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Research article

Exploring the antioxidant and antimicrobial potential of three common seaweeds of Saint Martin's Island of Bangladesh

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ABSTRACT

Antioxidants, which have long been deemed an indispensable guardian of human health, play a pivotal role in bolstering the body's defense against a plethora of diseases. Three well-recognized seaweeds in Bangladesh, including *Caulerpa racemosa*, *Padina tetrastromatica,* and *Hypnea musciformis*, were subjected to meticulous analysis to reveal their phytochemical composition, antioxidant activity, and antimicrobial efficacy using advanced spectroscopic and disc diffusion methods. Intriguingly, we observed that *C. racemosa* emerges as frontrunners, possessing a substantial arsenal of phenol (143.08 ± 18.51 mg gallic acid equivalent g^{-1}) and flavonoid (63.79 ± 2.16 mg rutin equivalent g^{-1}). More fundamentally, *C. racemosa* exhibits a notable enrichment in the content of tannin (73.58 mg RE g^{-1}) and chlorophyll (13.50 mg g^{-1}), as well as, antioxidant capacity (4457.67 μg g^{-1}). *P. tetrastromatica*, on the other hand, displayed commendable effectiveness in scavenging the DPPH radical, with percentages ranging from 53.98 to 62.17%. In terms of hydroxyl radical ($OH^{\bullet}$) scavenging activity, *C. racemosa* exhibited the highest efficacy at 400 g mL^{-1}. Fascinatingly, *C. racemosa* exhibited an impressive antioxidant potential, as evidenced by its exceptionally low IC_{50} value of 5.58 μg mL^{-1} for $OH^{\bullet}$ scavenging, whereas *P. tetrastromatica* showed impressively low value of 0.96 μg mL^{-1} for DPPH scavenging. Although the three seaweeds demonstrated limited efficacy against a spectrum of five human pathogenic bacteria, their potential as abundant sources of antioxidants remains unscathed. Notably, heatmap and PCA analysis revealed that *C. racemosa* and *P. tetrastromatica* emerge as the leading contender for studied antioxidant compounds, demonstrating their proclivity for antioxidant extraction, a trait that could be exploited for large-scale production of these valuable compounds.

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1. Introduction

The marine environment is widely recognized as one of the most ecologically diverse ecosystems on earth. The profusion of biotic and abiotic resources, including different fishes, shellfish, mollusks, univalves, cephalopods, crustaceans, echinoderms, and seaweed has made the ocean into a promising avenue for human investigation to make substantial progress in contemporary biological research [1,2]. Seaweeds, among the aforementioned resources, have been employed since ancient times for their therapeutic properties in the treatment of many ailments. Scientists nowadays pay impulsive attention to seaweeds as a crucial reservoir of antioxidants like ascorbate, phenol, flavonoid, glutathione, vitamins, and vitamin precursors such as chlorophylls and carotenoids, along with minerals encompassing over 54 trace elements essential for sustaining human body functions [2–5].

Although oxidation serves as an energy-generation process, excessive and imbalanced oxidation triggers the generation of free radicals, which may lead to chronic ailments within living organisms [6,7]. To counteract the diverse array of reactive oxygen species (ROS), including superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hydroxyl radical ($HO^{\cdot}$), and subsequent oxidative burden, a robust antioxidant defense system, comprising of both enzymatic and non-enzymatic antioxidants, play a decisive role. This, in turn, contributes to diminishing the susceptibility of diseases like cancer, Parkinson's disease, aging, and coronary heart disease in humans [8,9]. Enzymes like catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR), and superoxide dismutase (SOD), as well as non-enzymatic substances like bilirubin and albumin, are examples of endogenous antioxidants [10]. These enzyme groups protect against the initial stages of carcinogenesis by neutralizing ROS-induced DNA damage [11]. SOD, CAT, and Glutathione related antioxidant enzymes play a vital role to reduce tumor cell activity caused by oxidative stress in cancer-affected patients [12]. Mitochondria is one of the important organelles for the generation of ROS, and excessive ROS production due to dysfunction of mitochondria causes several damages in DNA, cell membrane, and protein which causes different diseases including Parkinson's, cancer, heart disease, cell death, etc. [13]. GR, GST, SOD, melatonin, etc. act as antioxidants to prevent Parkinson's disease caused by excessive ROS in the brain [13]. Under extreme stress conditions, the endogenous antioxidant supply is insufficient to protect the body and then it requires an exogenous supply of antioxidants through nutritional supplements, food, and pharmaceuticals. Phenol, flavonoids, anthocyanin, carotenoids, Vitamins, etc. are good examples of non-enzymatic exogenous antioxidants. SOD is a powerful antioxidant in the cell and it neutralizes ROS like $O_2^{\bullet-}$ to H_2O_2 and then CAT and GPx converted H_2O_2 into H_2O and O_2 [10]. Non-enzymatic antioxidant neutralizes the free radical mostly by donating electrons or acting as a cofactor of enzymatic antioxidants [14]. Although, artificially manufactured antioxidants, including butylated hydroxyanisole (BHA), tertbutylhydroquinone (TBHQ), and butylated hydroxyl toluene (BHT) have recently gained momentum; however, their potential to pose substantial health risks highlights the necessities of harnessing antioxidants from natural sources [15]. As an alternative, food, and drugs derived from seaweeds have recently gained popularity worldwide, including in Bangladesh, because of their multiferous medicinal properties, encompassing cholesterol reduction, providing antioxidant, preventing blood clotting, diabetic management, bolstering probiotic efficiencies and impacting estrogen metabolism [16–18].

Bangladesh has been endowed with the magnificent Bay of Bengal environment, one of the world's 64 Large Marine Ecosystems, which includes estuaries, coral reefs, mangrove forests, and vital areas for fish spawning [19]. The coastline of Bangladesh covers more than 30% of the entire country, including full or fragmented sections of 19 administrative districts and providing habitat for over 39 million people [20]. More fundamentally, within the coastal area of Bangladesh, around 335 diverse natural populations of seaweeds thrive, contributing to an annual availability of 5000 metric tons of seaweed biomass [21,22]. Despite the substantial abundance, seaweeds are currently insufficiently utilized, with only a small fraction of the available seaweed being harnessed by the Rakhyine or Mog ethnic community and the seaweed collectors of Saint Martins' Island [23]. Among diverse species of seaweeds, *Caulerpa racemosa*, *Padina tetrastromatica,* and *Hypnea musciformis* have been considered the promising seaweeds in Bangladesh coastal ecosystems. The green algae *C. racemosa* is well-recognized as sea lettuce; however, in Bangladesh, it is locally referred to as "*Sagor Angur or Sea Grapes*', growing profoundly at Saint Martin's Island [24]. The Brown *P. tetrastromatica* algae has been characterized by a flattened, thin structure, and is composed of four cell layers, with a soft and slightly leather-like texture. These algae are usually dried and processed into flakes, which are predominantly used as additives in diverse foods and dietary salts, intended for people use with hypertension. Notably, the presence of alginic acid within *P. tetrastromatica* renders it an invaluable natural resource with immense potential for antiviral and anticoagulant properties [25]. *H. musciformis*, on the other hand, is typified by its cylindrical, spreading, bushy, and purplish green in color [26].

Indeed, foods rich in therapeutic properties are gaining popularity in today's health-conscious society. Seaweeds, with their high nutritional value and cost-effectiveness, offer an attractive avenue to fulfill nutritional requirements. Surprisingly, while Bangladesh has been considered a potential hub for a variety of seaweed species, the potent antioxidant and antimicrobial properties of seaweeds in the country have been inadequately explored. Therefore, this study aims to explore the antioxidant and antimicrobial potential of three paramount seaweeds harvested from the shores of Saint Martin's Island in Bangladesh. Additionally, this research could pave the way for the development of the seaweed industry in Bangladesh, opening up exciting new opportunities in this sector.

2. Materials and methods

2.1. Sample collection and location of the sampling area

Three varieties of seaweeds, including *C. racemosa, P. tetrastromatica,* and *H. musciformis*, were procured from the rocky intertidal zone of Saint Martin's Island. This island is situated in the north-eastern region of the Bay of Bengal, between the longitudes of 92°18' and 92°21′E and the latitudes of 20°34' and 20°39′N. Seaweeds in Bangladesh experienced substantial growth during the winter

season, especially spanning from December to April, and these three types of sea algae were collected during that particular period. Collections would usually commence 2 h prior to the time of the lowest low tide. Sampling was systematically carried out, progressing from higher to lower levels of the tidal area. This approach ensured that seaweeds were recently exposed and retained the moisture during the sampling process. After collecting the samples, a marine biologist and professor from the Institute of Marine Science and Fisheries at the University of Chittagong, Bangladesh, verified the collected seaweeds (Fig. 1).

2.2. Seaweed crude extracts preparation

The freshly collected raw seaweeds were promptly rinsed with saline water to remove external materials like sand, stones, and fragments of coral. These rinsed seaweeds were then dried in an oven at 40 °C and processed into a coarse powder using a mechanical crusher. The resulting powder was stored in an airtight extraction jar. Subsequently, 1400 mL of ethanol was added to 80 g of the powder and left to incubate for 7 days at room temperature (25 ± 1)°C. Following the 7-day period, the ethanol extracts were filtered using Whatman no. 1 filter paper, and the filtered solution was concentrated below 50 °C (at around 40–45 °C) using a rotary vacuum evaporator (RE200, Bibby Sterling Ltd., England) under reduced pressure. The concentrated sample was air-dried in the Petri dish, and the resulting crude extracts were stored at 4 °C for further experiments. The yields of crude extracts from 80 g of dried powder were 4.07 g (blackish green), 3.22 g (dark brown), and 5.15 g (blackish red) for green, brown, and red algae, respectively. Refer to Fig. 2 for an illustration outlining the experimental procedures employed in this research.

2.3. Biochemical analysis of seaweeds

2.3.1. Determination of total phenol content (TPC)

TPC was determined using the Folin-Ciocalteu techniques outlined in Ref. [27] with minor modification. Initially, 200 μL of sample solution (400 μg mL^{-1} ethanol), as well as 200 μL of the gallic acid standard solution of different concentrations (25, 50, 100, 200, 400, and 800 μg mL^{-1} ethanol) were separately poured in screw cap tubes. Folin-Ciocalteu reagent (1.5 mL, prepared as a 1:4 reagent-to-water ratio) was added to each tube, and the mixture was kept at 25 °C for 3 min. Subsequently, 3 mL of sodium carbonate solution (2:3, sodium carbonate to water ratio) was added and the mixture was allowed to keep for 2 h at room temperature. The absorbance of the solution was then measured at 765 nm using a spectrophotometer (UV-1601 Shimadzu Corporation, Kyoto, Japan), considering ethanol as a blank. The total phenol was estimated as Gallic acid equivalents (GAE) by employing the calibration curve equation (Supplementary Fig. 1a). Finally, the TPC was calculated using the following equation: $C = (c \times V)/m$, where, C represents the total phenolic content (mg g^{-1} of plant extract in GAE), c denotes the concentration of gallic acid obtained from calibration curve (mg mL^{-1}), V signifies the volume of the sample solution (mL), m stands for the weight of the sample (g).

2.3.2. Determination of total flavonoid content (TFC)

TFC was determined following the method described in Ref. [28] with slight modifications. Initially, 1 mL of sample solution (400 μgmL^{-1} ethanol), as well as 1 mL of Rutin standard solution of different concentrations (25, 50, 100, 200, 400, and 800 μg mL^{-1} ethanol) were separately poured into a screw cap tube. Subsequently, 200 μL of 10% aluminium chloride solution was added into each tube and mixed properly, after which 3 mL of ethanol was added. Consecutively, 200 μL of 1 M potassium acetate solution and 5.6 mL of distilled water was added to each tube and the mixture was allowed to keep for 30 min at room temperature. The absorbance of the solution was then measured at 415 nm using a spectrophotometer (UV-1601 Shimadzu Corporation, Kyoto, Japan), considering ethanol as a blank. The total flavonoid was estimated as Rutin equivalents by employing the calibration curve equation (Supplementary Fig. 1b) and the formula: $C = (c \times V)/m$, where, C represents the total flavonoid content (mg g^{-1} of plant extract in Rutin), c denotes the concentration of Rutin obtained from the calibration curve (mg mL^{-1}), V signifies the volume of the sample solution (mL), m stands for the weight of the sample (g).

2.3.3. Determination of total tannin content (TTC)

The TTC of seaweed extract was quantified following the protocol outlined in Ref. [29], with minor modifications. Initially, 100 μL of sample solution (400 μg mL^{-1} ethanol), as well as 100 μL of Rutin standard solution of different concentrations (3.12, 6.25, 12.5, 25, 50, and 100 μg mL^{-1}) were separately poured in screw cap tubes. Subsequently, 3 mL of 4% vanillin solution was added and mixed thoroughly. Afterward, 1.5 mL of concentrated HCL solution was added into each tube, and the mixture was vortexed vigorously and kept under dark conditions at room temperature (25 °C) for 20 min. The absorbance of the solution was then measured at 500 nm using

Fig. 1. A pictorial view of studied seaweeds.

Fig. 2. An illustration of the experimental procedures of antioxidant and antimicrobial activity of seaweeds. Here, R, red algae (*Hypnea musciformis*); B, brown algae (*Padina tetrastromatica*) and G, green algae (*Caulerpa racemosa*). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

a spectrophotometer (UV-1601 Shimadzu Corporation, Kyoto, Japan), considering ethanol as a blank. The total tannin content was estimated as Rutin equivalents by employing the calibration curve (Supplementary Fig. 1c) and the formula: $C = (c \times V)/m$, where, C represents the total tannin content (mg g^{-1} of plant extract in Rutin), c denotes the concentration of Rutin obtained from the calibration curve (mg mL^{-1}), V signifies the volume of the sample solution (mL), m stands for the weight of the sample (g).

2.3.4. Determination of total antioxidant capacity (TAC)

The procedure outlined in Refs. [30,31] was employed to determine the TAC. In essence, 300 μL of extract sample solution and 300 μL of ascorbic acid (standard) solutions of different concentrations (12.5, 25, 50, 100, 200, and 400 μg mL^{-1}) were poured into separate screw cap tubes. Subsequently, 3 mL of reagent solution (comprising 4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid) was added into each tube. The mixture was then heated by keeping the tubes in a water bath at 95 °C for 90 min. Subsequently, after cooling, the absorbance of the solution was measured at 695 nm using a spectrophotometer (UV-1601 Shimadzu Corporation, Kyoto, Japan), considering 3 mL reagents and 300 μL ethanol as a blank. The TAC was estimated as ascorbic acid equivalents by employing the calibration curve (Supplementary Fig. 1d) and the same formula: $C = (c \times V)/m$, where, C represents the total antioxidant capacity, c denotes the concentration of ascorbic acid from calibration curve (mg mL^{-1}), V signifies the volume of the sample solution (mL), m stands for the weight of the sample (g).

2.3.5. Determination of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The procedure outlined in Ref. [32] was employed to evaluate the scavenging activity of the DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical. Using ethanol as a dissolving solvent, 3 mL of different concentrations (25, 50, 100, 200, and 400 μg mL^{-1}) of seaweed extracts and ascorbic acid were prepared in separate screw cap tubes. Afterward, DPPH solution (0.004%) was prepared in ethanol and 3 mL of this solution was added to each screw cap tube. Then the tubes were promptly placed in a dark environment for 30 min. Subsequently, the absorbance of the solution was measured at 517 nm using a spectrophotometer (UV-1601 Shimadzu Corporation, Kyoto, Japan), considering ethanol as a blank. The percentage of DPPH radical scavenging activities was calculated by employing the following formula: DPPH scavenging (%) = $[(A - B)/A] \times 100$, Where, A represents the absorbance of the control (DPPH solution without seaweed extract/ascorbic acid) and B correspondence to the absorbance DPPH solution with the seaweed extract or ascorbic acid.

2.3.6. Determination of hydroxyl (OH) radical scavenging activity

The methodology described in Refs. [31,33] was adopted to determine the scavenging activity of hydroxyl radical, with minor modifications. Initially, seaweed extracts and ascorbic acid (positive control) solutions (25, 50, 100, 200, and 400 μg mL^{-1}) were prepared in ethanol separately. From each concentration of seaweed extract and ascorbic acid, 500 μL were poured into a separate screw cap tube, and subsequently added 250 μL of orthophenanthrolin (7.5 mM) solution in each tube. Next, for each sample, a mixture of solution encompassing 1.25 mL of phosphate buffer (0.2 M; pH 6.6), 250 μL of ferrous sulfate (7.5 mM), 250 μL of H_2O_2 (0.5%), and 3.75 mL of deionized water was prepared. The mixture was then added to each tube to obtain a final volume of 6.25 mL, and subsequently, these tubes were vortexed vigorously and kept for 30 min at room temperature (25 °C). Solution mixture without seaweed extracts and seaweed extracts + H_2O_2 was also kept and incubated in the same way to measure the absorbance. The absorbance of the solutions was then measured at 490 nm using a spectrophotometer (UV-1601 Shimadzu Corporation, Kyoto, Japan), considering ethanol as a blank. Finally, the scavenging percentage of hydroxyl radical was computed using the following equation: OH radical scavenging percentage (%) = (A−A1/A2−A1)*100, Where, A represents the absorbance value of solutions mixture (including H_2O_2,

seaweed extract, and other chemicals), A1 correspondnce to the absorbance value without the seaweed extract, and A2 represents the absorbance value without H_2O_2 and seaweed extract.

2.3.7. IC_{50} (50% radical inhibition concentration) value of the seaweed extracts

The IC_{50} value denotes the minimum concentration of the sample necessary to reduce the radical levels (both OH and DPPH) by 50%. A regression equation plot was prepared using the seaweed extract concentrations (25, 50, 100, 200, and 400 μg mL^{-1}) and their corresponding radical scavenging capabilities to determine the IC_{50} value for the seaweed extract.

2.4. Pigment content of seaweeds

2.4.1. Determination of chlorophyll and carotenoid content

The content of chlorophyll and carotenoids in the studied seaweeds was determined using the techniques described in Refs. [34, 35]. Approximately 500 mg of fresh seaweed was placed in a mortar and pestle, followed by homogenization with 10 mL of 80% acetone. The samples were then subjected to centrifugation at 3000 rpm for 15 min. The absorbance of the collected supernatants was measured at 480, 645, and 663 nm using a spectrophotometer (UV-1601 Shimadzu Corporation, Kyoto, Japan), considering acetone as a blank. Chlorophyll and carotenoid content were measured by using the following formula:

$$\text{Chlorophyll “a”} \left(\text{mg } \text{g}^{-1} \text{ FW}\right) = \frac{12.7\ x\ A663 - 2.69\ x\ A645}{a\ x\ 1000\ x\ W}\ x\ V$$

$$\text{Chlorophyll “b”} \left(\text{mg } \text{g}^{-1} \text{ FW}\right) = \frac{22.9\ x\ A645 - 4.68\ x\ A663}{a\ x\ 1000\ x\ W}\ x\ V$$

$$\text{Total Chlorophyll} \left(\text{mg } \text{g}^{-1} \text{ FW}\right) = \frac{20.2\ x\ A645 + 8.02\ x\ A663}{a\ x\ 1000\ x\ W}\ x\ V$$

$$\text{Carotenoid} \left(\mu\text{g } \text{g}^{-1} \text{ FW}\right) = \text{A.480} + (0.114 \times \text{A. 663}) - (0.638 \times \text{A. 645})$$

Where, A = Absorbance at respective wavelength, FW = Fresh weight of the sample (g), V = Volume of extract (mL).

2.4.2. Determination of β-carotene and lycopene

In essence, 100 mg of dried ethanolic extract seaweed was intermixed with a 10 mL solution comprising 4 volumes of acetone and 6 volumes of hexane. Following 1-minute interval, the solution was filtered using Whatman filter paper. The absorbance of the collected supernatants was measured at 453, 505 and 663 nm using a spectrophotometer (UV-1601 Shimadzu Corporation, Kyoto, Japan), considering acetone-hexane as a blank. Finally, the concentration of β-carotene was measured using the following formula: β-carotene (mg 100 mL^{-1}) = 0.216 × A663 – 0.304 × A505 + 0.452 × A453. However, the concentration of lycopene was quantified using the following formula: Lycopene (mg 100 mL^{-1}) = (– 0.0458 × A663) + (0.372 × A505) – (0.0806 × A453).

2.5. Determination of antimicrobial activity of seaweeds

The study evaluated the antibacterial properties of specific algal extracts against human pathogenic bacteria obtained from the Biochemistry department of the University of Chittagong in Bangladesh. These pathogenic bacteria include *Vibrio cholerae* Gram (−), *Salmonella paratyphi* Gram (−), *Escherichia coli* Gram (−), *Bacillus subtilis* Gram (+), *Staphylococcus* Gram (+). Following the disk diffusion method described in Ref. [36], the researchers examined the antibacterial abilities of seaweed extracts. The bacteria were subcultured overnight in Nutrient agar media (Hi-Media, Mumbai-400086, India) at 37 °C, and their concentration was adjusted to McFarland (0.5) standard. The test samples were prepared with a known concentration (μgmL^{-1}). Using a forcep, sterilized Whatman paper disks (6 mm in diameter) were submerged in these sample solutions and then allowed to air-dry. Ethanol-soaked discs served as a negative control, while ready-made antibiotic disks (Gatifloxacin, 5 μg $disc^{-1}$, Hi-Media, Mumbai-400086, India) functioned as a positive control. The freshly grown bacteria were mixed with 10 mL of autoclaved distilled water, followed by mixing gently, spread evenly on nutrient agar petri plate. For maximal diffusion, the plates were initially incubated at 4 °C for 4–6 h, followed by 24 h at 37 °C. The diameter (mm) of the area where bacterial growth was prevented was measured to assess the samples' efficacy.

2.6. Statistical analysis

Biochemical results were displayed as mean ± standard deviation (SD) by using the Microsoft Excel (version 2016) program. Analysis of variance (ANOVA) and mean comparison analysis was done by Statistical Tools for Agricultural Research (STAR) software using the least significant difference test (LSD). To illustrate the association among the parameters and seaweeds, a heatmap was prepared by using the “pheatmap” package of R. Principal component analysis was done by using “ggplot2”, “factoMineR” and “factoextra” packages. Additionally, a correlogram plot was prepared using the “metan” and “ggplot2” packages to unravel the relationship among the studied parameters of seaweeds.

3. Result

3.1. Total phenol, flavonoids, tannin, and antioxidant capacity of the seaweeds

Our findings in terms of total phenolic content in seaweeds, quantified as gallic acid equivalent, reveal that *C. racemosa* displayed the highest phenolic content at 143.08 mg GAE g^{-1}. Conversely, the phenolic content of *P. tetrastromatica* was found to be 63.24 mg GAE g^{-1}, whereas *H. musciformis* exhibited a phenolic content of 41.51 mg GAE g^{-1} (Table 1). Furthermore, we also quantified the level of total flavonoid and total tannin, both expressed in terms of Rutin equivalent (RE). Among these three seaweeds, *C. racemosa* displayed the highest flavonoid content, measuring 63.79 mg RE g^{-1}, whereas *H. musciformis* exhibited the lowest at 25.70 mg RE g^{-1} (Table 1). Nonetheless, we did not observe any substantial differences in terms of tannin content among the seaweeds. Numerically, the utmost tannin content was found in *C. racemosa* (73.58 mg RE g^{-1}) followed by *P. tetrastromatica* (71.90 mg RE g^{-1}), and *H. musciformis* (70.08 mg RE g^{-1}) (Table 1). Similar to tannin content, the total antioxidant capacity among the seaweeds displayed no noteworthy differences. Numerically, *C. racemosa*, *P. tetrastromatica*, and *H. musciformis* exhibited total antioxidant capacity of 4457.67 μg g^{-1}, 4434.33 μg $g^{-1,}$ and 4427.67 μg g^{-1}, respectively (Table 1).

3.2. Analysis of β-carotene, lycopene, carotenoid, and chlorophyll content of the seaweeds

Our findings revealed that *P. tetrastromatica* displayed the highest β-carotene content at 0.86 mg 100 mL^{-1}, which was statistically similar to the β-carotene content observed in *C. racemosa*. By comparison, *H. musciformis* exhibited the lowest β-carotene content (0.57 mg 100 mL^{-1}) (Table 2). Lycopene content, on the other hand, was found to be higher in both *C. racemosa* and *P. tetrastromatica* (1.05 and 1.09 mg 100 mL^{-1}, respectively), whereas *H. musciformis* displayed substantially lower lycopene content (0.24 mg 100 mL^{-1}) (Table 2). The carotenoid content in *P. tetrastromatica* was notably higher at 1.75 mg g^{-1} FW, surpassing *H. musciformis* by around 71.42% and *C. racemosa* by approximately 45.71% (Table 2). *C. racemosa*, on the other hand, displayed the highest total chlorophyll content (13.50 mg g^{-1} FW), which was approximately 35.11 and 88.89% higher than what we observed in *P. tetrastromatica* and *H. musciformis* seaweeds, respectively (Table 2). In addition, *C. racemosa* also displayed higher levels of chlorophyll *a* (0.51 mg g^{-1} FW), which was approximately 99.41 and 99.60% higher than those found in *P. tetrastromatica* and *H. musciformis* seaweeds, respectively (Table 2). By contrast, *P. tetrastromatica* exhibited the utmost level of chlorophyll *b* (0.53 mg g^{-1} FW), which was around 19.69 and 95.45% higher than what we found in *C. racemosa* and *H. musciformis* seaweeds, correspondingly (Table 2).

3.3. DPPH and hydroxyl radical ($OH^{\bullet}$) scavenging activity of seaweeds

Our observations into DPPH scavenging activity revealed that the divergent concentrations of seaweeds did not substantially affect the DPPH scavenging activity (Fig. 3a). Nevertheless, *P. tetrastromatica* displayed utmost DPPH scavenging activity of 54.75%, 55.70%, 57.07% and 62.17% at the concentrations of 50, 100, 200 and 400 μg mL^{-1}, respectively (Fig. 3a) but at 25 μg mL^{-1} no significant difference was found among *C. racemosa* (49.17%), *H. musciformis* (41.23%) and ascorbic acid (48.60%). In contrast, DPPH scavenging activity was substantially lower in *H. musciformis*, with values of 48.97%, 50.49%, 52.04%, and 54% at the concentrations of 50, 100, 200, and 400 μg mL^{-1}, respectively. Contrarily, when appraising $OH^{\bullet}$ radical scavenging activity across the concentrations ranging from 25 to 200 μg mL^{-1}, we did not observe any substantial differences between *P. tetrastromatica* and *H. musciformis*. However, *C. racemosa* exhibited lower hydroxyl radical scavenging activity, with values of 8.93%, 22.16%, 28.46%, and 30.77% at concentrations of 25, 50, 100, and 200 μg mL^{-1}, respectively (Fig. 3b). It is interesting to note that at 400 μg mL^{-1}, *C. racemosa* displayed surprisingly higher hydroxyl radical scavenging activity (47.95%), whereas *H. musciformis* exhibited the lowest $OH^{\bullet}$ radical scavenging activity (35.07%) at the same concentration (Fig. 3b).

3.4. IC_{50} value of seaweeds for scavenging DPPH and $OH^{\bullet}$ radical

We observed that *P. tetrastromatica* displayed significantly lower IC_{50} value (0.96 μg mL^{-1}) for scavenging DPPH compared to *C. racemosa* (1.49 μg mL^{-1}) and *H. musciformis* (3.22 μg mL^{-1}). No significant difference was found between the IC_{50} value of standard ascorbic acid (0.87 μg mL^{-1}) and *P. tetrastromatica* (0.96 μg mL^{-1}) for DPPH scavenging (Fig. 4a). Similarly, the lowest IC_{50} value (5.58

Table 1
Antioxidant phytochemicals of three seaweeds.

Seaweed	TPC (mg GAE g^{-1})	TFC (mg RE g^{-1})	TTC (mg RE g^{-1})	TAC (μg g^{-1})
C. racemosa	143.08 ± 18.51 a	63.79 ± 2.16 a	73.58 ± 5.89 a	4457.67 ± 32.43 a
P. tetrastromatica	63.24 ± 9.89 b	58.41 ± 1.21 b	71.90 ± 4.31 a	4434.33 ± 35.28 a
H. musciformis	41.51 ± 1.46 b	25.70 ± 1.99 c	70.08 ± 3.94 a	4427.67 ± 31.47 a
P value	0.0001	0.0000	0.6863	0.5417
CV (%)	14.70	3.72	6.66	0.74

Note. The different letter indicates a significant difference (Least significance test analysis for mean comparison at $p \leq 0.05$) among the seaweeds whereas the same letter indicates no significant difference; GAE, Gallic acid equivalent; RE, Rutin equivalent; TPC, Total phenol content; TFC, Total flavonoid content; TTC, Total tannin content; TAC, Total antioxidant capacity.

Table 2
Pigmented antioxidant phytochemicals of three seaweeds.

Seaweed	β-carotene (mg 100 ml^{-1})	Lycopene (mg 100 ml^{-1})	Carotenoid (mg g^{-1} fresh wt.)	Total chlorophyll (mg g^{-1} fresh wt.)	Chl a (mg g^{-1} fresh wt.)	Chl b (mg g^{-1} fresh wt.)
C. racemosa	0.69 ± 0.09 ab	1.05 ± 0.08 a	0.95 ± 0.03 b	13.50 ± 0.89 a	0.51 ± 0.02 a	0.53 ± 0.02 b
P. tetrastromatica	0.86 ± 0.14 a	1.09 ± 0.04 a	1.75 ± 0.16 a	8.76 ± 0.59 b	0.003 ± 0.001 b	0.66 ± 0.03 a
H. musciformis	0.57 ± 0.02 b	0.24 ± 0.01 b	0.50 ± 0.30 c	1.50 ± 0.36 c	0.002 ± 0.001 b	0.03 ± 0.01 c
P value	0.0302	0.0000	0.0007	0.0000	0.0000	0.0000
CV (%)	14.20	7.11	18.64	8.23	6.75	5.42

Note. Different letter indicates the significant difference (Least significance test analysis for mean comparison at $p \leq 0.05$) among the seaweeds whereas the same letter indicates no significant difference. Chl a, Chlorophyll *a*; Chl b, Chlorophyll *b*.

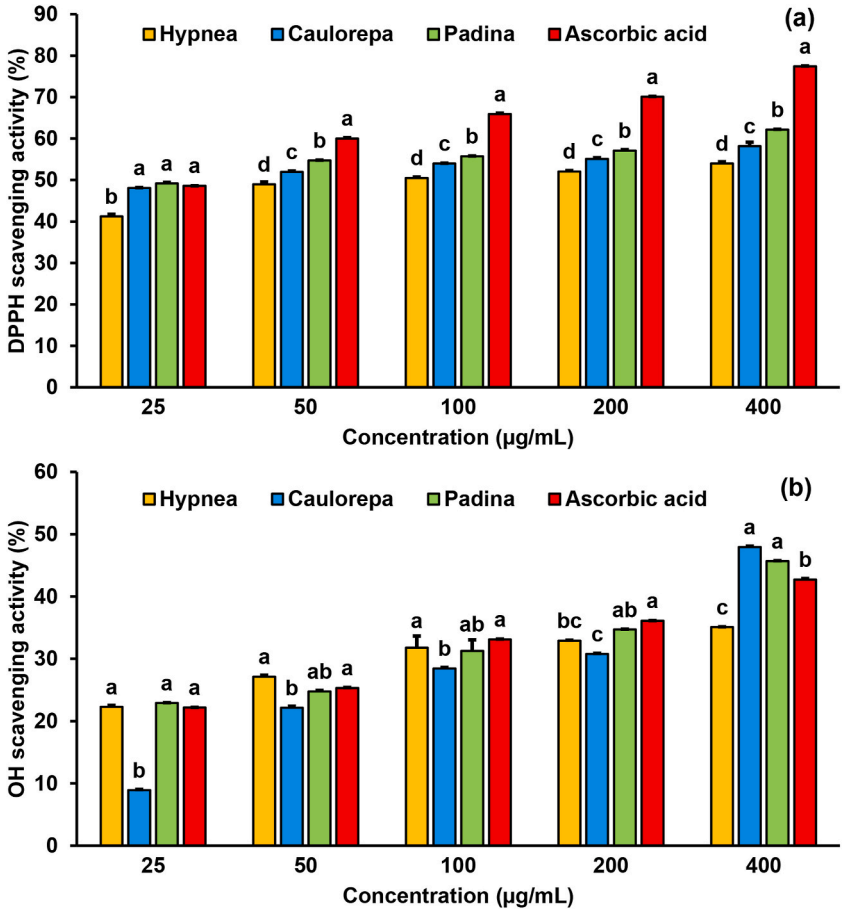

Fig. 3. (a) DPPH and (b) OH radical scavenging activity of seaweeds and positive control ascorbic acid at different concentrations. *P* value (at ≤ 5%) for DPPH and OH scavenging of seaweeds and concentrations are 0.0000, which indicates significant variations are present among the seaweeds and different concentrations.

µL mL^{-1}) for scavenging $OH^{\bullet}$ radical was observed in *C. racemosa*, which was lower when compared to the values observed in *P. tetrastromatica* (6.27 µg mL^{-1}), ascorbic acid (6.50 µg mL^{-1}) and *H. musciformis* (9.43 µg mL^{-1}), respectively (Fig. 4b).

3.5. Exploring associations of studied parameters and seaweeds through clustering heatmaps and principal component analysis

The performance of various parameters under various treatment conditions was displayed using a heatmap, and subsequently, the parameters were grouped into two major distinct clusters using the hierarchical clustering approach (Fig. 5a). The cluster-I parameters for *P. tetrastromatica* and *H. musciformis* showed a downward trend for TTC, total chlorophylls, chlorophyll *a*, TPC, and TAC, but intriguingly, *C. racemosa* showed an opposite trend for the above–mentioned parameters (Fig. 5a). Cluster-II variables displayed a considerably higher amount of beta-carotene, carotenoids, TFC, lycopene and chlorophyll *b* in *C. racemosa* and *P. tetrastromatica* compared to *H. musciformis* (Fig. 5a). Subsequently, to examine the relationship between different treatments and variables, a principal

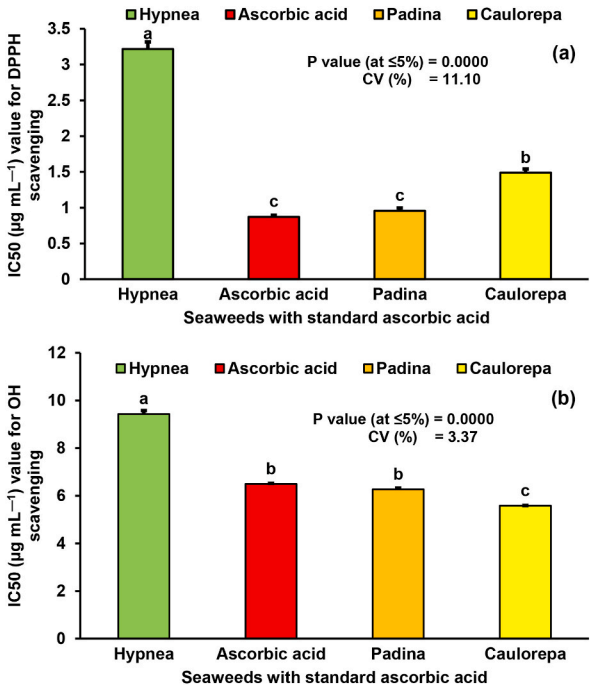

Fig. 4. IC_{50} value of the studied seaweeds for scavenging (a) DPPH and (b) OH radicals. Here, CV, Coefficient of variation.

component analysis (PCA) was executed (Fig. 5b). Together, PC1 (72.8%) and PC2 (27.2%) accounted for a substantial portion of the variability, accounting for 100.00% of the total variability observed in the data. Significantly, the parameters of this study showed a positive association with each other, and all of the variables were mostly influenced by the values of *C. racemosa* and *P. tetrastromatica* (Fig. 5a and b). Surprisingly, *P. tetrastromatica* and *C. racemosa* showed strong affinity with carotenoid, beta–carotene and TPC, TAC, and chlorophyll *a*, respectively (Fig. 5a and b). Correlation analysis revealed TPC significantly (1**) influenced the TAC whereas flavonoid (0.76), tannin (0.94), carotenoid, lycopene, chlorophyll *a* (0.98), chlorophyll *b* (0.51) and total chlorophyll (0.91) content positively related with TAC (Fig. 6).

3.6. Comparative effects of different seaweeds against different human pathogens

It is surprising to note that, all three seaweeds i.e., *H. musciformis* (Fig. 7a), *P. tetrastromatica* (Fig. 7b), and *C. racemosa* (Fig. 7c) did not exhibit any activity against the studied human pathogenic bacteria (Fig. 7).

4. Discussion

Seaweed exhibits noteworthy potential as a valuable oceanic nutrient source, boasting a wide range of therapeutic ingredients that offer numerous health benefits [37]. The marine ecosystem of south–eastern coastal area of Bangladesh hoards a diverse array of seaweeds; however, research endeavours focusing on the biochemical composition and functional properties of seaweed are still elusive. Therefore, we investigated three highly promising seaweeds, including *C. racemosa*, *P. tetrastromatica,* and *H. musciformis* to explore their phytochemical composition and evaluate their antioxidant potential through the application of spectroscopic and disc–diffusion methods.

Polyphenols, predominantly present in a wide range of plant–based foods, have recently gained momentum for their impressive positive effects on human health. These benefits include counteracting oxidative stress, mitigating the risk of chronic ailments like cardiovascular illnesses, cancer, and neurological disorders, facilitating the regulation of body weight, mitigating the risk of developing type 2 diabetes, and potentially alleviating inflammation–associated illnesses [38]. We also determined the total phenolic content within these seaweeds and observed that *C. racemosa* displayed the highest phenolic content, which implies that incorporating this seaweed into one's diet may play a pivotal role in promoting the recovery of the aforementioned health ailments. Consistent with our study, the consumption of seaweeds rich in phenolic compounds plays a crucial role in the recuperation from various human diseases, including neurodegenerative diseases, inflammatory disorders, cancer, diabetes, cardiovascular diseases (CVDs), and infectious illness [39,40]. Flavonoids, on the other hand, are phytochemical substances that are often present in a diverse range of plant-based foods and are widely employed because of their role as antibacterial, antiviral, anticancer, antiangiogenic, antioxidant, antimalarial, antitumor, neuroprotective, and anti-proliferative agents [41]. According to Dorman et al. [42], plant flavonoids that exhibit antioxidant activity in vitro also serve as antioxidants in vivo. Our findings revealed that *C. racemosa* exhibited high levels of

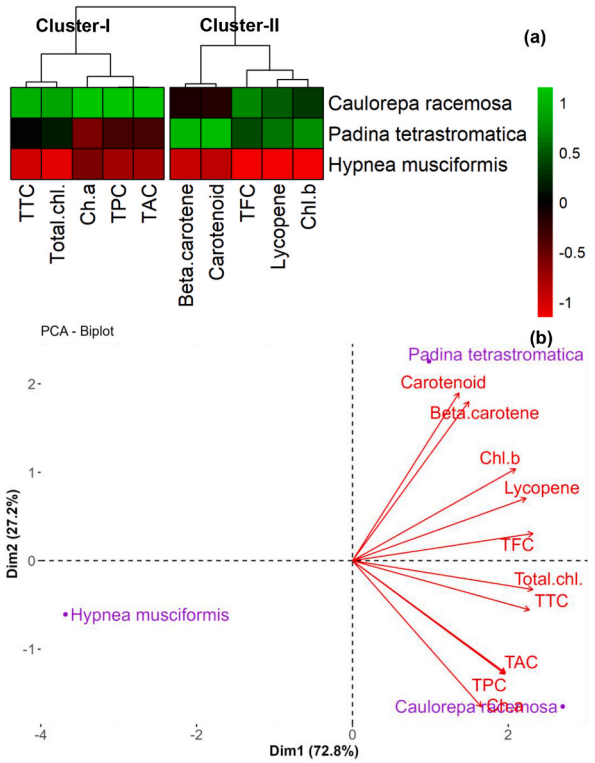

Fig. 5. (a) The clustering heatmap displayed a comprehensive visual overview of the responses of various parameters to different treatments, utilizing normalized mean values to facilitate effective comparisons (b) Principal Component Analysis (PCA) provides valuable insights into the intricate relationships between the treatments and parameters. Positive or negative associations between the parameters in respective of seaweeds are illustrated by the vector lines of the biplot. A small angle indicates a feeble association, whereas a large angle indicates a strong association. Here, TTC, total tannin content; Total chl., total chlorophyll; chl. a, chlorophyll *a*; TPC, total phenol content; TAC, total antioxidant capacity; TFC, total flavonoid content; and Chl. b, chlorophyll *b*.

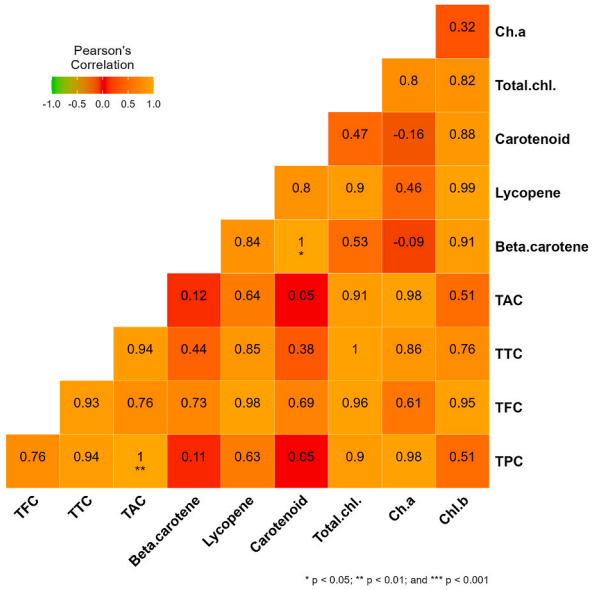

Fig. 6. Pearson's correlation study discloses the relationship among the studied parameters of seaweeds. Here, Ch. a, chlorophyll *a*; Ch. b, chlorophyll *b*; TAC, total antioxidant capacity; TTC, total tannin content; TFC, total flavonoid; TPC, total phenol content.

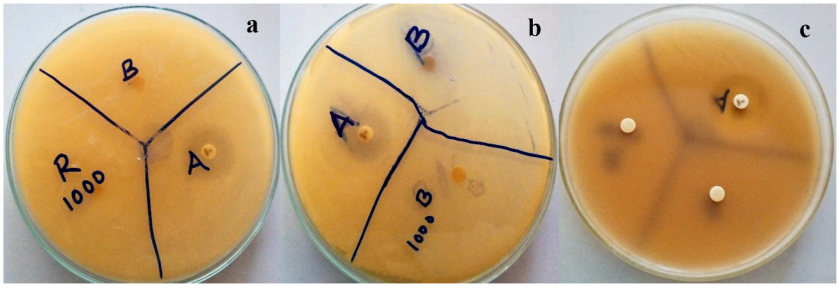

Fig. 7. A partial view of antimicrobial activity of seaweeds. Here, a. Red seaweed (*Hypnea musciformis*); b. Brown seaweed (*P. tetrastromatica*); c. Green seaweed (*C. racemosa*); A, antibiotic (Gatifloxacin); B, blank (Water). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

flavonoids, which might also play an additional role, along with total phenolic acids, in the recovery of the aforementioned diseases. Similar to our findings, a plethora of studies showed that seaweeds in diets rich in flavonoids help in the recovery of several human diseases [43]. Tannins, which are water–soluble phenolic compounds, have been well–recognized for their capacity to interact with the protein, and subsequently aid in the curing of inflammatory, aging, proliferative, cancer, and neurodegenerative diseases, such as cardiovascular diseases and Alzheimer's [44]. Although, our findings revealed no significant divergence in tannin content among the seaweeds; however, all three have the potential to be used in human diets as a source of tannin supplementation. It is important to note that we did not observe any significant variation in the total antioxidant capacity among the seaweeds, which implies that we can use these seaweeds as a source of daily diets to boost our antioxidant properties to fight against oxidative stress. To appraise the ability of the examined seaweeds to neutralize free radicals, we conducted DPPH and $OH^{\bullet}$ scavenging tests. Our findings revealed that among the various seaweeds studied, *P. tetrastromatica* exhibited superior DPPH radical scavenging capabilities, indicating its effectiveness in eliminating free radicals. Oxidative burden, predominantly caused by the excessive accumulation of reactive oxygen species (ROS), such as the $OH^{\bullet}$ radical, can have detrimental effects on a variety of health issues. Surprisingly, our research findings also highlighted that *P. tetrastromatica* displayed a significant level of $OH^{\bullet}$ radical scavenging activity, which implies its potential to be used in dietary components for minimizing the risk of diseases in humans caused by overaccumulation of ROS. IC_{50} value indicates the efficacy of a compound to scavenge 50% of the respective substrate [45]. Among the seaweeds, which displayed the lowest IC_{50} value indicates its high potentiality that means under low dose, it can scavenge 50% radicals. The stronger a substance is at scavenging DPPH and $OH^{\bullet}$, as indicated by a lower IC_{50} value, which also indicates a higher level of antioxidant activity [43,46]. To substantiate these findings, we calculated the IC_{50} value, and it was observed that *P. tetrastromatica* and *C. racemosa* exhibited the lowest IC_{50} value for DPPH and OH radical scavenging, respectively, signifying their exceptional antioxidant capacity.

Indeed, seaweed have been well–recognized as a pigment powerhouse, boasting a wide diversity of chlorophylls and carotenoids. These pigments have recently gained popularity across diverse industries, spanning from food and beverages to the frontiers of cosmetics and pharmaceuticals. These multifaceted pigments not only confer bioactive prowess, loaded with antioxidants and health benefits but also reign dominant as natural food colorants. The rapidly expanding market for food colors, which is projected to reach a staggering USD 3.75 billion by 2022, is driven by consumer demand for synthetic additives-free, resoundingly safe, and saturated in a spectrum of vibrant possibilities [47]. In our research, we also observed that seaweeds, notably *C. racemosa* and *P. tetrastromatica*, house an array of pigments in significant quantities. This underscores the potential of these seaweeds as a formidable natural colorant, given a strong focus on addressing stability concerns and meeting regulatory requirements. Their integration holds the promise of elevating both the visual allure and nutritional excellence of food products.

Marine algae can perhaps manage the numerous harmful microbes in addition to having medical value. It is commonly recognized that seaweed has antibacterial properties, however not all seaweeds are equally efficient against all microorganisms. In this experiment, three seaweeds did not show any antimicrobial activity. This event may have occurred as a result of environmental conditions or a decline in the activity of the crude extract. In addition, maybe these seaweeds are not effective against the studied pathogens of this experiment. The antimicrobial activity of marine algae depends on both species and the chemical solvents deployed for extraction [48]. Extraction is a vital step for obtaining extracts with acceptable yields and strong antioxidant activity [49,50]. The extraction efficiency is influenced by various factors such as the method of extraction, solvent concentration, and types, time of extraction, pH, temperature, and chemical compositions of the sample [51,52]. Researchers from many countries have tested the antioxidant and antibacterial properties of seaweeds using a variety of solvents, but it is still unclear which solvent is the best and most efficient for seaweed extraction [53,54]. The phytochemicals, antioxidants as well as antimicrobial results of this study, may differ from the result of other experiments because of different factors such as the climatic and geographical condition of Bangladesh, nutrient status of water, temperature, life stage, and physiological condition of the algae during harvesting, extraction process of the crude extract, etc.

5. Conclusion

Seaweeds have antioxidant potential to neutralize harmful reactive oxygen species and boost human health. This study demonstrates that the investigated seaweeds contain promising antioxidant compounds, which can be beneficial for human health. Among the seaweeds studied, *C. racemosa* and *P. tetrastromatica* were found to have the highest phenol, flavonoid, tannin content, total

antioxidant capacity and chlorophyll contents. The lowest IC_{50} value for $OH^{\bullet}$ and DPPH scavenging was observed in *C. racemosa and P. tetrastromatica*, respectively. However, none of the three seaweeds showed effectiveness against the five human pathogenic bacteria tested. Given the diversity among available antioxidant methods, the findings of a single method can not fully represent the actual antioxidant and antimicrobial properties of the seaweed extract. Therefore, the application of multiple methods is necessary for a more comprehensive comparison of the findings. Further studies are needed to investigate additional compounds using various solvents for the studied seaweeds, especially in the context of Bangladesh.

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Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Omma Honey: Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sheikh Arafat Islam Nihad:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Conceptualization. **Md. Atiar Rahman:** Writing – review & editing, Supervision, Resources, Methodology, Investigation. **Md. Mezanur Rahman:** Writing – review & editing, Visualization, Formal analysis. **Mahibul Islam:** Visualization, Methodology. **Mohammed Zahedur Rahman Chowdhury:** Writing – review & editing, Visualization, Supervision, Resources, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26096.

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