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Phytochemical analysis of *Turbinaria Ornata* and *Sargassum latifolium* collected from Red sea, Egypt+

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Abstract: Seaweeds are marine macroscopic algae, which play a vital role in marine living organisms. Secondary metabolites (phytochemicals) have been intensively studied as potential therapeutic agents. Brown seaweeds (Phaeophyceae) such as *Sargassum latifolium* and *Turbinaria Ornata* were collected in winter season from Dahab red seacoast of Egypt. *Sargassum latifolium* and *Turbinaria Ornata* are rich in carbohydrates being44.52 and 40.2 mg/g dry weight, respectively. *Sargassum Latifolium* recorded a higher protein content of 6.48 2 mg/g dry weight), whereas *Turbenaria Ornata* reported a higher lipid content being 1.9 2 mg/g dry weight. Moreover, the phenolic, flavonoids, alkaloids and tannin contents were quantitatively estimated in methanolic extract of algae under study. The Phenolic content was found in a higher amount in *Sargassum latifolium* being 2.71 ± 0.01 mg of GAE/gm dry wt weight while *the Turbinaria Ornata alga* recorded a higher content of flavonoids, tannins and alkaloids being41.5 mg quercetin acid – 81.55 mg TAE acid – 3.21 mg GAE acid / g dry weight, respectively. *Sargassum latifolium* showed a higher antioxidant activity with IC_{50} being1.9 mg/mL whereas *Sargassum latifolium* showed a higher antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* with an inhibition zone being13±0.024 mm and 15.66±0.3 mm, respectively. Conclusively, these results signify the vital role for those algae in the medical field.

Key words: brown algae, secondary metabolites, carbohydrates, proteins, Lipid, Antioxidant, Antibacterial

Introduction

The oceans are thought to have a unique collection of abundant bioactive metabolites which derived from algae, hydrophytes and so many microbial sources. With Especial concern macroalgae are the greatest producer for valuable secondary metabolites which have a great effect in medical field [1, 2]. About 10,000 metabolites have been isolated according to the investigation of new metabolites from aquatic environments, many of which having pharmacodynamics qualities. Algae are potential renewable, photosynthetic aquatic organisms [3], which found in different aquatic environments [2, 4].Macroalgae (Seaweeds) are divided according to their pigment pattern into 3 groups According to [5] ; Chlorophyceae (green algae), Phaeophyceae (brown algae) ,Rhodophyceae (red algae). They are very rich in proteins, carbohydrates, fats, fibers, vitamins (A, C, K,), and some essential minerals like Magnesium, selenium and potassium. Algae are regarded as an significant source for secondary metabolites which have a great impact on improvement of drugs production, Since they have a high pharmaceutical potential as antioxidant [6], antibacterial [7], anti-inflammatory, anti-viral([8]), anti-fungal [9] and anti-cancer([10]). Nowadays, algae are used widely in pharmaceutical sector because of the lowest side effects of those natural based products in comparison to the common chemical drugs.

Phaeophyceae have an abundance of bioactive compounds, including fucoxanthin

and golden-brown xanthophyll pigments. The massive amounts of fucoxanthin and carotenoid that cover the remaining pigments, carotenes and other xanthophylls, such as chlorophyll a and c, give the brown algae their brownish color. Alginic acid is the main component of their cell wall which is very important in the industrial field. As well as they are utilized in medical field in treatment of serious diseases such as cancer, fibromyalgia, arthritis, heart disease, high cholesterol and also stress, cough, stomach problems and so many other health problems [11, 12]. In addition to its use in skincare for the high antioxidant activity [13], brown seaweeds are also used to promote weight loss [14].

This study aims to investigate the phytochemical analysis, antioxidant and antibacterial potentiality of the two-brown algal species, *i.e.*, *Sargassum latifolium* and *Turbenaria Ornata*.

Materials and methods

Macroalgae collection and preparation

The *Sargassum latifolium* and *Turbinaria Ornata* were collected at summer season from the shallow water of Dahab city from the 3 pools area and blue lagoon. Macroalgae samples were taken to the algae biotechnology and water quality lab at the Botany Department, Faculty of Science, Mansoura University, Egypt. Right away for cleaning and extraction. The algae were washed several times to remove shells or sand residues and then air-dried for several days. The samples were ground into a fine powder for the extraction step. The macroalgae samples were identified according to Aleem and Bhavanath Jha [15] and [16].

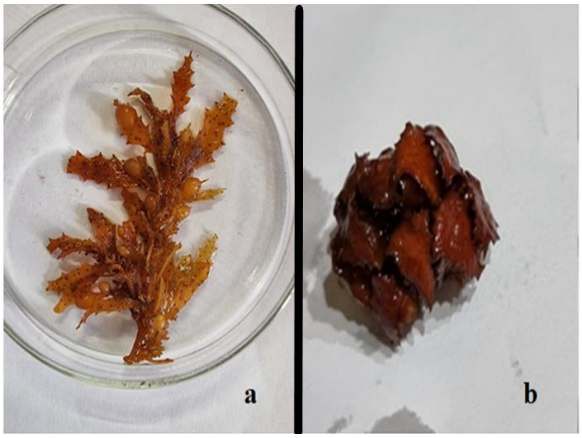

Fig (1): *Sargassum latifolium* (a) *Turbinaria Ornata* (b)

Macroalgae Samples

Biochemical analysis for the investigated macroalgae

Total carbohydrate content

Total carbohydrate content was estimated using colorimetric phenol-sulphuric acid method [17].The absorbance were read at 490 nm using a spectrophotometer and glucose was used as standard.

Total soluble protein content

The estimation of total soluble protein content was determined according to the protocol of Lowry [18] using Bovine Serum Albumin (BSA) as a standard.

Total lipid content

The total lipid content was estimated gravimetrically according to Bligh and dyer [19]. Lipids were extracted by a

mixture of chloroform: methanol 2:1 ratio solvent and leaving overnight, collect the supernatant and repeat this process for total lipid extraction (3 times), then the total supernatants were taken and NaCl (0.9N) was added to separate the upper layer (methanol + NaCl) from the lower layer (chloroform + lipid content), then the lower layer was collected and left to dry to estimate lipid content.

Crude Extraction

A mass of 20 g air-dried powdered algal biomass was soaked in 200 ml of 80 % methanol for 5 days in dark with continuous shaking using rotary shaker at 200 rpm to ensure homogeneity. Then the mixture was filtered and concentrated using rotary evaporator (Stuart RE300) at 40 ºC under reduced pressure. The resultant residue was weighed and kept at -20 ºC for further analysis.

Quantitative phytochemical analysis

Total phenolic content

Folin-Ciocalteu (F-C) reagent was used to estimate the total phenolic content in the prepared algal extracts according to the method of [20] and [21].

Gallic acid was used as a standard, whereas data was calculated as milligram Gallic acid equivalents/grams of dry biomass.

Total Flavonoid Content

Total flavonoid content was performed using the aluminum chloride colorimetric assay according to the method described by [22].A mixture of 100 µL algal extract and 100 µL of aluminum Chloride solution (2 % w/v) was prepared and incubated at room temperature for 20 min. The total amount of flavonoids in each algae extract was calculated as mg of catechin equivalent / g of the dry biomass

Total Tannin Content

Total tannin content of macroalgae methanolic extract was estimated following **t**he vanillin-hydrochloride assay [23], which measures the sample's absorbance following treatment with freshly prepared vanillin-hydrochloride, was used to analyze the tannin content. mg tannic acid equivalents per gram of dry algal biomass were used to express the determined tannin content values for the tested samples. The standard curve for tannins was used to calculate the quantity of tannins present in the samples under study.

Total alkaloids content

After dissolving 0.3 ml of the algal extract sample in 2% dimethyl sulfoxide (DMSO), 1 ml of 2 N HCl was added, and the mixture was filtered. Move the filtrate into a separating funnel and rinse with 10 milliliters of chloroform, 1 milliliter of 0.1 N NaOH, 5 milliliters of bromocresol green solution, and 5 milliliters of phosphate buffer (PH 4.7). After giving the mixture a good shake, the complex was extracted using 1, 2, 3, and 4 milliliters of chloroform. The atropine reference standard solutions (20, 40, 60, 80, and 100 µg/ml) were produced concurrently with the sample to generate the calibration. After 30 minutes, the absorbance was measured at 470 nm using a UV-Visible spectrophotometer (Genway, Japan). By averaging three readings, the total alkaloid content was calculated in milligrams [24] [25].

DPPH (1, 1 diphenyl 2- picryl hydrazyl) assay

The DPPH colorimetric method [26, 27]was used to assess the antioxidant capacity of the test algae methanolic extracts. Ascorbic acid was used as a standard. Using methanol, successive dilutions of each sample were created. Each sample received a 1 mL addition of a 0.135 mM DPPH solution. The samples were kept at room temperature in the dark for 30 min. At 517 nm, changes in sample absorbance were detected. The extract was replaced with methanol to create a control reading. The free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula:

$$\text{Inhibition } (\%) = \left[\frac{(\text{A0} - \text{At})}{\text{A0}}\right] \text{X } 100$$

Where A_0 and A_t are the absorbance of the control (DPPH) and test samples, respectively. To determine the IC_{50} value for each algae sample, the values of % DPPH remaining were plotted against mg extract/mL using an exponential curve.

Antibacterial Activity

The antibacterial effects of two algal extracts against a Gram-negative bacterial pathogen., *Escherichia coli* ATCC8739 and a Gram-positive bacterial pathogen., *Staphylococcus aureus* ATCC 25923 *in vitro*. The compound effect was carried out by the disc diffusion method following US CLSI (Clinical and Laboratory Standards Institute, 2015) and [28], [29], [30]. The algal extracts were dissolved in 0.5% dimethyl sulfoxide (DMSO, Sigma-Aldrich, USA) to a final concentrations (250 µg/ml) algal extract in 1mL of DMSO. The bacterial strains were grown on Muller-Hinton broth medium for 7 days at 37 °C and pH7. Müeller-Hinton agar (MHA) plates were prepared and inoculated with bacterial pathogens (approximately 10^5 CFU/mL). After solidifying, wells were punched out using 0.6 cm cork-borer. The algal extract was sterilized by filtration on 0.45 µm Millipore filters. Then, 30 µL (250µg/ml DMSO) of this extract was pipetted into each well and left for one hour in refrigerator for compound diffusion. The DMSO was used as a negative control, as compared with 10 µg/30 µL (0.5% DMSO) gentamicin standard antibiotics per 5mm paper disc. The plates were used in triplicate for each treatment. All plates were incubated at 37±2 °C for 24 h. After the incubation period, the diameter of the clear

zone around each well was linearly measured in millimeters (mm).

Statistical analysis of results

All determinations were performed in triplicates. All data were expressed in terms of mean ± SD and analyzed for variance and the least significant difference (LSD) using One-way ANOVA ($P < 0.05$). SPSS version 18.0 for windows was used in this study.

Results and discussion

Biochemical analysis

The carbohydrates fraction (mg/g dry wt) of each investigated macroalgae are presented in figure (3). *Sargassum latifolium* documented the highest carbohydrate content with ± concentration of **44.5** mg/g dry wt, while *Turbinaria Ornata* recorded the lowest concentration (**40** mg/g dry wt).

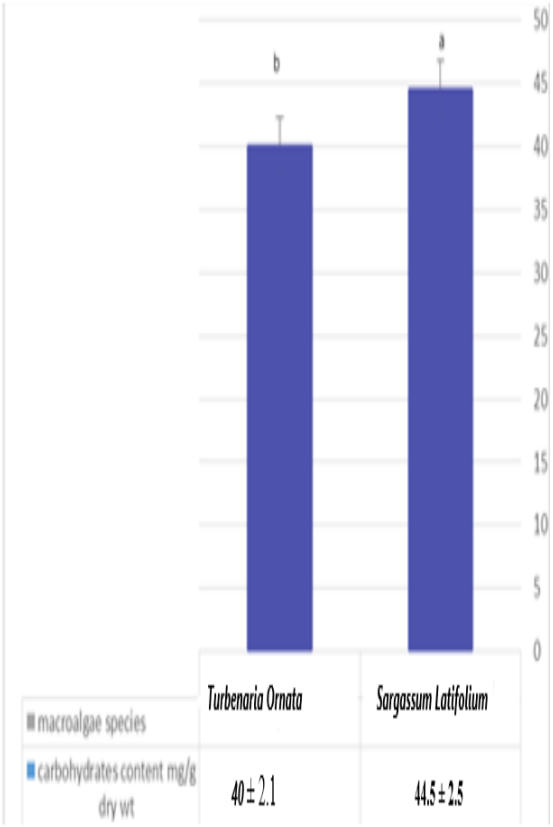

Fig (2): Carbohydrate's content of *Sargassum latifolium and Turbinaria Ornata (mg/g dry weight)*. Data are presented as Mean ± SD of three replications, different letters indicate significant differences at (P≤ 0.05)

Total soluble proteins (mg/ g dry weight) for the two algae *Sargassum latifolium and Turbinaria Ornata* demonstrated in figure (4). *Sargassum latifolium* recorded the highest protein content **6.38** mg/g dry biomass whereas *Turbinaria Ornata* documented **5.5** mg/g dry weight.

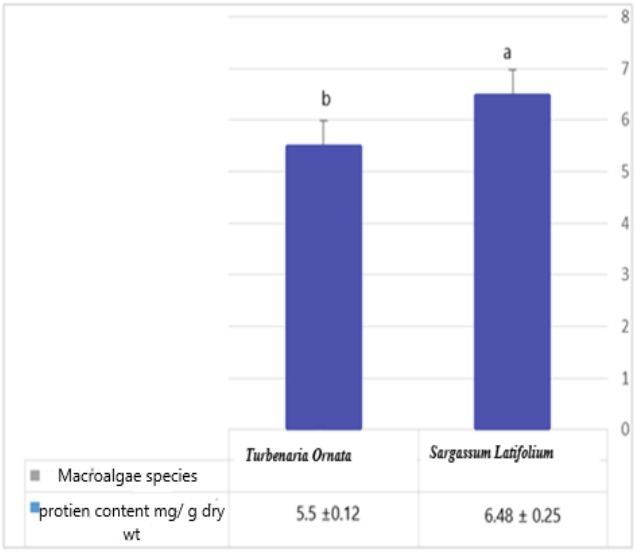

Fig (3): Total soluble protein content (mg/g dry wt) for *Turbinaria Ornata and Sargassum latifolium*. Data are presented as Mean ± SD of three replications, different letters indicate significant differences at (P≤0.05)

Total lipid content (mg/ g dry weight) for both *Sargassum latifolium* and *Turbinaria Ornata* illustrated in figure (5). *Turbinaria Ornata* recorded the highest lipid content **1.9** mg/g dry wt, Whereas *Sargassum latifolium* documented **1.12** mg/g dry wt.

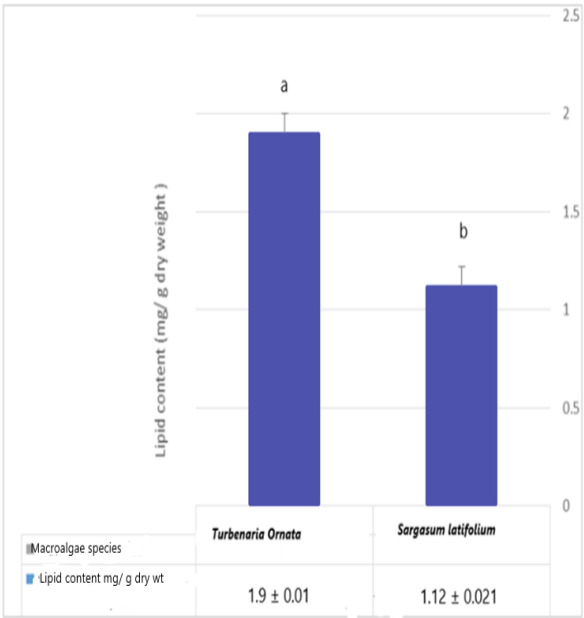

Fig (4): Total lipid content (mg/g dry wt) Sargassum latifolium and Turbinaria Ornata. Data are presented as Mean ± SD of three

replications, different letters indicate significant differences at (P≤0.05)

The total carbohydrate content for *Sargassum latifolium* was reported 45 mg/g dry wt according to [31] , while *sargassum vulgare* documented 41.33 45 mg/g dry wt according to [2] and *Sargassum muticum* reported 27.32 ± 7.25 mg /g dry wt in the study [32], while the carbohydrates content of *Turbenaria triquetra* recorded 163 mg/g dry wt as described by [33].

Protein content of *sargassum latifolium* recorded 6.38 mg/g dry wt [31] while the protein content for *Sargassum muticum* recorded 8.29 ± 0.67 % as [32] whereas protein content for *Turbenaria triquetra* recorded 40 mg/g dry wt according to [33].

Lipid content for *sargassum muticum* was recorded 0.94 ± 0.06% [32] while *Turbenaria Ornata* reported 2.5 mg/g dry wt lipid content according to [34].

Quantitative phytochemical analyses of the tested macroalgae

Phytochemical composition for the methanolic extracts of the two tested macroalgae are genus dependent. The total amount of phenolic, flavonoids, tannins and alkaloids (mg/g dry weight) for investigated macroalgae are listed in table (3) and demonstrated in figure (8) and (9).

Sargassum latifolium verified the highest quantity of phenols (2.7 mg GAE/g dry wt), while the lowest amount recorded to *Turbinaria Ornata* (1.2 mg GAE/gm dry wt). *Turbinaria Ornata* recorded higher level of flavonoids (41.5 mg of Quercetin /g dry wt) than *Sargassum latifolium* which recorded (38.33 ± 0.3 mg of CAE/gm dry wt). *Turbenaria Ornata* recorded a higher level of tannins recorded 81.55 mg of TAE/gm dry wt than *Sargassum latifolium* which recorded 77.5 ± 0.65 mg of TAE/gm dry wt. Concerning alkaloids content, T*urbenaria Ornata* recorded the higher level of alkaloids (3.2 mg GAE/gm dry wt) while *Sargassum latifolium* recorded (2.65 ± 0.11 mg of GAE/gm dry wt.

Table (3): Phytochemical analysis of methanolic extracts for *Turbinaria Ornata* and *Sargassum latifolium*

Macroalgae species	**Phenolic Content** (mg GAE/gm dry wt)	**Flavonoids Content** (mg quercetin /gm dry wt)	**Tannins Content** (mg TAE/gm dry wt)	**Alkaloids content** (mg GAE/gm dry wt)
T. Ornata	1.2 ± 0.1	41.5 ± 0.4	81.55 ± 0.87	3.21 ± 0.12
S. latifolium	2.71 ± 0.17	38.33 ± 0.3	77.5 ± 0.56	2.65 ± 0.11

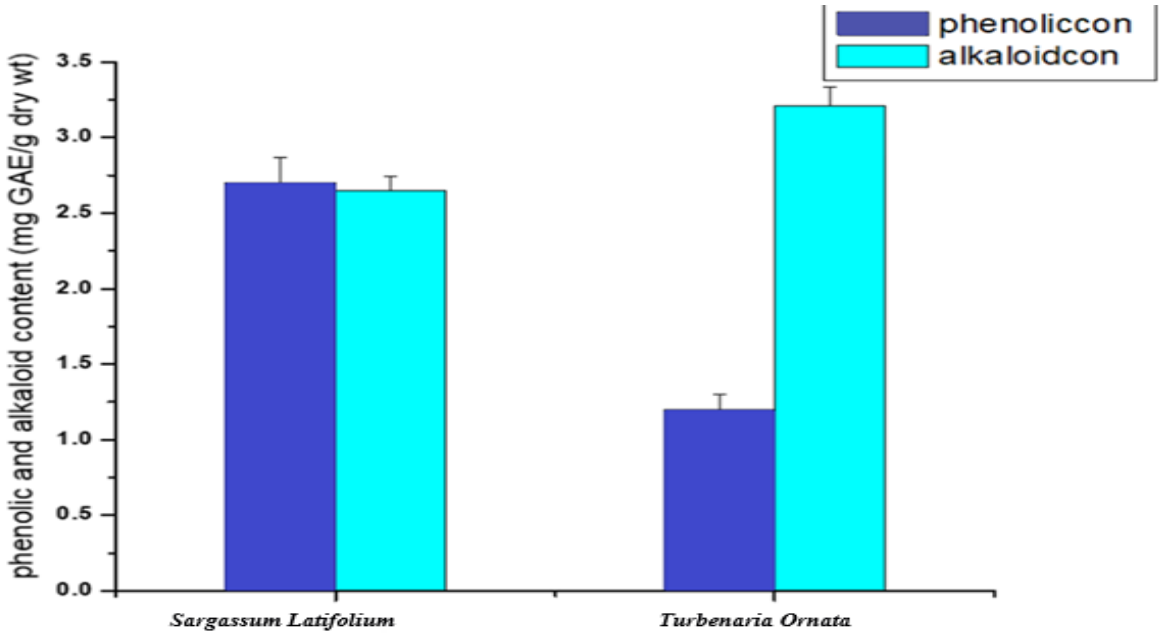

Fig (5): phenolic and alkaloid contents for *sargassum latifolium* and *Turbinaria Ornata* (mg GAE/g dry wt). Data are presented as Mean ± SD of three replications, different letters indicate significant differences at (P≤ 0.05)

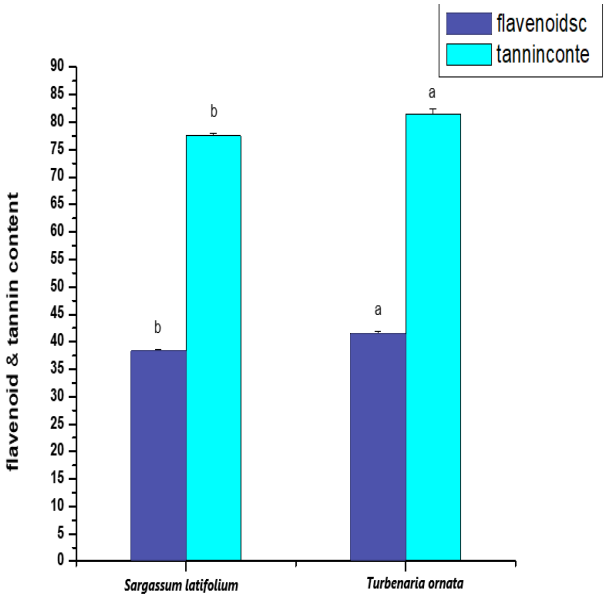

Fig (6): Flavonoid and tannin content for *Sargassum latifolium* and *Turbinaria Ornata*. Data are presented as Mean ± SD of three replications, different letters indicate significant differences at (P≤ 0.05).

Studies shows that the phenolic content for methanolic extract of *Turbenaria Ornata* being 2.18 mg catechin/g dry seaweed, while the *Sargassum polycystum* recorded 0.59 mg catechin/g dry seaweed according to [35] and in other study for [36] shows that methanolic extract for *T.Ornata* recorded 43.72 ± 1.63 mg GAE/g . Flavonoids content of *Sargassum fusiforme* was 19% of dry biomass as described by [37] while the flavonoids content for the ethyl acetate extract of *turbinaria decurrens* was obtained of 4.8 mgEQ/g (mg Equivalent quercetin/g) extract according to [38]. Alkaloid content for *Sargassum fusiforme* recorded 0.33% of dry biomass according to [37] while the alkaloid content in methanolic extract of T.Ornata found to be absent as described in [39].Tannin content of Sargassum sp. recorded 0.464 ± 0.136% according to [40]

Biological activity of *S. latifolium* and *T. Ornata* extracts

Applying DPPH free radical scavenging assay to investigate the antioxidant activity of the two tested macroalgae expressed as IC50 (mg/mL) for both *S. latifolium* and *T. Ornata* was depicted in fig (6). *Turbenaria Ornata* recorded a higher antioxidant activity 1.9 ± 0.055 mg/mL than *Sargassum latifolium* which recorded 2.5 ± 0.078 mg/mL when compared with ascorbic acid 0.0222±0.0007 mg/mL.

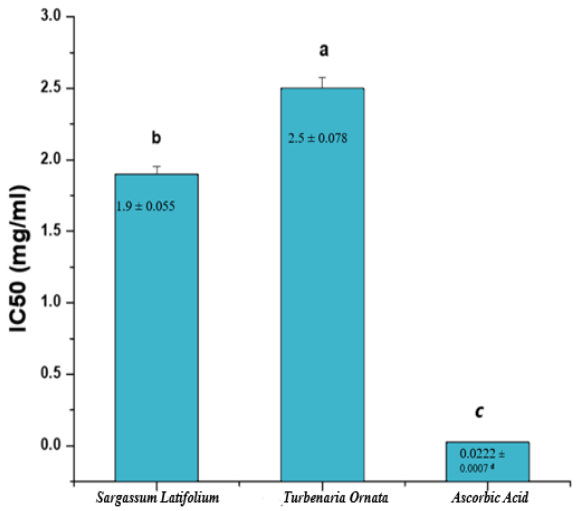

Fig (7): IC50 values (mg/mL dry wt) for the two investigated algae. Data are presented as Mean ± SD of three replications, different letters indicate significant differences at (P≤0.05)

It was found that IC50 for *Sargassum binderi* was 4.053 mg/L according to [41], While ethyl acetate extract of *Turbenaria conoides* recorded IC50 12.22 ± 0.17 % [42].

Antibacterial Activity

Both macroalgae species recorded high antibacterial activity against the two human pathogens *Escherichia coli* and *staphylococcus aureus* compared to the used standards (antibiotics) demonstrated in table (2) and figure (7) .*Sargassum latifolium* recorded higher antibacterial potentiality against both E.coli (gram negative bacteria) and *staphylococcus aureus (*gram positive bacteria) with inhibition zone 13.5 ,15.66 mm, respectively while the inhibition zones for *Turbenaria Ornata* recorded 12.8 , 13.5 mm respectively.

Table (2): the antibacterial activity against two different bacterial species expressed in (mm)

Samples	Bacterial strains	
	S. aureus	***E. coli***
Sargassum latifolium	15.66 ± 0.3mm	13 ± 0.024 mm
Turbenaria Ornata	13.5 ± 0.51 mm	12.8 ± 0.02 mm
DMSO	+ve	-ve
CTX (Cefotaxime) 30mg	10.6 ± 0.43	-
CAZ (Ceftazidime) 30mg	-	11.3 ± 0.65

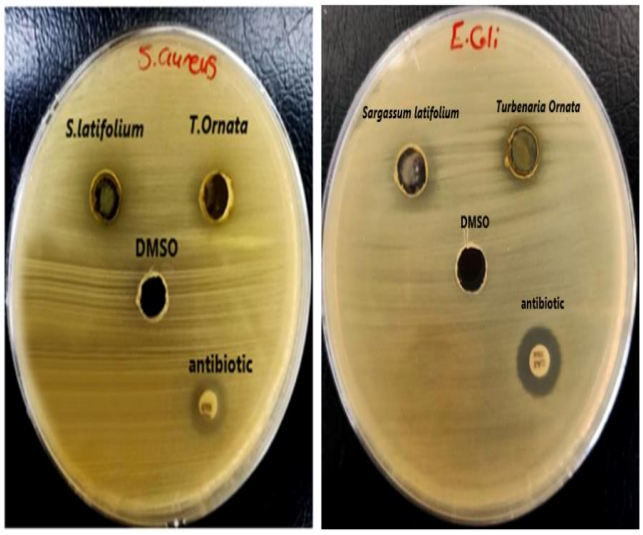

Fig (8): Antibacterial activity of *Sargassum latifolium* and *Turbenaria Ornata* methanolic extract against *E. coli* and *Staphylococcus aureus*. Data are presented as Mean ± SD of three replications, different letters indicate significant differences at (P≤0.05).

Previous Studies of *[43]* showed that *Turbenaria Ornata* recorded 16 mm inhibition zone against *E.coli* and recorded 15 mm inhibition zone against *s.aureus*, while *sargassum wightii* recorded 18 mm inhibition zone against *E.coli* and 9 mm inhibition zone against *s.aureus*

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