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Evaluation of bioactive phytochemical characterization, antioxidant, antimicrobial, and antihemolytic properties of some seaweeds collected from Red Sea coast, Egypt.

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ABSTRACT

This study aimed to evaluate total phenolics (TPC) and flavonoid (TFC) contents, antioxidant, antihemolytic, and antimicrobial properties of different seaweed ethanolic crude extracts that were collected from the Red Sea coast, Egypt. These extracts were obtained from ten different species of seaweeds including *Padina boergesnii, Sargassum subrepandum M Alam, Sargassum subrepandum Hur, Sargassum aquifolium, Sargassum cinerum M Alam, and Sargassum cinerum Mangroove* fromPheaophytae, *Codium tomentosum, Dictyota dichotoma,* and *Halimeda opunta* from Chlorophytae, and *Laurancia papilosa* from Rhodophytae. Furthermore, The ethanolic extracts were analyzed for total phenolic (TPC) and total flavonoid content (TFC), the antioxidant activities including; 1,1-diphenyl-2-picryl-hydrazil (DPPH), Nitric Oxide (NO), and Hydrogen Peroxide (H_2O_2) scavenging activities. In addition, the antihemolytic and antimicrobial activities were estimated and the phenolic compounds (phenolic acids and flavonoids) were evaluated by HPLC. Among the ten species, extract of *C. tomentosum* and *S. aquifolium* has recorded the highest value of TPC (18.83±1.62 and 18.51±2.22 mg equivalent/g dry extract, respectively) and the TFC was 3.77±0.41 and 3.45±0.41 mg equivalent/g dry extract, respectively. Also, the two species showed significantly greater radical scavenging ability in DPPH radicals (IC50 5.41 and 5.51 mg/ml, respectively), NO (IC50 5.31 and 8.24 mg/ml, respectively) and H_2O_2 (IC50 0.425 and 0.332 mg/ml, respectively). Interestingly, the crude extract of *C. tomentosum* and *S. aquifolium* showed the lowest hemolytic activities however, they showed strong antimicrobial activity. Conclusively, this study highlights the interesting candidates for the pharmaceutical and medicinal applications owing to their antioxidant and antimicrobial activities, which may lay the foundation for developing a new therapeutic intervention from seaweeds.

INTRODUCTION

For decades, natural products are used to treatment of several diseases. These products are proven to be effective, economic, and available. As a consequence of an

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increasing demand for using of the natural products in the therapeutically drugs manufacture, there is a greater interest in marine organisms, especially algae **(Suleiman *et al*., 2019)**.

Seaweeds or marine macroalgae are primitive non-flowering plants without true root, stem, and leaves. These macroalgae are classified into three major groups including Pheaophytae (brown algae), Chlorophytae (green algae), and Rhodophytae (red algae) **(Moubayed *et al*., 2017; Rashad & El-Chaghaby, 2020).** Seaweeds are considered as a source of a great variety of bioactive secondary metabolites with potent biological activities such as antioxidant, anticoagulant, antiviral, antibacterial, antifungal, antimutagenic, and anticancer activities **(Greenwell & Rahman, 2015; Alagan *et al*., 2017).** Under sever harmful environments; seaweeds produce powerful secondary metabolites as a protective defense system **(Kumar *et al*., 2009; Chanda *et al*., 2010; Nagshetty *et al*., 2010; Poore *et al*., 2013).**

The potent medicinal antioxidant and antimicrobial efficacies of seaweeds in dried samples considered greater than these of fresh forms. Furthermore, marine macroaglae included several biomedical antioxidant and antimicrobial agents such as acrylic acid, chlorellin derivatives, carotenoids, terpenoids, xanthophylls, and halogenated aliphatic and sulfur-containing heterocyclic compounds as well as alginate, carrageenan, and agar as phycocolliods, which had free radicals scavenging activities and bacteriostatic and bactericidal properties **(Rattaya *et al*., 2015; El-Sheekh *et al*., 2020)**. Also, seaweeds contained many bioactive phytochemicals such as vitamins, riboflavin, minerals, polyunsaturated fatty acids, sterols, proteins, polysaccharides, tocopherols, and pigments **(Alagan *et al*., 2017)**. Previous studies were demonstrated that seaweeds had high natural phytochemical antioxidant agents such as phenolics, tannins, glycosides, flavonoids, and alkaloids that associated in treatment of different chronic diseases **(Suleria *et al.*, 2015; Moubayed *et al*., 2017; El-Sheekh *et al*., 2020).** The treatment of infectious diseases by using of the antimicrobial medicine shown limitations because of the altering patterns of the resistance pathogens and the side effects of they made. These limitations required searching for new natural antimicrobial compounds with improved pharmacokinetic properties from traditional and ancient sources **(Takó *et al*., 2020).** The macroalgal bioactive metabolites have powerful antioxidant and anti-inflammatory efficacies that may protect and support of the human and animal health **(Varijakzhan *et al*., 2021).**

Oxidative stress responses are stimulated by overproduction of free radicals and proinflammatory mediators and cause intracellular damage and the pathogenesis of human chronic diseases such as coronary heart disease, atherosclerosis, diabetes, hepatocytotoxicity, cancer, and aging **(Alagan *et al*., 2017; Alemany-Cosme *et al*., 2021).** Reactive oxygen and nitrogen species (RONS) are free radicals and oxidative stress biomarkers **(Hussain *et al*., 2016).** Polyphenolics are considered bioactive

secondary metabolites, which considered as free radicals eliminators and metal chelators **(Ali *et al*., 2021).** These metabolites had potent scavenging activities towards nitric oxide and hydrogen peroxide, as natural antioxidants, which lowered the lipid peroxidation **(Takó *et al*., 2021).** This study aimed to evaluate of the qualitative and quantitative phytochemical screening (total phenolics, total flavonoids by HPLC analysis), as well as antioxidant, antihemolytic, and antimicrobial activities of marine brown, green, and red seaweeds that collected from Red Sea coast, Egypt.

MATERIALS AND METHODS

- **Seaweeds collection and processing:**

Marine macroalgal samples were collected from three different study sites along the north western coast of the Red Sea, Egypt. These locations are beside of the National Institute of Oceanography and Fisheries (NIOF) located in between latitudes 27° 17\ 13\\ N and longitudes 33° 46\ 21\\ E, about 17 km south of Safaga near to the mangrove forest located between latitudes 26° 36\ 59\\ N and longitudes 34° 00\ 41\\ E, and Wadi El Gemal National Park, Marsa Alam located between latitudes 24° 16\ 51\\ N and longtudes 35° 23\ 12.7\\ E. Furthermore, the collected seaweed species were *Padina boergesnii, Sargassum subrepandum M Alam, Sargassum subrepandum Hur, Sargassum aquifolium, Sargassum cinerum M Alam, and Sargassum cinerum Mangroove* from Pheaophytae, *Codium tomentosum, Dictyota dichotoma,* and *Halimeda opunta* from Chlorophytae, and *Laurancia papilosa* from Rhodophytae.

Marine macroalgal species were identified according to their morphological characterizations including pigments and reproductive structure of the vegetative thallus long by using taxonomic references **(Aleem, 1978; Coppejans & Beeckman, 1990; Sahoo *et al*., 2001)**. Healthy macroalgal samples were harvested manually and washed with seawater to remove all impurities and sand particles. These samples were transported in an icebox to the laboratory in sterilized bags. In the laboratory, macroalgal samples were washed using tap water and then washed again with sterilized distilled water. The different algal samples were placed in a dryer with a good air circulating system and a temperature controlling thermostat for one week to prevent photolysis and thermal degradation, then all samples were dried in a hot oven at 60-65 °C. These dried samples were ground with a mechanical grinding mill to produce a fine powder. This powder was stored in sealed sterilized polyethylene bags at -20 °C until usage **(Moubayed *et al*., 2017).**

- **Marine macroalgal samples extraction:**

The dried macroalgal samples were extracted by soaking of their fine powder in 70% ethanol in sealed containers at a rotator shaker with continuous shaking and stirring for 7 days at 200 rpm and 25 °C. Macroalgal alcoholic samples were filtered by using

filter papers and cotton. Each different alcoholic algal sample extract solution was concentrated and evaporated by using a rotary evaporator under vacuum at 45-65 °C. Also, each concentrated hydrated algal extract solution was lyophilized using lyophilizer and the resulted freeze dried algal forms weighted and stored at -20 °C until assayed **(El-Sheekh *et al.*, 2020)**. The stock solutions of all different seaweed dried forms were prepared to final concentration of 10 mg/ml. Dilutions were made to obtain concentrations 8, 6, 4, and 2 mg/ml. Ascorbic acid was introduced as a reference standard.

- **Determination of the antioxidant activities of the different marine macroalgal ethanolic extracts:**

The total phenolic contents:

Total phenolic contents (TPC) of the different marine macroalgal ethanolic extracts were determined by Folin-Ciocalteu colorimetric method described by **Taga *et al.* (1984)** with minor modifications. Briefly, 20 µl of each marine macroalgal extract or catechin at different dilutions, was mixed with 100 µl of 1:10 Folin-Ciocalteu reagent followed by addition of Na_2CO_3 solution (80 µl, 7.5%). After incubation at room temperature for 2 hrs in the dark, the absorbance was recorded with microplate reader at 760 nm. Catechin was introduced as a reference standard. TPC was expressed as mg catechin equivalents/g of dried extract (mg CAE/g extract) **(Antolovich *et al.*, 2002).**

The total flavonoid contents:

Total flavonoid contents (TFC) were detected by aluminium chloride colorimetric method, which described by **Zhishen *et al.* (1999)** with minor modifications. Briefly, 20 µl of each marine macroalgal extract or gallic acid at different dilutions, was mixed with aluminium chloride (20 µl, 10%), potassium acetate (20 µl, 1 M), and distilled water (180 µl), and then left the mixture at room temperature for 30 min. The absorbance of the reaction was recorded with microplate reader at 415 nm. Gallic acid was used as a reference standard. TFC was expressed as mg gallic acid equivalents/g of dried extract (mg GAE/g extract) **(Silva & Paiva, 2012)**.

DPPH scavenging activity (%) assay:

1,1-dipheny-2-picrylhydrazyl (DPPH) scavenging activity (%) of all different seaweed extracts was estimated according to the method of **Zhang *et al.* (2007)** with minor modifications. Briefly, 100 µl of each marine macroalgal extract at different dilutions, was mixed with 100 µl of 0.16 mM DPPH solution .The mixture was vortexed for 1 min, kept for 30 min in the dark and then, the absorbance was measured at 517 nm spectrophotometrically in microplate reader to determine the degree of inhibition (%) for DPPH free radicals **(Zhang *et al.*, 2007).**

Nitric oxide scavenging activity (%) assay:

Nitric oxide ($NO^{\cdot}$) scavenging activity (%) was measured by Griess reaction method with slight modifications (**Marcocci *et al*., 1994**). One milliliter from each several dilutions of all different marine macroalgal extracts was treated with 3 ml of 10 mM sodium nitroprusside in phosphate buffer. The resulting solution was incubated at 25°C for 150 min. From this solution, 0.5 ml was taken and 1 ml of 0.33% Sulphanilic acid was added and incubated at 25 °C for 5 min. Furthermore, 1 ml of 0.1% Napthylethylenediamine dihydrochloride was added and incubated at 25°C for 30 min. The absorbance of the formed pink chromophore azo dye was determined at 546 nm. Also, sample blank tubes were prepared without adding of 10 mM sodium nitroprusside buffered solution in the mixture. Moreover, control tubes were performed with distilled water or phosphate buffer instead of seaweed samples. All tubes were confirmed in triplicates (**Vadnere *et al*., 2012; Narasimhan *et al*., 2013**).

Hydrogen peroxide scavenging activity (%) assay:

Hydrogen peroxide (H_2O_2) scavenging activity was determined by using the standard H_2O_2 free radical reaction method with minor modifications **(Domitrović *et al*., 2013; Afsar *et al*., 2016).** One milliliter from each several dilutions of all different marine macroalgal extracts was treated with 0.6 ml of 40 mM H_2O_2 solution that prepared in phosphate buffer solution pH 7.4. After incubation at 37 °C for 10 min, the absorbance of all tubes was measured at 230 nm. Sample blank tubes used phosphate buffer solution instead of H_2O_2 solution (0.6 ml, 40 mM). Furthermore, control tubes were performed with distilled water or phosphate buffer solution instead of seaweed extracts. A decrease in the absorbance was indicated an increase in the free radical scavenging activity. The percentage of H_2O_2 scavenging activity was calculated **(Ebrahimzadeh *et al*., 2010).**

Red blood cells hemolysis (%) assay:

To determine the antihemolytic efficacy of all different seaweeds extracts at serial dilutions on H_2O_2-induced red blood cells hemolysis as described previously with slight modifications (**Mathuria & Verma, 2007**). Briefly, the following sets of spectrophotometric tubes were prepared. Control tubes were contained 1.0 ml of RBC suspension and 3.0 ml of 0.9% normal saline. Seaweed tubes were included 1.0 ml of different marine macroalgal extracts at serial dilutions, 1.0 ml of RBC suspension, and 2.0 ml of 0.9% normal saline. Hemolysis tubes were contained 0.5 ml of 8 mM H_2O_2 solution, 1.0 ml of RBC suspension, and 2.5 ml of 0.9% normal saline. Seaweed-treated tubes (seaweeds+H_2O_2) were included 0.5 ml of 8 mM H_2O_2 solution, 1.0 ml of RBC suspension, 1.0 ml of different marine macroalgal extracts at serial dilutions, and 1.5 ml of 0.9% normal saline. Furthermore, all tubes were incubated in a shaking incubator at 37 °C for 4 hr. The absorbance of the supernatants was obtained after centrifugation of all

the incubated tubes at 1000 rpm for 10 min spectrophotometrically at 540 nm. **(Salehiabar *et al*., 2018)**.

- **Antimicrobial activities of the different marine macroalgal ethanolic extracts:**

Antimicrobial activity assay:

The human pathogenic microorganisms were obtained from the National Institute of Oceanography and Fisheries (NIOF), Red Sea branch, Hurghada city, Egypt. Two tested gram positive bacteria were *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633, as well as two gram negative bacteria were *Escherichia coli* ATCC 19404 and *Vibrio alginolyticus* MK 170250 and one yeast isolate *Candida albicans* ATCC 10231. The antimicrobial activities of the different marine macroalgal ethanolic extracts at serial dilutions were assayed by agar well diffusion technique by using nutrient agar medium **(Almalki, 2020).** The plates were inoculated with 0.1 ml containing 10^6 cfu/ml of fresh culture pathogenic microbes. Also, the wells of diameter 6mm were performed on the inoculated agar plates by using sterilized cork borers.

Minimum inhibitory concentration (MIC):

Serial dilutions from each seaweed ethanolic crude extract (1, 0.8, 0.6, 0.4, 0.2 and 0.1 g/ml) were added on wells of each target agar plate that inoculated with specific pathogenic microbe. The minimum inhibitory concentration (MIC) was introduced as the lowest specific concentration value of specific target seaweed extract that inhibited the growth of the pathogenic microbe after 24 h of incubation at 37ºC. The microbial growth was determined by measurement of the diameter (mm) of the inhibition zone area **(Velmurugan *et al*., 2012)**.

- **Phytochemical screening of selected target seaweeds:**

High performance liquid chromatography analysis:

High performance liquid chromatography (HPLC) analysis was carried out by using an Agilent 1260 series. The separation was performed by using Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm). The injected volume was 10 μl for each marine macroalgal sample solution. The mobile phase consisted of water and 0.05% trifluoroacetic acid in acetonitrile at a flow rate 1 ml/min. Also, the mobile phase was programmed consecutively in a linear gradient. The column temperature was maintained around 35 °C and a multi-wavelength detector was monitored at 280 nm. Furthermore, gallic acid, chlorogenic acid, catechin, methyl gallate, coffeic acid, syringic acid, pyrocatechol, rutin, ellagic acid, coumaric acid, vanillin, and ferulic acid were used as standard phenolics **(Vinoth *et al*., 2014).**

- **Statistical analysis:**

Data values are statistically expressed as means ± standard deviation (SD), (n=3). Statistical significance (P<0.05) was evaluated by using the analysis of one-way ANOVA with the post hoc test of SPSS Windows Version 19.0 (SPSS, Inc., Chicago, IL, USA). For the *in vitro* analyses, the differences were estimated by the Student's T-test (Microsoft Excel 2010 software). The concentration of sample can be provided 50% inhibition or effectiveness (IC50/EC50) that obtained by the interpolation from the linear regression analysis.

RESULTS

- **Total phenolic and flavonoid contents of the different marine macroalgal ethanolic extracts:**

As reported in Table 1, most tested seaweed extracts were included markedly high values of the bioactive secondary metabolites (polyphenolics, flavonoids). Furthermore, TPC and TFC of the marine macroalgal ethanolic extracts as *Dictyota dichotoma*, *Codium tomentosum, Sargassum subrepandum M Alam, Sargassum aquifolium, Sargassum cinerum M Alam, Sargassum subrepandum Hur,* and *Laurancia papilosa* were 19.11±2.11 and 4.11±0.38; 18.83±1.62 and 3.77±0.41; 18.61±1.11 and 3.54±0.33; 18.51±2.22 and 3.45±0.41; 13.72±1.12 and 2.93±0.35; 13.42±0.45 and 2.88±0.22; 13.22±0.77 mg CAE/g dry extract and 2.73±0.33 mg GAE/g dry extract, respectively.

- **The antioxidant and the antihemolytic activities of the different marine macroalgal ethanolic extracts:**

As demonstrated in Fig. 1 and Table 2, the tested seaweed ethanolic crude extracts showed free radicals scavenging activities (%) that increase with increasing their concentrations (bioactive antioxidant secondary metabolities). Furthermore, *Dictyota dichotoma, Codium tomentosum, Sargassum subrepandum M Alam, Sargassum cinerum M Alam, Laurancia papilosa, Sargassum subrepandum Hur,* and *Sargassum aquifolium* seaweed ethanolic crude extracts induced great inhibition (%) efficacies toward DPPH free radicals, which demonstrated IC_{50} values as 4.84±0.18; 5.31±0.41; 5.40±0.21; 7.86±0.28; 8.10±0.37; 8.11±0.41; 8.24±0.32 mg/ml, respectively. Moreover, *Codium tomentosum, Sargassum aquifolium, and Dictyota dichotoma* seaweed ethanolic crude extracts described marked inhibition (%) activities toward NO free radicals, which represented IC_{50} values as 5.41±0.33; 5.51±0.26; 8.84±0.31 mg/ml, respectively. In addition, *Sargassum aquifolium, Codium tomentosum, Sargassum subrepandum M Alam, Dictyota dichotoma,* and *Sargassum cinerum M Alam* seaweed ethanolic crude extracts introduced powerful inhibition (%) properties toward H_2O_2 free radicals, which indicated IC_{50} values as 0.33±0.03; 0.43±0.04; 0.87±0.08; 1.89±0.16; 1.94±0.12 mg/ml, respectively.

Table 1. Total phenolic and flavonoid contents (TPC and TFC) of the different marine macroalgal ethanolic crude extracts.

Algae ethanolic extracts	TPC (mg CAE/g dry extract)	TFC (mg GAE/g dry extract)
Padina boergesnii	5.11±0.41[a]	0.59±0.13[a]
Sargassum subrepandum M Alam	18.61±1.11[b]	3.54±0.33[b]
Sargassum subrepandum Hur.	13.42±0.45[c]	2.88±0.22[c]
Sargassum aquifolium	18.51±2.22[b]	3.45±0.41[b]
Sargassum cinerum M Alam	13.72±1.12[c]	2.93±0.35[c]
Sargassum cinerum Mangroove	5.53±0.35[a]	0.72±0.11[a]
Codium tomentosum	18.83±1.62[b]	3.77±0.41[b]
Dictyota dichotoma	19.11±2.11[b]	4.11±0.38[b]
Halimeda opunta	9.11±1.21[d]	1.66±0.22[d]
Laurancia papilosa	13.22±0.77[c]	2.73±0.33[c]

Data values are expressed as means ± SD (n=3). The different letters (a, b, c, d, e, f, g, h) mean statistically significant differences ($P<0.05$) and the similar letters consider statistically non-significant differences ($P>0.05$).

Hemocompatibility and cytotoxicity of the different seaweed ethanolic crude extracts toward the integrity of red blood cells homeostasis were illustrated in Fig. 2 and Table 3. *Sargassum aquifolium* and *Codium tomentosum* seaweed ethanolic crude extracts significantly ($P<0.05$) introduced the lowest IC_{50} values toward the red blood cells lysis rates (%) as 0.93±0.05 and 1.11±0.08 mg/ml, respectively.

Table 2. IC_{50} values of the different marine macroalgal ethanolic crude extracts as sources of bioactive antioxidant secondary metabolites toward DPPH, NO, and H_2O_2 free radicals**.**

Algae ethanolic extracts	DPPH IC_{50}	NO IC_{50}	H_2O_2 IC_{50}
		(mg/ml)	
Padina boergesnii	22.81±2.10[a]	11.51±1.20[a]	2.72±0.17a[b]
Sargassum subrepandum M Alam	5.40±0.21[b]	11.82±0.82[a]	0.87±0.08[c]
Sargassum subrepandum Hur.	8.11±0.41[c]	17.97±1.11[b]	2.48±0.21[af]
Sargassum aquifolium	8.24±0.32[c]	5.51±0.26[c]	0.33±0.03[d]
Sargassum cinerum M Alam	7.86±0.28[c]	13.75±0.81[d]	1.94±0.12[e]
Sargassum cinerum Mangroove	21.64±1.80[a]	13.11±0.66[d]	2.17±0.14[ef]
Codium tomentosum	5.31±0.41[b]	5.41±0.33[c]	0.43±0.04[g]
Dictyota dichotoma	4.84±0.18[b]	8.84±0.31[e]	1.89±0.16[e]
Halimeda opunta	11.23±0.88[d]	18.71±0.81[b]	2.95±0.13[b]
Laurancia papilosa	8.10±0.37[c]	19.12±0.63[b]	18.20±0.71[h]

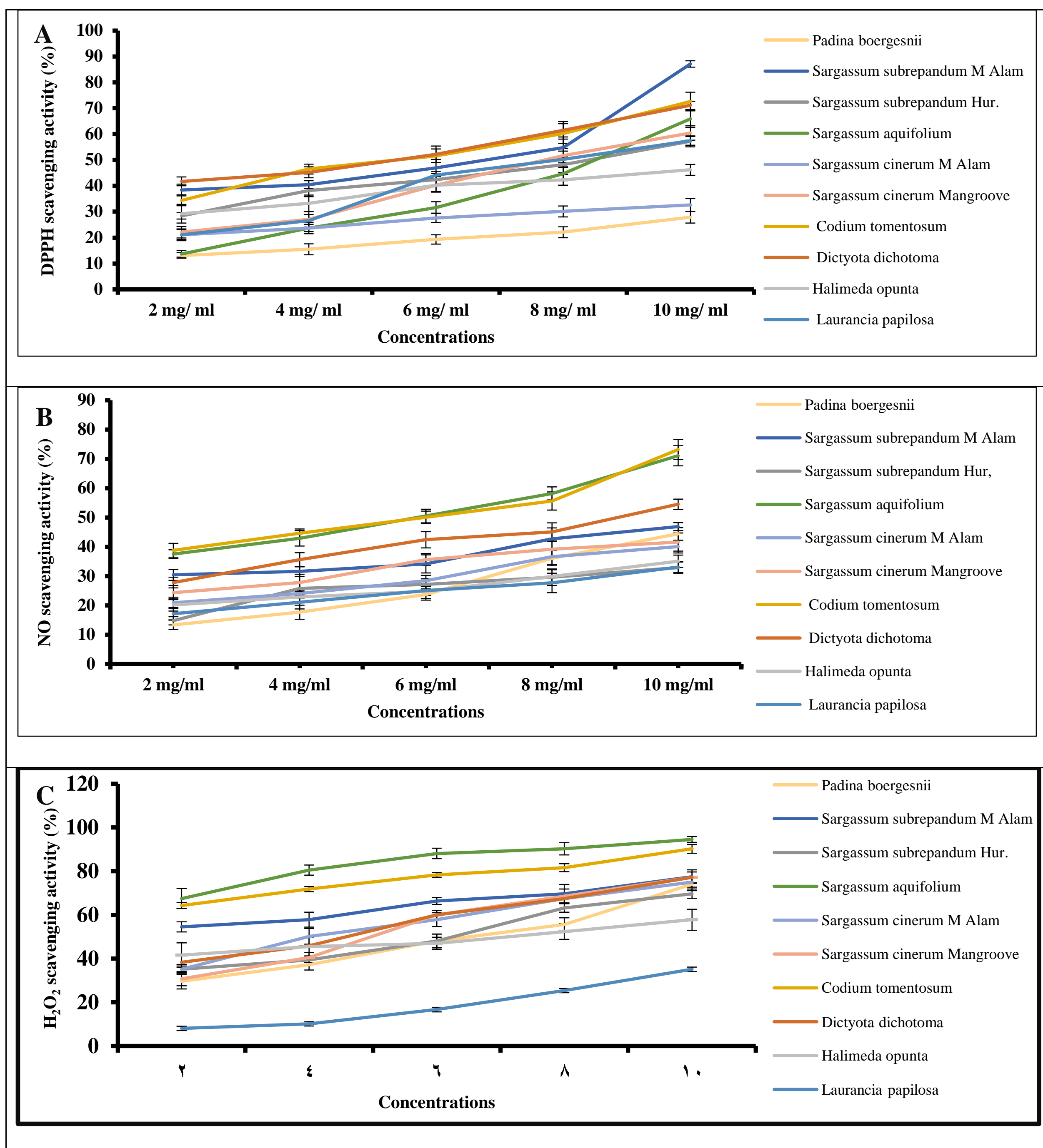


Fig. 1. Several scavenging activities (%) of the different marine macroalgal ethanolic crude extracts. DPPH (A), NO (B), H_2O_2 (C). Data values are expressed as means ± SD (n=3).

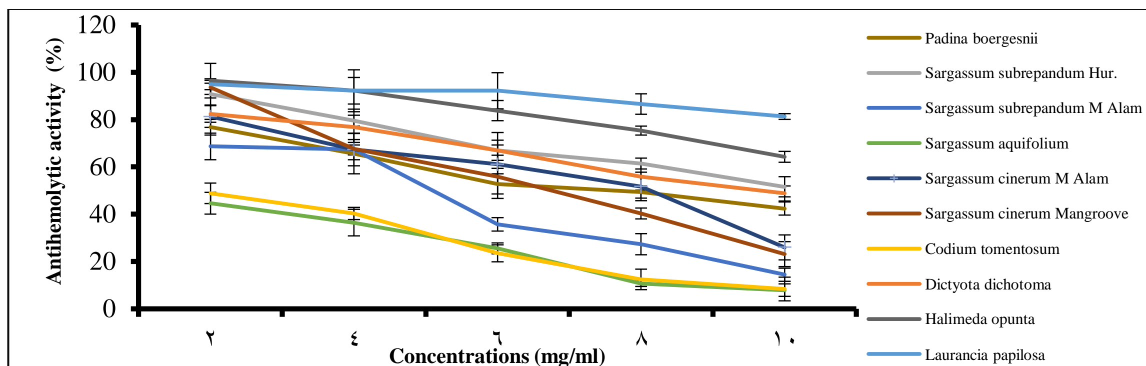


Fig. 2. The effect of different marine macroalgal ethanolic extracts on the red blood cells hemolysis rates (%). Data values are expressed as means ± SD (n=3).

Table 3. Red blood cells lysis IC_{50} of the different marine macroalgal ethanolic extracts.

Algae ethanolic extracts	RBCs lysis IC_{50} (mg/ml)
Padina boergesnii	3.68±0.13^{a}
Sargassum subrepandum M Alam	2.12±0.14^{c}
Sargassum subrepandum Hur.	6.10±0.31^{d}
Sargassum aquifolium	0.93±0.05^{e}
Sargassum cinerum M Alam	3.35±0.24^{ab}
Sargassum cinerum Mangroove	3.11±0.14^{b}
Codium tomentosum	1.11±0.08^{f}
Dictyota dichotoma	5.61±0.21^{d}
Halimeda opunta	14.37±1.11^{g}
Laurancia papilosa	486.58±22.3^{h}

Data values are expressed as means ± SD (n=3). The different letters (a, b, c, d, e, f, g, h) mean statistically significant differences ($P<0.05$) and the similar letters consider statistically non-significant differences ($P>0.05$).

- **Antimicrobial activity:**

Fig. 3 and Tables 4, 5 demonstrated diameter zones of inhibition and MICs of the different marine macroalgal ethanolic crude extracts against several human pathogenic microbes. *Sargassum aquifolium* and *Codium tomentosum* seaweed ethanolic extracts showed a significant ($P<0.05$) zones of inhibition (20.0±1.0 and 20.0±1.0 mm, respectively) toward *S. aureus* ATCC6538 compared to other seaweed species. Also, *Codium tomentosum* and *Sargassum aquifolium* macroalgal extracts demonstrated a significant ($P<0.05$) zones of inhibition (18.0±1.0 and 16.0±1.0 mm, respectively) toward *E. coli* ATCC 19404 compared with other seaweed ethanolic extracts. Furthermore, *Sargassum aquifolium* and *Codium tomentosum* macroalgal ethanolic crude extracts indicated a significant ($P<0.05$) zones of inhibition (20.0±1.0 and 14.0±1.0 mm, respectively) toward *V. alginolyticus* MK170250 when compared to other marine macroalgal species. Also, *Halimeda opunta* and *Sargassum aquifolium* marine macroalgae represented a significant ($P<0.05$) zones of inhibition (20.0±1.0 and 18.0±1.0

mm, respectively) toward *B. subtilis* ATCC 6633 compared to other seaweed species. Moreover, *Sargassum aquifolium*, *Halimeda opunta*, and *Codium tomentosum* seaweed ethanolic extracts showed a significant ($P<0.05$) zones of inhibition (20.0±1.0; 20.0±1.0; 18.0±1.0 mm, respectively) toward *C. albicans* ATCC 10231 when compared to other seaweed ethanolic crude extracts. According to MIC results, *Sargassum subrepandum Hur* (brown macroalgae) ethanolic crude extract indicated a significant ($P<0.05$) MIC against *S. aureus* ATCC6538 and *C. albicans* ATCC 10231 pathogenic microbes (0.2±0.05 and 0.4±0.01 mg/ml, respectively) compared to other seaweeds. Also, *Codium tomentosum* (green macroalgae) ethanolic extract demonstrated a significant ($P<0.05$) MIC against *E. coli* ATCC 19404 species (0.4±0.05 mg/ml) compared with other macroalgal ethanolic extracts. In addition, *Sargassum subrepandum Hur* (brown macroalgae) and *Codium tomentosum* (green macroalgae) seaweed extracts introduced a significant ($P<0.05$) MIC against *V. alginolyticus* MK170250 pathogenic bacteria (0.6±0.05 and 0.6±0.05 mg/ml, respectively) compared to other seaweeds. Also, *Sargassum cinerum Mangroove* (brown macroalgae) and *Halimeda opunta* (green macroalgae) seaweeds represented a significant ($P<0.05$) MIC against *B. subtilis* ATCC 6633 pathogen (0.2±0.01; 0.2±0.01 mg/ml, respectively) compared to other seaweed ethanolic extracts.

Table 4. Pathogenic microbes diameter zones of inhibition of the different marine macroalgal ethanolic crude extracts.

Macroalgal crude extracts 1g/ml	Pathogenic microbes/Zones of inhibition (mm)				
	S. aureus ATCC6538	*E. coli* ATCC 19404	*V. alginolyticus* MK170250	*B. subtilis* ATCC 6633	*C. albicans* ATCC 10231
Padina boergesnii	12.0±1.0[a]	10.0±1.0[a]	10.0±1.0[a]	12.0±1.0[a]	16.0±1.0[a]
Sargassum subrepandum M Alam	10.0±0.5[b]	8.0±0.5[b]	0.0	0.0	0.0
Sargassum subrepandum Hur.	0.0	10.0±1.0[a]	0.0	0.0	0.0
Sargassum aquifolium	20.0±1.0[c]	16.0±1.0[c]	20.0±1.0[b]	18.0±1.0[b]	20.0±1.0[b]
Sargassum cinerum M Alam	10.0±1.0[b]	10.0±1.0[a]	12.0±1.0[c]	16.0±1.0[c]	14.0±1.0[c]
Sargassum cinerum Mangroove	10.0±0.5[b]	10.0± 1.0[a]	10±0.5[a]	0.0	0.0
Codium tomentosum	20.0±1.0[c]	18.0±1.0[d]	14.0±1.0[d]	16.0±1.0[c]	18.0±1.0[d]
Dictyota dichotoma	0.0	8.0±0.5[b]	0.0	0.0	0.0
Halimeda opunta	10.0±1.0[b]	14.0±1.0[e]	12.0±1.0[c]	20.0±1.0[d]	20.0±1.0[b]
Laurancia papilosa	0.0	0.0	0.0	0.0	0.0

Data values are expressed as means ± SD (n=3). The different letters (a, b, c, d, e, f, g, h) mean statistically significant differences ($P<0.05$) and the similar letters consider statistically non-significant differences ($P>0.05$).

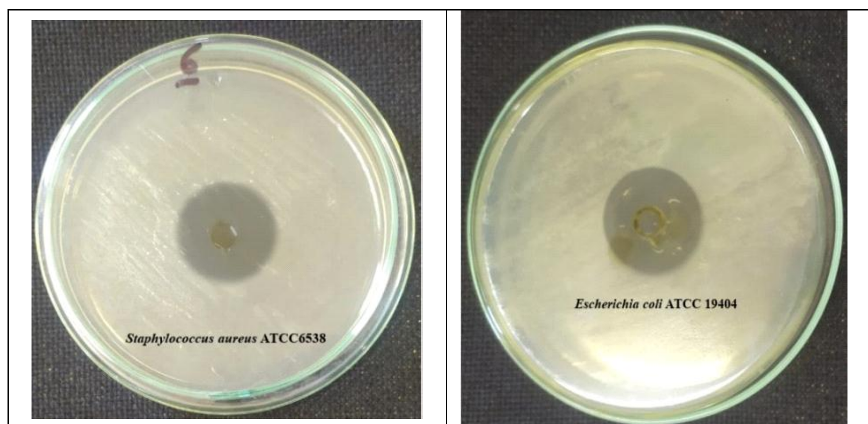


Fig. 3. Pathogenic microbes diameter Zone of inhibition of *Sargassum aquifolium* against *Staphylococcus aureus* ATCC6538 (20.0±1.0 mm) and *Escherichia coli* ATCC 19404 (16.0±1.0 mm).

Table 5. Minimum inhibitory concentration (MIC) of seaweed ethanolic crud extracts against different human pathogenic microbes.

Marine macroalgal ethanolic extracts	MIC (mg/ml)				
	S. aureus ATCC6538	*E. coli* ATCC 19404	*V. alginolyticus* MK170250	*B. subtilis* ATCC 6633	*C. albicans* ATCC 10231
Padina boergesnii	0.8±0.05[a]	1.0±0.05[a]	0.8±0.01[a]	0.8±0.01[a]	0.6±0.01[a]
Sargassum subrepandum M Alam	1.4±0.11[d]	2.4±0.16[e]	0.0	0.0	0.0
Sargassum subrepandum Hur.	0.2±0.05[c]	0.8±0.05[b]	0.6±0.05[b]	0.6±0.01[b]	0.4±0.01[b]
Sargassum aquifolium	0.4±0.1[b]	0.6±0.05[c]	0.8±0.05[a]	0.8±0.05[a]	0.8±0.01[c]
Sargassum cinerum M Alam	1.2±0.11[e]	0.6±0.05[c]	1.4±0.14[c]	1.8±0.18[d]	1.2±0.11[d]
Sargassum cinerum Mangroove	0.8±0.05[a]	0.6±0.01[c]	0.8±0.01[a]	0.2±0.01[c]	0.6±0.05[a]
Codium tomentosum	0.4±0.05[b]	0.4±0.05[d]	0.6±0.05[b]	0.6±0.01[b]	0.8±0.01[c]
Dictyota dichotoma	0.0	1.0±0.02[a]	0.0	0.0	0.0
Halimeda opunta	0.8±0.05[a]	0.6±0.01[c]	0.8±0.01[a]	0.2±0.01[c]	0.6±0.05[a]
Laurancia papilosa	0.0	0.0	0.0	0.0	0.0

Data values are expressed as means ± SD (n=3). The different letters (a, b, c, d, e, f, g, h) mean statistically significant differences (P<0.05) and the similar letters consider statistically non-significant differences (P>0.05).

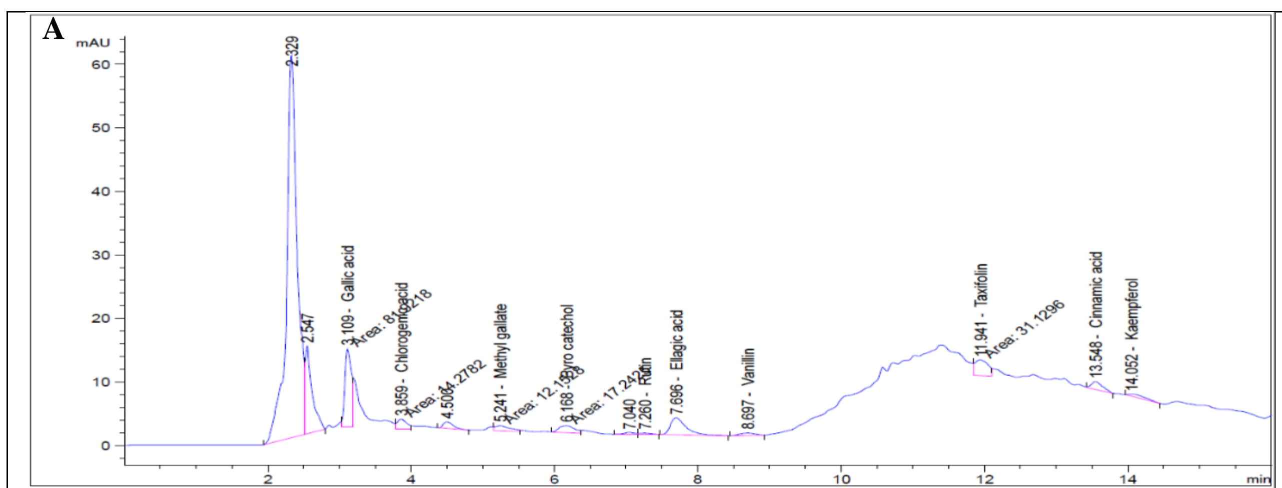
- **HPLC analysis of selected target specific seaweed ethanolic extracts:**

Table 6 represented the potent bioactive ingredients and their concentrations that included in *Codium tomentosum* and *Sargassum aquifolium* seaweed ethanolic crude extracts. Several phenolics were detected in their crude extracts by HPLC analysis. The *Codium tomentosum* seaweed extract chromatogram profiles (Fig. 4A, 4C) explained the presence of gallic acid (317.9 µg/g), ellagic acid (133.8 µg/g), taxifolin (98.3 µg/g), pyrocatechol (51 µg/g), and chlorogenic acid (49.7 µg/g) as its some bioactive secondary metabolities. Also, *Sargassum aquifolium* ethanolic crude extract chromatogram profiles

(Fig. 4B, 4C) represented the presence of ellagic acid (219.9 µg/g), rutin (75.3 µg/g), gallic acid (71.7 µg/g), taxifolin (38.8 µg/g), kaempferol (29.4 µg/g), and catechin (28.8 µg/g) as its some powerful antioxidant metabolities. These active compounds reflected the antioxidant, antihemolytic, and antimicrobial properities of some seaweed ethanolic extracts toward DPPH, NO, and H_2O_2 free radicals as well as human pathogenic microbes.

Table 6. Phtochemical characterization of *Codium tomentosum* and *Sargassum aquifolium* seaweeds by HPLC technique.

Standards	*Codium tomentosum* Conc.(µg/g)	*Sargassum aquifolium* Conc.(µg/g)
Gallic acid	317.96	71.72
Chlorogenic acid	49.74	17.32
Catechin	0	28.85
Methyl gallate	7.69	12.11
Coffeic acid	0	0
Syringic acid	0	5.16
Pyrocatechol	51.01	7.42
Rutin	10.97	75.35
Ellagic acid	133.83	219.91
Coumaric acid	0	0
Vanillin	5.68	4.92
Ferulic acid	0	1.51
Naringenin	0	23.24
Taxifolin	98.87	38.89
Cinnamic acid	5.74	11.91
Kaempferol	13.11	29.45
Total	694.58	547.74



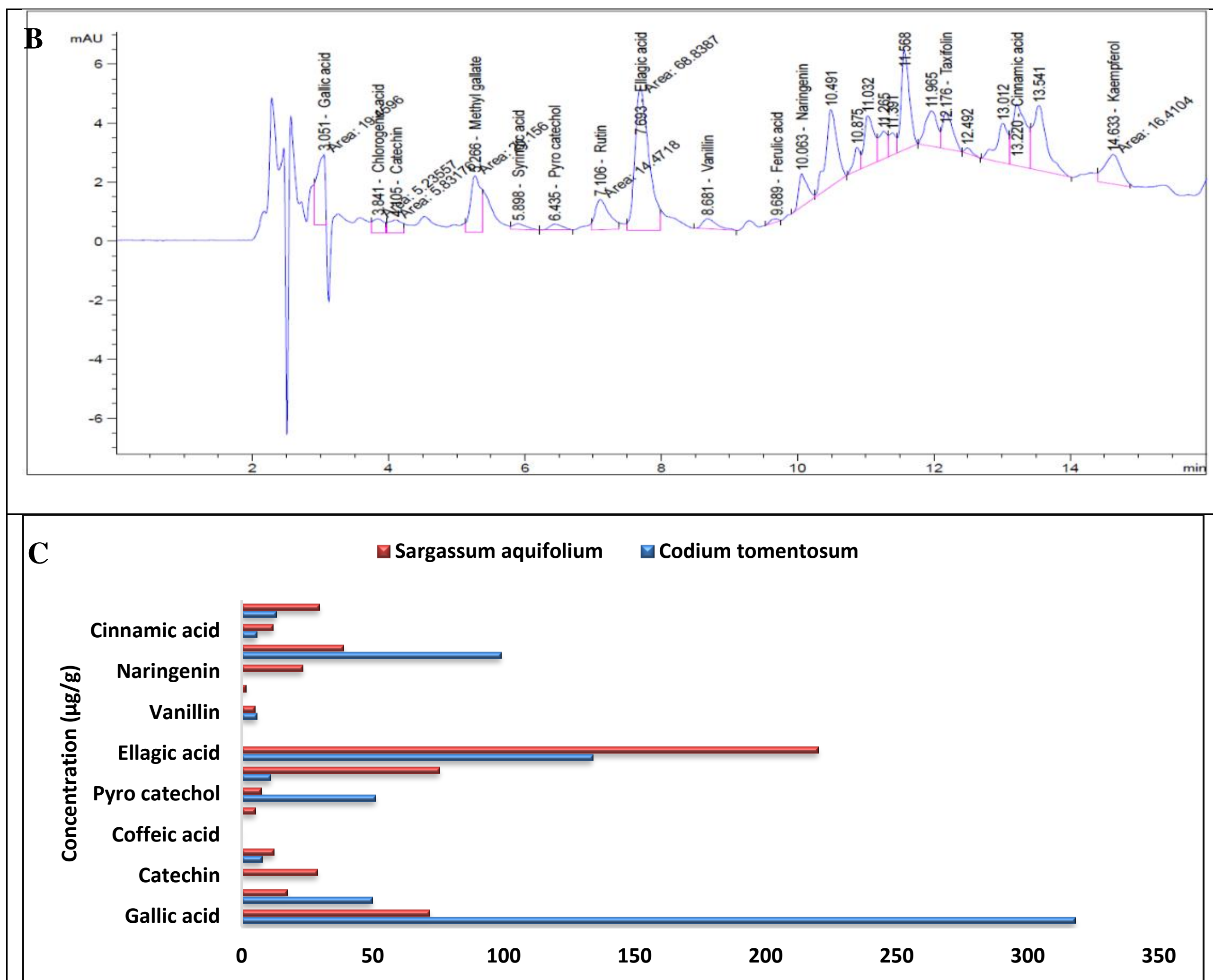


Fig. 4. HPLC chromatograms of seaweed ethanolic crude extracts. *Codium tomentosum* as a green algae (A), *Sargassum aquifolium* as a brown algae (B) , and their polyphenolic concentrations (C).

DISCUSSION

Marine organisms are a rich source of bioactive chemical constituents that use in the clinical pharmaceutical industry. The different marine macroalgal ethanolic crude extracts and their potent active ingredients had several *in vitro* antioxidant and antimicrobial activities **(Sundaram *et al*., 2016)**. Previous study demonstrated that phenolic compounds (antioxidant metabolites) were considered the major chemical components of marine macroalgae with a potent inhibitory effect toward the microbial growth **(Manivannan *et al*., 2011)**. Also, phenolics as bioactive secondary metabolites are produced by plants and marine macroalgae as a defense system under different environmental stress conditions including wounding, infection, excessive light or UV irradiation **(Moubayed *et al*., 2017)**. Our study was aimed to evaluate the

pharmacological properties and the therapeutic efficacies of the different seaweed ethanolic extracts through *in vitro* antioxidant and antimicrobial studies. Previous studies indicated that the phytochemical screening of the marine macroalgae methanolic extracts included flavonoids, terpenoids, steroids, alkaloids, phenols, tannins, saponins, glycosides, essential oils, carbohydrates, and proteins **(Selim *et al*., 2015; Abotaleb *et al*., 2019).** Different solvents such as aqueous, acetone, ethanol, methanol, petroleum ether, hexane, chloroform, and benzene were used to extract bioactive ingredients from seaweeds (brown, green, and red algae) **(Govindan *et al*., 2014)**. The present study was carried out on ethanolic extracts.

In biological systems, antioxidant modulators were considered the intracellular defense responses against oxidative stress and reactive oxygen species generation, which regulated the cellular homeostasis. Mozhi *et al*. (2009) demonstrated that the methanolic crude extracts of some seaweeds introduced significant increases in the yield of their total phenolics and flavonoid contents and scavenging activities toward DPPH free radicals **(mozhi *et al*., 2009).** Flavonoids as powerful secondary metabolites have antioxidant activities, which restore the cellular homeostasis and inhibit the microbial growth as well as protect against chronic diseases **(Kumar *et al*., 2013).**

The red blood cells lysis rate and the released hemoglobin were considered as a critical harmful sign of the erythrocytes cytotoxicity. Hydrogen peroxide (H_2O_2) as a potent oxidant inhibited the integrity and stability of the human erythrocytes membrane, which damaged their cellular membranes and released hemoglobin. Also, inhibition and/or effectiveness concentrations (IC_{50}/EC_{50}) were identified as the sample concentration that inhibited the red blood cells lysis rates by 50% and called anti-hemolytic powerful activity **(Lakshmi *et al*., 2014).** The present study examined the effect of different seaweeds extracts and their bioactive components on the stability of human erythrocytes in presence and/or absence of H_2O_2 as a potent cellular oxidant. According to our results, brown, green, and red marine macroalgae ethanolic extracts showed significant antioxidant and antihemolytic properities against DPPH, NO, and H_2O_2 free radicals due to their bioactive constituents and phytochemicals.

As reported by Hamza *et al.* (2015), *Codium tomentosum* crude extracts introduced antibacterial activity against *S. typhimurium* and *S. boydii* pathogens **(Hamza *et al*., 2015).** Also, Poosarla *et al*. (2013) demonstrated that, *Codium tomentosum* ethanolic, chloroform, and diethyl ether crude extracts represented marked antimicrobial characterizations against gram positive bacteria (*Streptococcus sps*; *Staphylococcus aureus; Bacillus subtilis)* and gram negative bacteria (*Escherichia coli*; *Proteus vulgaris*) **(Poosarla *et al.,* 2013).** In addition, *Sargassum aquifolium* petroleum ether crude extract was described a significant potent antimicrobial efficacy against some human pathogenic microbes **(Moni *et al*., 2019).** In this study, the antimicrobial activities of the different

seaweed ethanolic crude extracts were performed by the well diffusion method against several human pathogenic microbes. Also, our study demonstrated that the most selected marine macroalgae crude extracts described potent degrees of diameter zones of inhibition and MICs against several pathogenic microbes.

HPLC analysis results of *Codium tomentosum* (green macroalgae) and *Sargassum aquifolium* (brown macroalgae) crude extracts confirmed the *in vitro* antioxidant and antimicrobial studies on several seaweeds, which demonstrated their biochemical components including gallic acid, chlorogenic acid, methyl gallate, pyrocatechol, rutin, ellagic acid, vanillin, taxifolin, cinnamic acid, and kaempferol. Also, these active components considered the main reason of free radicals scavenging activities of our target seaweeds

CONCLUSION

In the current study, the most potent antioxidant, anti-hemolytic, and antimicrobial seaweed ethanolic crude extracts were *Codium tomentosum* (green macroalgae) and *Sargassum aquifolium* (brown macroalgae) toward DPPH, NO, and H_2O_2 as oxidants and several human pathogenic microbes.

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