). TPC of hexane, dichloromethane, 2-butanol and methanol extracts was around 23, 264, 14 and 63 mg GAE/g of dry extract, respectively (Fig. 2). The results showed that the dichloromethane extract exhibited the highest TPC as compared to the hexane, 2-butanol and methanol extracts. Higher phenolic content is responsible for bioactivity and expected to exhibit good results in antioxidant and antibacterial activities (Johari et al., 2019).

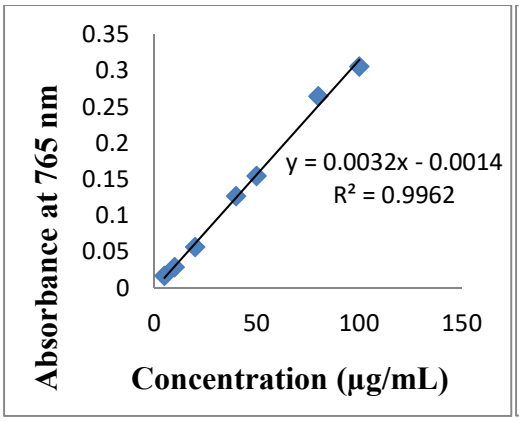

Fig. 1: Calibration curve of gallic acid

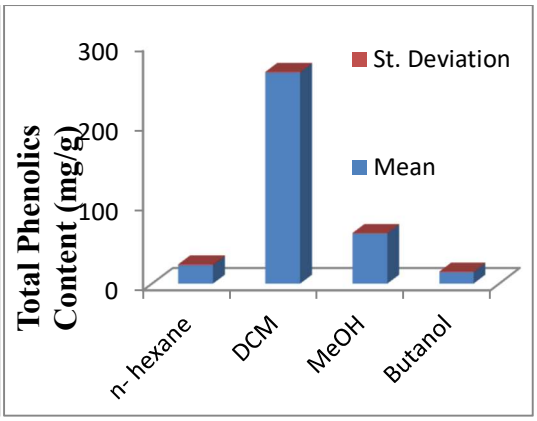

Fig. 2: TPC of the extracts of *S. flavicans*

Total antioxidant capacity: To determine antioxidant capacity ascorbic acid solution as standard ranging from (5-100) ppm was used to draw a calibration curve (Fig. 3). The total antioxidant capacity of dichloromethane extract was found to be 388.0±9.55 mg AAE/g of dry extract, which the highest was compared with other extracts. On the other hand, the lowest amount containing 61.5±4.05 mg was present in the 2-butanol extract (Fig. 4).

Antioxidant activity: The DPPH assay was used as a reproducible parameter to measure the antioxidant activity of plant extracts (Burda et al., 2001, Ara et al., 2009). Due to the reduction capacity of DPPH with hydroxyl groups of the antioxidant molecule, absorbance at 517 nm of DPPH was decreasing with the increasing of the concentration of extract (Cotelle et al., 1996, Basnet et al.,

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1997). The result revealed that the n-hexane, dichloromethane, 2-butanol, and methanol extracts exhibited

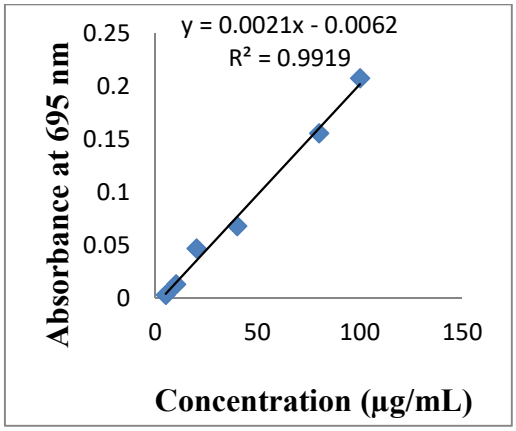

Fig. 3: Calibration curve of Ascorbic Acid

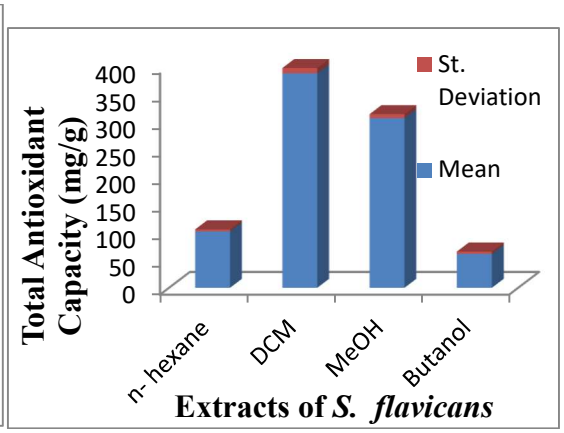

Fig. 4: TAC of the extracts of *S. flavicans*.

29.98±0.42, 86.04±0.43, 22.08±0.47 and 74.02 ± 0.59 at 400 µg/mL (Fig. 5), whereas 87.11 ± 0.12 at 10 µg/mL was for ascorbic acid (Fig. 6). The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological function. The lowest IC_{50} value indicates the highest activity as antioxidant molecule. The IC_{50} value of dichloromethane (211.23±0.06 µg/mL) extract was the lowest compared with n-hexane (655.87±0.13 µg/mL), 2-butanol (1064.85±0.36 µg/mL) and methanol (219.02±0.25 µg/mL) (Fig. 7).

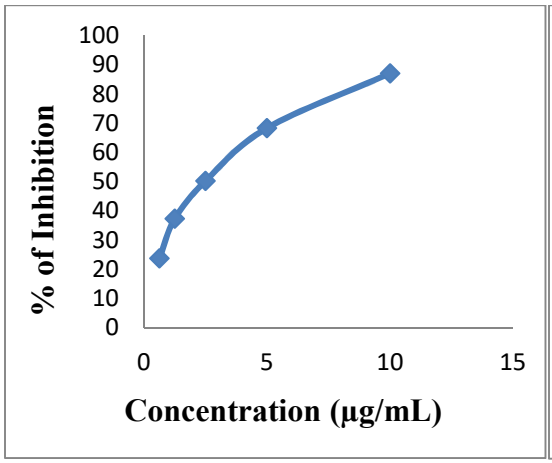

Fig. 5: DPPH radical scavenging activity of ascorbic acid

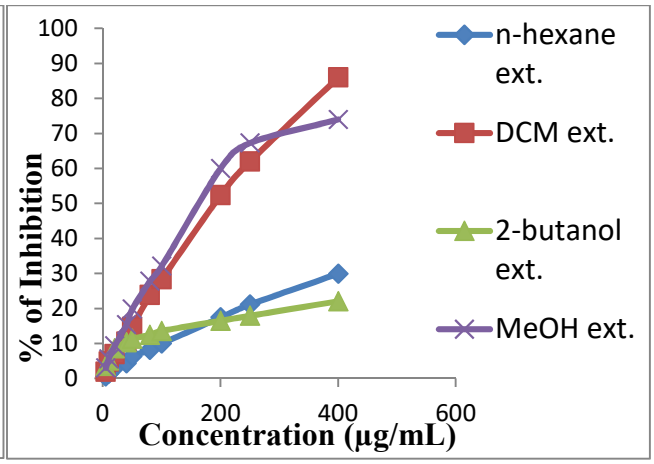

Fig. 6: DPPH free radical scavenging activity of the extracts of *S. flavicans*

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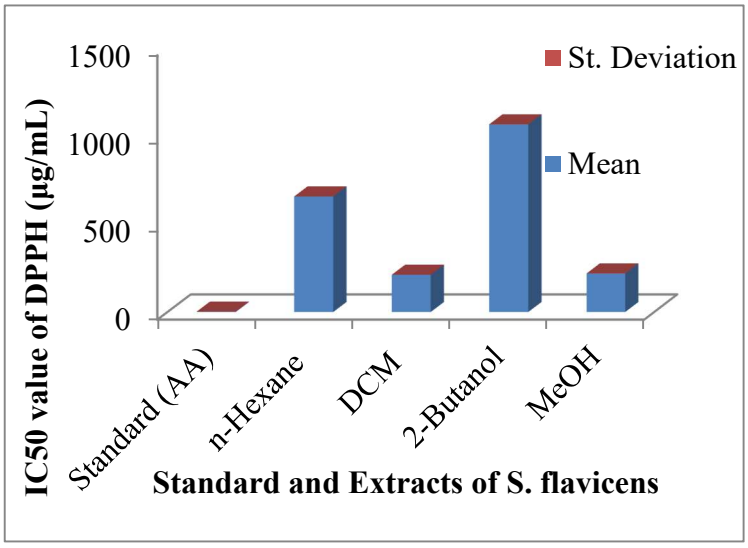

Fig. 7: IC_{50} value of standard AA and extracts of *S. flavicans*

Relative fatty acid compositions: The relative fatty acid compositions in the oil from hexane extract were made into their methyl ester by saponification with methanolic NaOH followed by esterification with BF_3-methanol complex, and analyzed by GC-FID. The relative percentage of methyl ester of fatty acids in oil was identified by comparing their retention time with that of methyl ester of fatty acid standard. It was found that palmitic acid (46.75%), stearic acid (18.45%), oleic acid (19.93%) and myristic acid (0.95%) were major acids in the extracts of *S. flavicans*. The present of unsaturated oleic acid may be helpful for the consumer to use *S. flavicans* as a vegetable.

Cytotoxicity assay: Cytotoxicity assay of n-hexane, dichloromethane, and methanol extracts of *S. flavicans* were assessed against HeLa, a human cervical carcinoma cell line. However, none of the extracts was found to be cytotoxic against Hela cell lines (Fig. 8).

CONCLUSION

The dichloromethane extract of *S. flavicans* was found to have significant phenolic content (264.67 ± 0.87 mg gallic acid equivalent per gram of dry extract) and antioxidant capacity (388.0 ± 9.55 mg ascorbic acid equivalent per gram of dry extract) compared to other extracts. Analysis of fatty acid compositions in oil revealed that palmitic acid (46.75%), stearic acid (18.45%) and oleic acid (19.93%) were major acids in the oils of *S. flavicans.*

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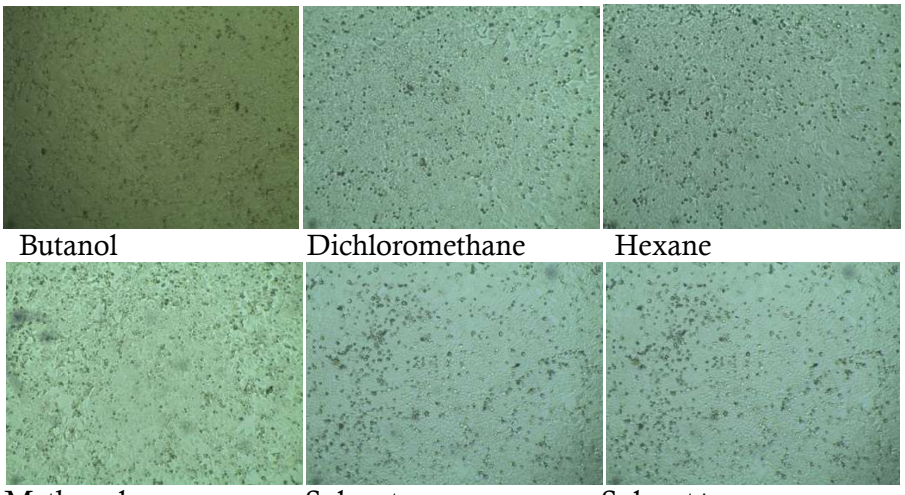

Fig. 8: Cytotoxicity assay of the extracts of *S. flavicans*

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare

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