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Exploring Bioactive Compounds, Antioxidant, and Antimicrobial Properties of Seaweed Extracts for Alleviating Aluminium Stress in *Trigonella foenum-graecum* Seedlings

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SEAWEEDS contain huge amounts of bioactive compounds that can be used for medicinal, nutritional, and agricultural purposes. This study analysed the antioxidant and antimicrobial properties as well as the content of bioactive compounds in twelve seaweeds and highlighted their use against aluminium toxicity in *Trigonella foenum-graecum* (fenugreek) seedlings. Among the tested seaweeds, the brown species *Eisenia bicyclis* and *Sargassum horneri* had the highest total antioxidant activities. They could scavenge 74% and 77% of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) free radicals, respectively. Thus, gas chromatography/mass spectrometry (GC/MS) analysis was performed for these two algal species, and reports showed some valuable compounds, such as phthalic acid, stigmasterol, and palmitic acid which are medically important for human health and stimulators for plant growth. The amounts of polyphenols (maximum 303.28 mg GAE/g DW) and flavonoids (maximum 45.44 mg CE/g DW) were higher in brown seaweeds than in green or red ones. Our results revealed that extracts of *Eisenia bicyclis* and *Sargassum horneri* exhibited antimicrobial effects against four microorganisms. Also, a bioassay was done using fenugreek seedlings subjected to aluminium stress and/or seaweed extracts. It was found that seedling growth was inhibited by Al treatment, while they enhanced when germinated in extracts of *S. horneri* and *E. bicyclis*, alleviating Al toxicity in the used concentrations. The results of our study shed light on the use of seaweed extracts as sources of bioactive compounds against aluminium stress in fenugreek seedlings and as natural antimicrobials in food preservation, pharmaceuticals, and other industries; their use is safe, low cost, and eco-friendly.

Keywords: Antioxidant activity, Antimicrobial activity, Aluminium, Bioactive compounds, Brown macroalgae, Fenugreek, *Trigonella foenum-grecum*.

Introduction

Seaweeds, red (Rhodophyta), green (Chlorophyta), and brown (Phaeophyta) are complex multicellular algae found in shallow marine ecosystems. Edible seaweeds are full of valuable and nutritional components (Kumar et al., 2011). Many studies reported seaweeds as rich sources of antioxidants, including phenolic compounds, flavonoids, sulfated polysaccharides, vitamins, phytohormones, mineral nutrients, proteins, terpenes, lipids, fats, omega-3 polyunsaturated fatty acids PUFAs, and many other bioactive compounds (Ismail & El-Shafay, 2015, Pacholczak et al., 2016, Schwartz et al., 2017; Hassan et al., 2021; El-Sheekh et al., 2023). The presence of seaweeds in the marine environment and exposure to salt stress has led to the synthesizing several osmo-protective compounds, such as proline, betaines, polyamines, and sorbitol (El Shoubaky & Salem, 2016; Morsy et al., 2018). The presence of such a wealth of bioactive compounds in seaweeds is considered the main reason that encourages researchers to search and explore more and more

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for these compounds and to study the benefits of using seaweeds in numerous fields, such as food, cosmetics, and agricultural and medical applications (Battacharyya et al., 2015).

Free radicals are reactive molecules or atoms containing an unpaired electron, and they tend to react with other molecules to gain an electron and stabilize themselves. Therefore, the formation of free radicals is a risk for all tissues. Antioxidants are compounds that can scavenge free radicals and inhibit oxidation within the cells. Previous studies indicated the presence of antioxidant compounds in seaweeds, such as some pigments (i.e., fucoxanthin, astaxanthin, and carotenoid) and polyphenols (i.e., phenolic acid, flavonoid, and tannins) that are widely distributed in seaweeds and have been reported to exhibit high antioxidative activities (Luo et al., 2010, Du Jardin, 2015, Shanmuganathan & Pandima Devi, 2016, Gomez-Zavaglia et al., 2019). They also inhibit mutation and scavenge active oxygen and free radicals.

Seaweeds have antimicrobial properties; they can inhibit the growth of microorganisms, such as bacteria and fungi (EL-Sheekh et al., 2022). Seaweed extracts exhibit antibacterial activity against a variety of bacterial strains, including *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa* (Ravisankar et al., 2013). The antimicrobial properties of seaweeds are due to the presence of bioactive compounds (Singh et al., 2015; Mohammed, 2023). These compounds have been shown to disrupt bacterial cell membrane integrity, inhibit bacterial growth and biofilm formation, and prevent the adhesion of bacteria to host cells (Pinteus et al., 2020). Over the past few years, the massive use of antibiotics has led to the presence of multiple drug-resistant bacterial isolates. This has raised scientific public concerns about developing novel antimicrobial agents with higher potential and exhibiting no harmful side effects (Pérez et al., 2016). Seaweeds are a promising source of natural antimicrobial agents, and ongoing research is exploring their potential for use in different applications as natural and sustainable antimicrobial agents in various industries, including food, pharmaceuticals, and cosmetics.

Several studies reported a wide range of benefits from using seaweeds as sources of organic bioactive compounds in agriculture, including enhanced seed germination rates and plant development, improved crop production and yield, and increased biotic and abiotic stress tolerance (Sharma et al., 2014; Battacharyya et al., 2015), the effect of seaweed extracts as biostimulators is related to the combination of many bioactive compounds in the extracts that may work synergistically at different concentrations (Fornes et al., 2002). The advantages of seaweed extracts as biofertilizers are that they are biodegradable, eco-friendly, safe, and non-toxic (Dhargalkar & Pereira, 2005).

Farmers operating organic farms are eager to use natural stimulants to boost crop productivity (Bradshaw et al., 2012). Additionally, the increasing demand for healthy food by consumers encourages the increasing interest in organic farming (Kyriacou & Rouphael, 2018).

One of the major environmental problems in agriculture and natural ecosystems is the toxicity of heavy metals such as aluminium and their accumulation in the food chain. Aluminium is one of the most significant reasons for preventing plant growth and lowering the effectiveness of agricultural production in acidic soils (Karimaei & Poozesh, 2016).

Aluminium toxicity distorts the root system by the inhibition of root cell elongation and cell division, perturbs several metabolic processes, and induces programmed cell death, leading to significant crop growth reduction, yield loss, or total crop failure (Kochian et al., 2015; Bojórquez-Quintal et al., 2017; Ofoe et al., 2022). It binds to root membrane surfaces, disrupts cell integrity by crystallizing cell walls, and restricts water and nutrient flow (Kochian et al., 2015; Ofoe et al., 2022).

Consequently, aluminium exposure triggers the accumulation of reactive oxygen species (ROS) that causes oxidative damage to cellular components, including membrane lipids, proteins, and nucleic acids, that lead to several metabolic alterations (Ofoe et al., 2022).

Seaweed extracts have been reported to improve plant resistance to abiotic stresses, such as salinity and drought (Khan et al., 2009; Elansary et al., 2016), but there are no reports in relation to *Trigonella foenum-grecum*'s resistance to heavy metals when applying seaweed extracts in the culture conditions. Seaweed biomass has proven to have a high sorption capacity of heavy metals, which is mainly attributed to polysaccharides found in the cell walls of seaweeds (Ortiz-Calderon et al., 2017). Hence,

this research includes the effect of using seaweed extracts on the germination of fenugreek seeds as a model plant in the presence of aluminium as an abiotic factor in the culture conditions. Fenugreek is significant for various cultural, culinary, and medicinal contexts. Originating from the Mediterranean region, this herbaceous plant has been cultivated and used for centuries due to its diverse properties and applications. *Trigonella foenum-graecum*'s cultural, culinary, medicinal, and agricultural significance, coupled with its sensitivity to aluminium stress, makes it a valuable and practical choice for scientific investigation in the context of this study.

This research aims to evaluate the antioxidant and antimicrobial capability of some seaweed extracts in relation to their contents of polyphenols, flavonoids, and other bioactive compounds. It identifies the bioactive compounds of some different seaweeds as the most functional ingredients used in many applications. Also, this investigation aims to study the capability of using seaweed extracts to avoid aluminium toxicity in *Trigonella foenum-grecum*'s seedlings.

Materials and Methods

Seaweeds material and extraction

Twelve types of edible seaweeds were purchased for this study from a food store in Tokushima, Japan: the green algae *Monostroma nitidum* (Japanese name Aosa) and *Ulva linza* (Usuba-aonori), the brown algae *Undaria pinnatifida* (Wakame), *Nemacystus decipiens* (Mozuku), *Sargassum horneri* (Akamoku), *Saccharina japonica* (Kombu), *Eisenia bicyclis* (Arame), *Hizikia fusiforme* (Hijiki), and the red algae *Compylaephora hypnaeoides* (Egosou), *Gloiopeltis tenax* (Funori), *Gelidium elegans* (Tengusa) and *Chondracanthus tenellus* (Akanori).

Extraction for antioxidant analysis

Air dried seaweeds were ground to fine powder. Seaweed powders (5 g) were extracted in 50 mL of methanol 95% in a shaker for 24 h and then filtrated. The remaining materials were re-extracted in 25 mL of methanol 95% in shaker for another 24 h and then filtrated. The filtrates were collected, methanol was evaporated, and the final volume was adjusted to 25 mL and used for antioxidant experimental analysis.

Extraction for GC/MS analysis

Powdered seaweeds were homogenized, then macerated in a stoppered container with 50 mL methanol and allowed to stand at room temperature for 3 days. The extract stands in a sonicator at 40 °C for 60 min. Then, this extract was filtered and concentrated under a vacuum at 40 °C by using a rotary evaporator to provide crude extract.

Extraction for plant bioassay

Seaweed extracts were prepared using 1 g of dry powder in 100 mL of distilled water and were incubated in a water bath at 50 °C for 3 hrs. The final extract was used as 100% concentration and was used for preparing other concentrations for the bioassay.

Determination of phenolics, flavonoids contents and total antioxidant activity of seaweeds

Folin-Ciocalteau reagent was used for total phenolics determination in methanolic seaweed extracts according to the method of Singleton & Rossi, 1965. Gallic acid was used as a standard. Methanolic extracts of seaweeds were mixed with Folin reagent. After 5 minutes, sodium bicarbonate (7.5% w/v) was added to the mixture. After standing for 60 min at room temperature, absorbance was measured at 765 nm. Phenolics content is expressed as mg Galic Acid Equivalent per g dry weight (mg GAE/g DW).

The flavonoid content of seaweeds was measured according to the method of (Quettier-Deleu et al., 2000) with some modifications. Extracts in methanol were mixed with 1 mL 5% sodium nitrite ($NaNO_2$) after 5 min, 1.0 mL of 10% $AlCl_3.6H_2O$ was added. The mixture was allowed to stand for 10 min, and then 5 mL of 4% sodium hydroxide was directly added to the mixture. The absorbance was measured after 15 min at a wavelength of 510 nm. The content of flavonoids was calculated based on the calibration curves of Rutin trihydrate and was expressed as mg Catechin equivalent per g dry weight (mg CE/g DW).

Total antioxidant activity (TAA) of seaweed methanolic extracts was analyzed using a DPPH scavenging assay according to the method of Shimada et al., 1992. Methanolic extracts were mixed with DPPH (1,1-diphenyl-2-picrylhydrazyl) freshly prepared with a concentration of 80 ppm in methanol. The mixture was kept in the dark for 30 min. Absorbance was measured by a spectrophotometer at 517 nm. TAA was calculated as (%) of DPPH scavenging activity according to the following equation:

TAA (%) = (1-(Ai-Aj)/Ac) *100

Ai: abs (extract+ DPPH), Aj: abs (extract+ methanol) and Ac: abs (DPPH+ methanol).

Antimicrobial effect of aqueous extracts of Eisenia bicyclis and Sargassum horneri

Aqueous extracts (5 %) of *S. horneri* and *E. bicyclis* were tested against the following microbes: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213 (Gram-positive), *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027 (Gram-negative), *Candida albicans* ATCC 10231, and *Candida albicans* ATCC 18804 (fungi). Agar-well diffusion method was employed for the antibacterial test according to Valgas et al. (2007), where bacterial isolates were re-inoculated on Nutrient Agar (NA) (peptone 5 g/L; yeast extract 3 g/L; sodium chloride 3 g/L; agar 15 g/L; final pH 6.5 ± 0.2) at 35 ± 2 ℃ for 2 days. Fresh bacterial cultures were swapped over the Mueller-Hinton agar (MHA) (beef extract 2 g/L; starch 1.5 g/L; agar 18 g/L) separately. Bores of 0.3 mm diameter were developed using the sterile back side of polypropylene micropipette tips 10-200 µL. Ampicillin was used as a positive control (50 µg/100 µL). Bores were loaded with 100 µL of extracts and positive controls. For the antifungal test, fresh slants of fungal isolates were used to spread over Sabouraud Dextrose Agar (SDA) (dextrose, 40 g/L; peptone, 10 g/L; agar 18 g/L) using a sterile swap. Fluconazole was used as a positive control (25 µg/100 µL). All plates were incubated at 37 ℃. After 24 h, plates were checked for the appearance of a clear zone. All plates were carried out in triplicates.

Protein and carbohydrate contents of seaweeds

Protein contents of seaweeds were determined according to Lowry et al., 1951 using Bovine serum albumin as a standard. Seaweeds were extracted for 2 h at 90 ºC in distilled water for analysis of soluble proteins and in NaOH for total protein. The extract was mixed with 5 mL of alkaline reagent (50 mL 2% Na_2CO_3 prepared in 0.1 N NaOH 1 mL 0.5% $CuSO_4.5H_2O$ prepared in 1% sodium potassium tartrate) and allowed to stand for 10 min. Folin reagent diluted 1:1 (v/v) was then added and mixed immediately. After 30 min, the blue colour was measured at 700 nm. Results were expressed as mg of protein per gram dry weight (mg/g DW). Insoluble proteins were calculated as the difference between the amounts of total and water-soluble proteins.

Using anthrone sulfuric acid reagent, carbohydrate content in seaweeds was determined by the method of Fales (1951) and Schlegel (1956). To extract soluble carbohydrates, the dry powder was boiled for 1 h in distilled water and total carbohydrates, the dry powder was boiled in 1 N HCl in a water bath for 1 h. The extract was mixed with anthrone reagent (0.2 g anthrone, 8 mL absolute ethyl alcohol, 30 mL distilled water, and 100 mL concentrated sulfuric acid (D=1.84). The mixture was then boiled in a water bath for 7 min. After cooling, the developed blue green colour was measured spectrophotometrically against blank at 620 nm.

Chemical composition of seaweed by GC/MS analysis

The chemical composition of seaweed extracts was performed using a GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 2m film thickness). The column oven temperature was initially held at 60 ℃ and then increased by 6 ℃ /min. to 250 ℃ withhold 1 min. After that, it was increased to 300 with 3 ℃ min^{-1}. The injector temperature was kept at 270 ℃. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The solvent delay was 4 min. and diluted samples of 1 mL were injected automatically using Autosampler AS3000 coupled with GC in the split mode. El mass spectra were collected at 70 eV ionization voltages over the range of m/z 50-650 in full scan mode. The ion source and transfer line were set at 200 ℃ and 280 ℃, respectively. The components were identified by comparison of their mass spectra with those of WILEY 09 and NIST 14 mass spectral databases (Abd El-Kareem et al., 2016).

Seed germination and aluminium stress experiment

Seeds of fenugreek were sterilized in ethyl alcohol 70% for 5 min and then washed three times with sterilized distilled water. Ten seeds per treatment were placed in a sterilized glass petri dish. Two seaweed extracts were chosen for this bioassay; they were extracts of *S. horneri* and *E. bicyclis* with two concentrations: 10 and 20% (w/v) in distilled water. Sterilized fenugreek seeds were treated with solutions of seaweeds and/or $AlCl_3$, as shown below. After 7 days, growth parameters, including seedling length (cm) and dry weights(g), were recorded.

The used treatments:

1. 0.0 (control),
2. 10% *S. horneri* seaweed extract,
3. 20% *S. horneri* seaweed extract,

4. 10% *E. bicyclis* seaweed extract,
5. 20% *E. bicyclis* seaweed extract,
6. 0.06 mM $AlCl_3$ (low),
7. 0.3 mM $AlCl_3$ (mid),
8. 1.5 mM $AlCl_3$ (high),
9. 0.3 mM $AlCl_3$ (mid)+ 10% *S. horneri* seaweed extract,
10. 0.3 mM $AlCl_3$ (mid)+ 20% *S. horneri* seaweed extract,
11. 0.3 mM $AlCl_3$ (mid)+ 10% *E. bicyclis* seaweed extract,
12. 0.3 mM $AlCl_3$ (mid) + 20% *E. bicyclis* seaweed extract.

Statistical analyses

Statistical tests were carried out using SPSS software (ver. 22) for Windows. Results were subjected to ANOVA and Duncan (Duncan, 1951) for similarities of contents in seaweeds. Also, Dunnett's test (Dunnett, 1955) was used for comparing treatments with the control in the bioassay test.

Results

Phenolic, flavonoids contents and total antioxidant activity of seaweeds

The results of the total phenolic content of seaweed extracts are shown in Figure (1a). The contents of phenolics ranged from 21.54 to 303.28 mg GAE/g DW of seaweeds in the studied edible seaweeds. It was noticed that brown seaweeds had the highest content of phenolics compared with other studied seaweeds while the red ones had the lowest values of phenolics. In detail, the highest phenolic content was detected in extracts of *S. horneri*, *E. bicyclis*, and *U. pinnatifida* (303.28, 236.27, and 226.54 mg GAE/g DW, respectively). On the other hand, the lowest phenolics content was noticed in extracts of *C. hypnaeoides*, *G. tenax*, *G. elegans*, and *C. tenellus* (31.1, 30.56, 21.54, and 27.04 mg GAE/g DW, respectively).
The flavonoid content of seaweeds followed similar behaviour as phenolics content (Fig. 1b). Extracts of two types of seaweeds had the highest values of flavonoids; they were brown seaweeds, *S. horneri* and *U. pinnatifida* (45.44 and 44.52 mg CE/g DW, respectively). Green seaweeds had contents of flavonoids lower than brown seaweeds but higher than red ones. The tested red seaweeds had the lowest contents of flavonoids, especially *C. hypnaeoides* and *C. tenellus* (8.33 and 7.40 mg CE/g DW, respectively).

The DPPH scavenging assay was used for analyzing the total antioxidant activity of seaweed extracts. The assay revealed variable antioxidant activities ranging from 22.17% up to 77.15%. The antioxidant activities of tested groups of seaweeds followed this order: brown > green> red seaweeds. In detail, *S. horneri* and *E. bicyclis* extracts of brown seaweeds showed the highest abilities to scavenge DPPH molecules: 77.15 and 73.68%, respectively. Contrarily, the red seaweed extracts had fewer antioxidants. Thus, they recorded lower percentages of DPPH scavenging assay. For example, *G. tenax*, *G. elegans*, and *C. tenellus* extracts had values of 24.81, 22.17, and 23.32%, respectively (Fig. 1c).

Antimicrobial activity of S. horneri and E. bicyclis seaweed

Results of antimicrobial tests revealed that the extract of *S. horneri* showed more antimicrobial activity than *E. bicyclis* (Fig. 2). Formed clear zones (mm) of seaweed extracts were closer to the size of positive controls or higher (Table 1). The aqueous extract of *E. bicyclis* showed the highest effect against *Bacillus subtilis* ATCC 6633. The inhibition zone size against *Staphylococcus aureus* ATCC 29213 was almost equal, using ampicillin and extract of *S. horneri*. Both algal extracts exhibited more inhibition for *Pseudomonas aeruginosa* ATCC 9027 than the positive control. Neither the tested algal extracts nor the positive controls showed any inhibition for the growth of *E. coli* ATCC 25922 or *Candida albicans* ATCC 18804.

Proteins and carbohydrates content of seaweeds

The soluble, insoluble, and total protein content of different seaweeds are shown in Table (2). Variable amounts of proteins were detected among seaweeds. Except for *U. pinnatifida* and *H. fusiforme*, all brown seaweeds recorded the highest soluble and total protein content. On the other hand, green and red seaweeds had lower values of soluble proteins compared with those of brown seaweeds. For example, *M. nitidum* and *C. hypnaeoides* had values of 147.45 and 138.04 mg/g DW, respectively. Similarly, the total proteins of red seaweeds were lower than those of brown seaweeds. The total protein content of red seaweeds ranged from 310.6 to 454.91 mg/g DW, while the protein content of brown seaweeds ranged from 629.42 to 721.59 mg/g DW. Moreover, it was noticed that in some cases, the total protein content was doubled in brown seaweeds compared with red seaweeds.

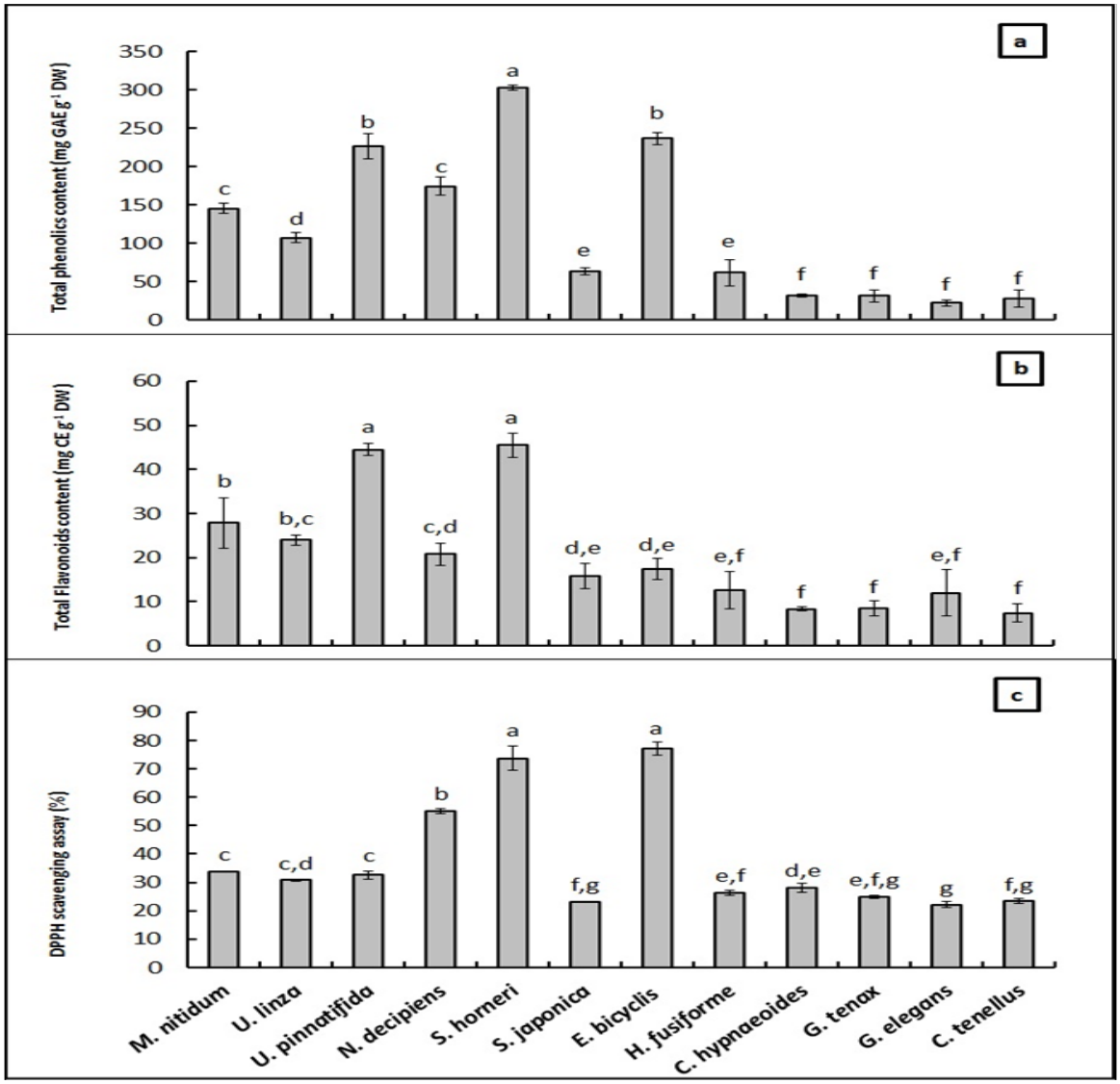

Fig. 1. Phenolics', flavonoids' contents, and total antioxidant activity of tested seaweeds. (a) total phenolic content (mg GAE g-1DW), (b) total flavonoids content (mg CE g-1DW), and (c) total antioxidant activity (%). Duncan's composition between variants at α = 0.05. The variants with the same letters showed the same reaction.

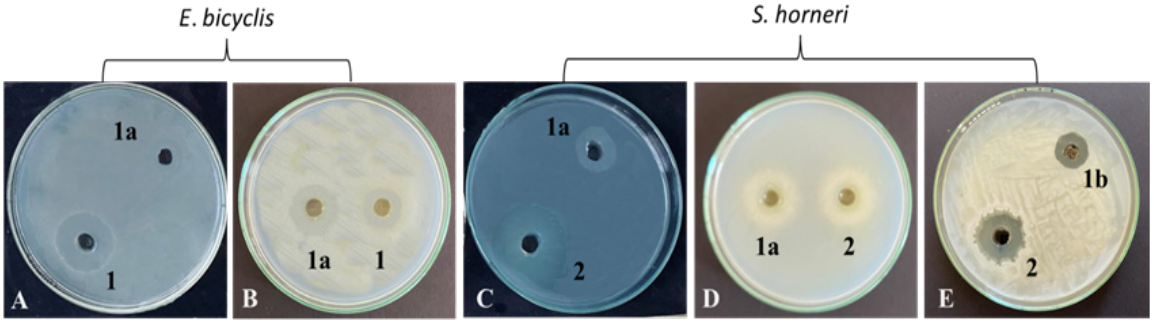

Fig. 2. Antimicrobial test of seaweeds; A and C, *Pseudomonas aeruginosa* ATCC 9027; B, *Bacillus subtilis* ATCC 6633; D, *Staphylococcus aureus* ATCC 29213; E, *Candida albicans* ATCC 10231. 1a, positive control (Ampicillin)1b, positive control (Fluconazole).

TABLE 1. Antimicrobial activities of *S. horneri and E. bicyclis*

Microbe	*E. bicyclis*			*S. horneri*			Ampicillin			Fluconazole		
	M	±	*SD*	*M*	±	*SD*	*M*	±	*SD*	*M*	±	*SD*
Bacillus subtilis ATCC 6633	13.67	±	0.33	0	±	0	11.53	±	0.47	NA	±	NA
Staphylococcus aureus ATCC 29213	0	±	0	12.7	±	0.33	13.93	±	0.12	NA	±	NA
E. coli ATCC 25922	0	±	0	0	±	0	0	±	0	NA	±	NA
Pseudomonas aeruginosa ATCC 9027	14.5	±	0.25	19.33	±	0.33	10.5	±	0.5	NA	±	NA
Candida albicans ATCC 10231	0	±	0	11.5	±	0.25	NA	±	NA	12.47	±	0.50
Candida albicans ATCC 18804	0	±	0	0	±	0	NA	±	NA	0	±	0

NA: no activity; values represent the mean±SD, n=3; inhibition zone measured in millimeters (mm).

TABLE 2. Proteins content (mg g^{-1} DW) of different edible seaweeds.

Seaweeds		(Proteins content (mg/g DW									
		Soluble			**Insoluble**			**Total**			
		M	±	*SD*	*M*	±	*SD*	*M*	±	*SD*	
Green	*M. nitidum*	147.45^{d}	±	10.87	94.12^{e}	±	18.82	241.57^{e}	±	10.87	
	U. linza	144.32^{d}	±	13.47	536.48^{a}	±	16.55	680.80^{a}	±	16.55	
Brown	*U. pinnatifida*	134.91^{d}	±	19.19	586.68^{a}	±	16.62	721.59^{a}	±	14.38	
	N. decipiens	222.75^{b}	±	13.05	406.67^{b}	±	11.74	629.42^{b}	±	11.03	
	S. horneri	288.63^{a}	±	12.44	398.44^{b}	±	19.97	687.08^{a}	±	17.25	
	S. japonica	244.71^{b}	±	19.97	429.81^{b}	±	16.59	674.53^{a}	±	13.31	
	E. bicyclis	295.70^{a}	±	13.24	$363.93^{b,c}$	±	19.97	$659.63^{a,b}$	±	12.44	
	H. fusiforme	$197.65^{b,c}$	±	17.06	432.95^{b}	±	13.28	630.60^{b}	±	13.28	
Red	*C. hypnaeoides*	138.04^{d}	±	19.59	301.18^{c}	±	13.24	439.23^{c}	±	16.59	
	G. tenax	$207.85^{b,c}$	±	13.28	175.69^{d}	±	13.31	383.54^{c}	±	13.31	
	G. elegans	165.42^{c}	±	9.41	145.18^{d}	±	15.21	310.60^{d}	±	16.36	
	C. tenellus	169.42^{c}	±	6.66	285.50^{c}	±	16.08	454.91^{c}	±	6.661	

Variants with same letters showed statistically similar content by Duncan's test between variants at α=0.05. values represent the mean±SD, n=3; DW=Dry weight.

The results of analysing carbohydrate content (soluble, insoluble, and total) in different seaweeds are presented in Table (3). The studied seaweeds had a lower content of carbohydrates compared to their protein content. Soluble carbohydrate contents were variable among the tested seaweeds. *M. nitidum*, *C. tenellus*, and *N. decipiem* had the highest values of soluble carbohydrates (108.27, 92.07, and 89.68 mg/g DW, respectively). Contrarily, *U. linza*, *S. japonica*, *U. pinnatifida*, and *H. fusiforme* had the lowest soluble carbohydrate content. Moreover, seaweeds recorded total carbohydrate contents ranging from 52.62 to 171.08 mg/g DW. Except for *E. bicyclis* and *N. decipiens*, all tested brown seaweeds were low in total carbohydrates when compared with red seaweeds. The seaweeds that recorded the highest content of total carbohydrates were *M. nitidum,* which belongs to green seaweeds, *E. bicyclis*; brown seaweed, and *C. hypnaeoides,* which belongs to red seaweed (171.08, 160.02, and 137.85 mg/g DW, respectively). Others that recorded the lowest total carbohydrates were *H. fusiforme* and *U. pinnatifida,* which belongs to brown seaweeds and *U. linza,* which belongs to green seaweeds (52.62, 86.11, and 71.82 mg/g DW, respectively).

TABLE 3. Carbohydrates content (mg g^{-1} DW) of different edible seaweeds

Seaweeds		Carbohydrates									
		Soluble			**Insoluble**			**Total**			
		M	±	*SD*	*M*	±	*SD*	*M*	±	*SD*	
Green	*M. nitidum*	108.27[a]	±	1.35	62.81[a]	±	10.89	171.08[a]	±	0.46	
	U. linza	27.34[c]	±	3.07	40.79[c]	±	8.88	71.82[d]	±	5.30	
Brown	*U. pinnatifida*	6.44[c]2	±	1.25	59.67[a]	±	7.60	86.11[c]	±	6.57	
	N. decipiens	89.68[a]	±	2.00	30.16[d]	±	3.38	118.68[b,c]	±	3.38	
	S. horneri	63.25[b]	±	0.46	28.31[e]	±	1.69	91.56[c]	±	2.15	
	S. japonica	51.79[b]	±	1.19	6.65[d]3	±	1.11	98.44[c]	±	1.89	
	E. bicyclis	84.08[a,b]	±	7.34	56.63[b]	±	9.12	160.02[a]	±	1.59	
	H. fusiforme	23.65[c]	±	1.08	8.97[e]٢	±	8.07	52.62[e]	±	9.14	
Red	*C. hypnaeoides*	84.29[a,b]	±	2.43	51.75[b,c]	±	6.60	137.85[b]	±	5.18	
	G. tenax	87.37[a]	±	4.03	44.99[c]	±	7.34	132.35[b]	±	1.95	
	G. elegans	45.13[b]	±	6.43	70.59[a]	±	16.40	115.72[b,c]	±	5.66	
	C. tenellus	92.07[a]	±	3.22	26.76[e]	±	2.32	119.33[b,c]	±	2.45	

Variants with same letters showed statistically similar content by Duncan's test between variants at α=0.05. values .represent the means, n=3; DW=Dry weight

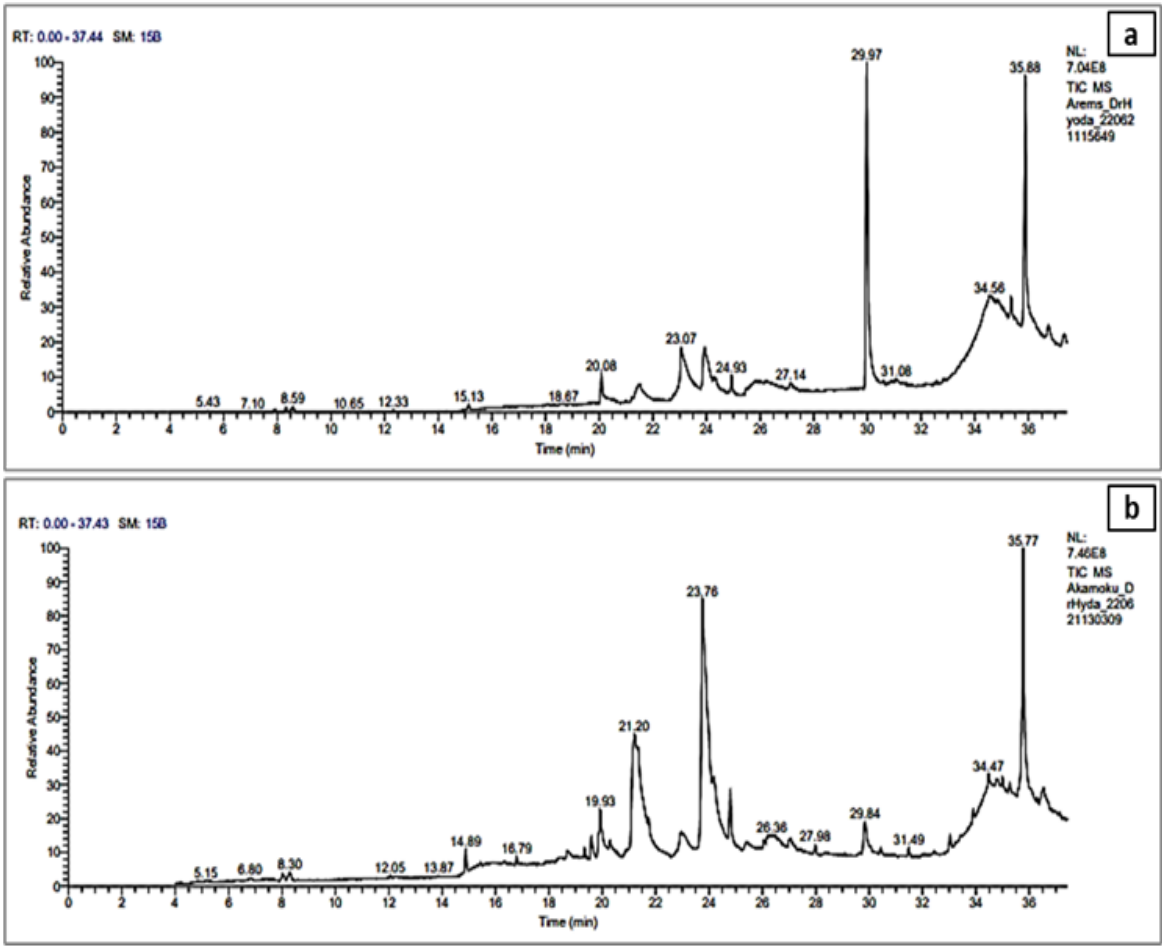

Fig. 3. GC/MS analysis of tested seaweeds. (a) Eisenia bicyclis, (b) Sargassum horneri extracts.

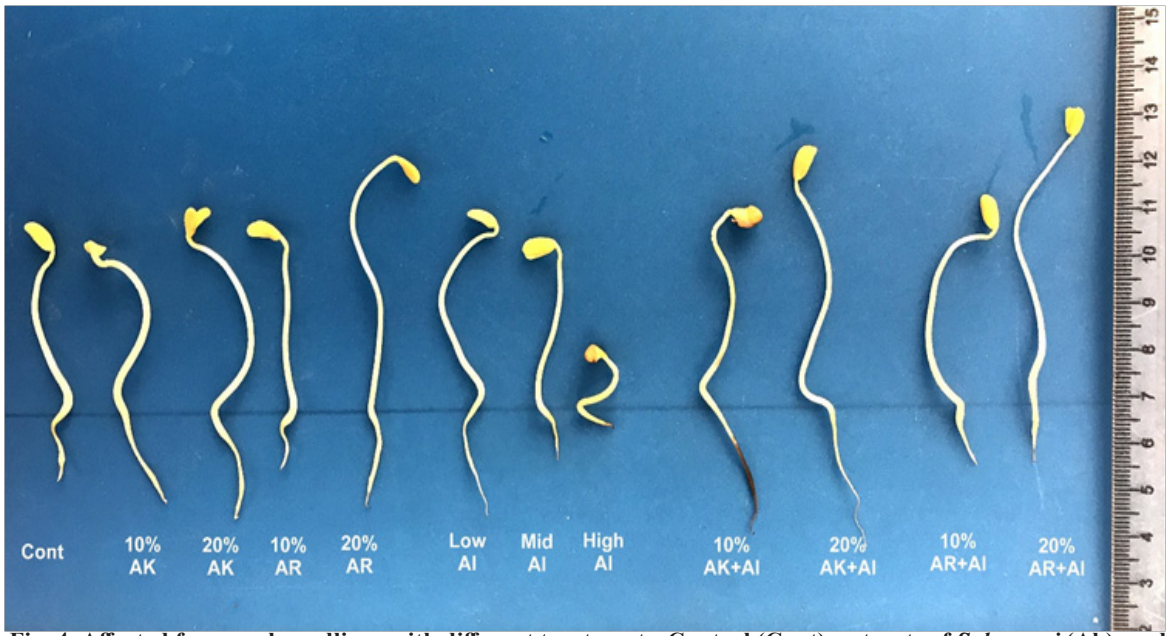

Fig. 4. Affected fenugreek seedlings with different treatments. Control (Cont), extracts of *S. horneri* (Ak), and Al seaweed treated fenugreek seedlings (Al); and extracts of *E. bicyclis* (AR).

TABLE 4. Identified compounds using GC/MS analysis in *Eisenia bicyclis* extract.

Peak No.	RT	Compound name	Molecular formula	Molecular weight	Area %
1	8.32	Geraniol, TMS derivative	C13H26OSi	226	0.47
2	8.59	Isopulegol, [1R-(1à,2á,5à)]-, TMS derivative	C13H26OSi	226	0.67
3	15.13	5-Hydroxy-6-(1-hydroxyethyl)-2,7-dimethoxy-1,4-naphthoquinone	C14H14O6	278	0.56
4	20.08	á-D-Galactopyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic methylboronate ((Remoxipride	C14H31BO6Si2	362	2.94
5	21.49	Hexadecanoic acid, methyl ester	C17H34O2	270	2.58
6	23.06	Palmitic Acid, TMS derivative	C19H40O2Si	328	6.57
7	23.92	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	296	7.21
8	24.32	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	C19H34O6	358	0.97
9	24.93	Phytol, TMS derivative	C23H48OSi	368	1.77
10	25.74	Oleic acid, (Z)-, TMS derivative	C21H42O2Si	354	1.32
11	25.84	TRIMETHYLSILYL(9E)-9-OCTADECENOATE	C21H42O2Si	354	0.70
12	27.13	1,25-Dihydroxyvitamin D3, TMS derivative	C30H52O3Si	488	1.44
13	29.97	Diisooctyl phthalate	C24H38O4	390	34.96
14	34.56	9,12,15-OCTADECATRIENOIC ACID, 2-[(TRIMETHYLSILYL)OXY]-1-[[(TRIMETHYLSILYL)OXY]METHY (L]ETHYL ESTER, (Z,Z,Z	C27H52O4Si2	496	8.38
15	35.35	TRISTRIMETHYLSILYL ETHER DERIVATIVE OF 1,25-DIHYDROXYVITAMIN D2	C37H68O3Si3	644	4.68
16	35.88	Stigmasterol, TMS derivative	C32H56OSi	484	24.78

TABLE 5. Identified compounds using GC/MS analysis in *Sargassum horneri* extract

Peak No.	RT	Compound name	Molecular formula	Molecular weight	Area %
1	8.02	Geraniol, TMS derivative	C13H26OSi	226	0.62
2	8.30	α-Linolenic acid, TMS derivative	C21H38O2Si	350	0.84
3	14.89	4-PENTEN-1-ONE, 2-[BIS(METHYLTHIO)METHYLEN E]-4-METHYL-1-PHENYL	C15H18OS2	278	1.31
4	18.43	-ALANINE, 3-(BENZYLOXY)-, L	C10H13NO3	195	0.62
5	18.70	,-(Octadecanoic acid, 9,10-epoxy-18-(trimethylsiloxy methyl ester	C22H44O4Si	400	0.78
6	19.34	9,12-OCTADECADIENOIC ACID (Z,Z)-, 2,3-BIS[(TRIMETHYLSILYL)OXY]PROPYL ESTER	C27H54O4Si2	498	0.63
7	19.59	17-Octadecynoic acid	C18H32O2	280	1.58
8	19.93	alpha-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic methylboronate	C13H26BNO6Si	331	3.74
9	20.29	TRIDEUTERIOMETHYL 10-EPOXY-7-ETHYL-3,11-DIMET HYLTRIDECA-2,6-DIENOATE	C18H27D3O3	297	1.02
10	21.21	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	C17H34O2	270	20.82
11	23.01	Palmitic Acid, TMS derivative	C19H40O2Si	328	2.15
12	23.75	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	C19H36O2	296	26.43
13	24.79	Phytol, TMS derivative	C23H48OSi	368	3.23
14	25.40	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl] methyl]cyclopropyl] methyl]-, methyl ester	C25H42O2	374	1.29
15	26.27	cis-5,8,11,14,17-Eicosapentaenoic acid	C20H30O2	302	2
16	26.48	(5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z	C21H34O2	318	1.44
17	27.98	4H-1-BENZOPYRAN-4-ONE, 2-(3,4-DIMETHOXYPHENYL)-3,5- DIHYDROXY-7-METHOXY	C18H16O7	344	0.59
18	29.84	Diisooctyl phthalate	C24H38O4	390	3.03
19	34.47	9,12,15-OCTADECATRIENOIC ACID, TRIMETHYLSILYL)OXY]-1-[[(TRIMETHYL-)]2--(SILYL)OXY]METHYL]ETHYL ESTER, (Z,Z,Z	C27H52O4Si2	496	12.69
20	35.77	Stigmasterol, TMS derivative	C32H56OSi	484	15.19

GC/MS analysis

The phytochemical components of the methanolic crude extracts of two seaweeds (*E. bicycles* & *S. horneri*) were analyzed using Gas chromatography/ mass spectrometry (GC/MS), the results shown in Fig. (3 a & b) and the identified compounds with their molecular formula, molecular weight, and area percentage were presented in Tables (4 & 5). A total of 16 different and 20 different compounds were identified in *E. bicyclis* and *S. horneri* respectively; the major detected components in *E. bicyclis* extract were, Diisooctyl phthalate as the first major compound (34.96%), and Stigmasterol, TMS derivative (24.78%) was the second major one, followed by 9,12,15-octadecatrienoic acid, 2-[(trimethylsilyl) oxy[-1-[(trimethylsilyl)oxy[methyl[ethyl ester, (z,z,z), 9-Octadecenoic acid (Z)-, methyl ester, Palmitic Acid, TMS derivative, and tris tri methyl silyl ether derivative of 1,25-dihydroxy vitamin D2, that are registered as 8.38, 7.21, 6.57, and 4.68%, respectively.

Regarding *S. horneri* extract, four major components were detected, 9-octadecenoic acid (Z)-, methyl ester, pentadecanoic acid, 14-methyl-, methyl ester, Stigmasterol, TMS derivative, and 9,12,15-octadecatrienoic acid, 2-[(trimethylsilyl) oxy[-1-[(trimethylsilyl)oxy[methyl[ethyl ester, (z,z,z) that are registered as 26.43, 20.82,15.19, and 12.69%, respectively. Four compounds were detected in both extracts: Stigmasterol, palmitic acid, phthalic acid, and 9,12,15-octadecatrienoic acid,2-[(trimethylsilyl)oxy[-1-trimethylsilyl) oxy[methyl[ethyl ester, (z,z,z) but with different concentrations in each extract.

The use of seaweed extract against aluminium stress in fenugreek seedlings

Figure (4) depicts the effect of different doses of Al on germination and growth of fenugreek seedlings, as well as the use of seaweed extracts to alleviate Al toxicity in plants. An obvious inhibition in the growth of seedlings was noticed due to Al stress, as shown in Fig. 4. Moreover, seedlings germinated in high Al concentrations were highly reduced. This reduction could be noticed in fenugreek seedlings dry weights (Fig. 5a), as well as seedlings' lengths of both shoots and roots (Fig. 5b & c). Mid and high doses of Al caused a reduction of shoot lengths to 75.5 and 34.6% and root lengths to 62.7 and 38.7% of the corresponding controls, respectively. Roots of fenugreek seedlings were more sensitive to Al stress than shoots. More inhibition was recorded in roots than shoots due to Al toxicity. It was found that the use of seaweed extracts to alleviate Al toxicity was efficient when used with a mid-concentration of aluminium (0.3 mM $AlCl_3$).

The growth of seedlings was enhanced when germinated in extracts of *S. horneri* and *E. bicyclis*. *E. bicyclis* extract was more efficient than *S. horneri* in the seedlings' growth and elongation of shoot and root, as well as alleviating Al toxicity in its used concentrations. For example, treatment with 20% *E. bicyclis* extract induced shoot and root lengths to be 135.22 and 104% of their controls, respectively. On the other hand, treatment of mid-Al dose + 20% *E. bicyclis* extract could overcome the reduction caused by Al alone. In detail, the lengths of shoot and root treated with Al+ *E. bicyclis* recorded 147.8 and 103.6% of the corresponding controls, while the lengths were reduced by Al alone to be less than their controls by 24.5 and 37.3%, respectively. It is better to use 20% extract of either *E. bicyclis* or *S. horneri* for treatment where more enhancement of seedling growth was noticed. Moreover, 20% *S. horneri* extracts caused induction of shoot and root lengths to be 114.5 and 106.7% of the corresponding controls. On the other hand, 20% *S. horneri* extracts with mid-Al caused enhanced shoot length (138.4% of control) and root length (126.7% of control). From the obtained results, seaweed extracts were able to induce germination and growth of fenugreek seedlings and could be used to alleviate Al toxicity at certain concentrations (Fig. 4).

Seedling dry weights were highly affected by Al and seaweed extract treatments (Fig. 5a). A noticeable reduction occurred in both mid and high doses of Al. This reduction reached 17% up to 58% less than the control with a mid and high concentration of Al. The use of 20% of either *S. horneri* or *E. bicyclis* extracts could induce more dry material weights of the produced seedlings to be 114.3 and 122.1% of the untreated control. The treatment of fenugreek seedlings with seaweed extracts (especially 20% concentration) mixed with a mid-dose of Al could not only protect the seedlings from Al's harmful effects but also enhance the growth of those seedlings. In detail, dry weights were increased by 32 and 46.5% more than control when treated with *S. horneri* and *E. bicyclis* seaweed extracts mixed with a mid-concentration of Al (Fig. 5a). In most cases, *E. bicyclis* seaweed extract was more efficient in enhancing germination and growth of seedlings and Al toxicity alleviation.

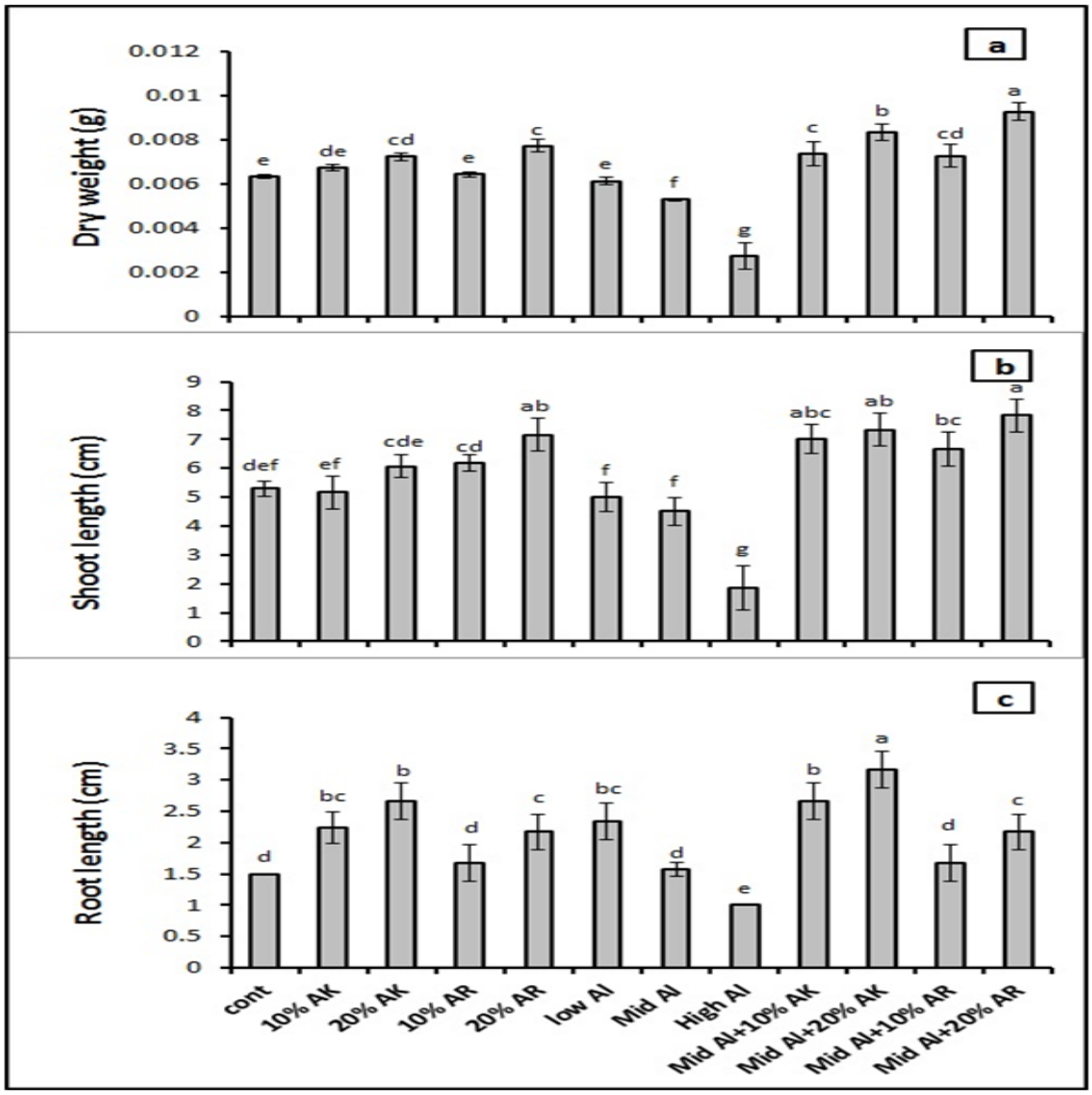

Fig. 5. Effect of seaweeds extraction on fenugreek germination under Al stress. (a) seedling dry weight, (b) shoot length, and (c) root length. Error bars indicate the standard deviation from the mean. Duncan's composition between variants at α = 0.05. The variants with the same letters showed the same reaction.

Discussion

The results of this study demonstrated that brown seaweeds showed high antioxidant capacity, especially extracts of *S. horneri* and *E. bicyclis,* which had the highest ability to scavenge DPPH free radicals. These results were in agreement with those of Heo et al. (2005), who reported the high antioxidant potential of brown seaweeds. Moreover, the brown seaweed *Padina pavonica* was reported to be a source of natural antioxidant compounds (Nabil-Adam & Shreadah, 2021). In this work, the antioxidant activities of tested groups of seaweeds followed this order: brown > green> red seaweeds. These results were in accordance with the results of (Cox et al., 2010, Kindleysides et al., 2012); they reported that brown seaweeds contained higher amounts and more active antioxidant compounds than green and red seaweeds.

Many researchers reported a significant correlation between total phenolic content and the antioxidant activity of extracts (Matanjun et al., 2008; Radwan et al., 2020;Yahia et al., 2020). In seaweeds, the antioxidant activity may be due to their contents of pigments, such as carotenoids and chlorophyll, vitamin precursors, e.g., carotene,

ascorbic acid, thiamine and niacin, as well as the phenolic compounds, such as hydroquinone, polyphenols, and flavonoids (Shahin et al., 2022), that play a role in the protection of seaweeds from oxidative stress (Yan et al., 1999, Shahin et al., 2022).

Earlier studies revealed that marine algal polyphenols had antioxidant activity (Yan et al., 1999; Kuda et al., 2005).

In the current study, the evaluated seaweeds showed variable content of polyphenols. Some contained high amounts of polyphenols, such as *S. horneri*, *E. bicyclis* and *U. pinnatifida* (303.28, 236.27, and 226.54 mg GAE/g DW), while others contained lower polyphenols, such as *C. hypnaeoides*, *G. tenax*, *G. elegans*, and *C. tenellus* (31.1, 30.56, 21.54, and 27.04 mg GAE/g DW, respectively). Moreover, it was noticed that brown seaweeds had the highest contents of polyphenols among the tested groups of seaweeds. Based on the study results, there is a strong correlation between the total antioxidant activity of extracts and their contents of polyphenols. The greatest the content of polyphenols in seaweed extract, the higher the antioxidant activity detected in this extract. Therefore, polyphenols in seaweed extracts determine how well they can scavenge free radicals. Many polyphenols have substantial antioxidant activity and are strong free-radical scavengers due to their redox properties, which can facilitate the adsorption of free radicals (Abdel-Wareth et al., 2019, Shahin et al., 2022). The results showed that the analysis of flavonoid contents in the studied seaweeds was in accordance with the contents of polyphenols. It is well known that flavonoids are part of the polyphenols present in seaweeds (Abbas et al., 2017, Garg et al., 2019). Hence, the antioxidant activity detected in *E. bicyclis* and *U. pinnatifida* seaweeds might be related partially to the presence of a high content of flavonoids. Flavonoids are non-nutritional but help in protection against some diseases, and they can act as anti-inflammatory, anti-hepatotoxic, and anticancer (Gutiérrez-Rodríguez et al., 2018). Due to the presence of high content of flavonoids in seaweeds and their abundance, they can be used as a rich source of flavonoids for medical use.

The antimicrobial activity of seaweed may vary within algal species due to environmental aspects, the region of the thallus or physiological status, growth conditions, and other factors (Freile-Pelegrin & Morales, 2004). El-Shaibany et al. (2022) reported no antibacterial activity of the aqueous extract of *Sargassum mangarevense* against any of the four tested bacteria except a mild effect against *Staphylococcus aureus* (9.5 mm inhibition zone), while in our study *S. horneri* showed an inhibitory effect against three out of the six tested microbes. Also, *E. bicyclis* showed an inhibitory effect against two isolates out of six. The inhibition effect against bacterial growth could be due to increased membrane permeability, imbalance of fluidity, and cytoplasm leakage, causing the lysis of bacterial cells (El-Shaibany et al., 2022). Three antimicrobial compounds (Diisooctyl phthalate, triterpenoids, and diterpene) were reported in this research through the GC/MS analysis, which could explain the antimicrobial activity shown from extracts of *E. bicyclis* and *S. horneri*. Moreover, Pérez et al. (2016) reported that the antimicrobial activity of seaweed does not indicate the presence of a single compound, but it might be attributed to the presence of a combination of compounds and factors at the same time. The emergence of resistant bacteria against various antibiotics led to the discovery of new sources from marine living organisms (Ravikumar et al., 2002). In this study, we reported the antibacterial effect of *E. bicyclis* and *S. horneri* against *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 9027, and the antifungal efficiency against the yeast *Candida albicans* ATCC 10231. Several studies showed that seaweed extracts contain compounds with the potential as natural antimicrobial agents in food preservation, pharmaceuticals, and other industries. For example, seaweed extracts inhibit the growth of harmful bacteria in food and may help reduce the need for chemical preservatives (Sipahutar et al., 2019).

Data obtained from GC/MS analysis showed the presence of Diisooctyl phthalate (34.96 %) and Stigmasterol (24.78%) in major amounts. Others present in minor amounts, such as 9- octadecenoic acid (Z)-methyl ester palmitic acid, tris tri methyl silyl ether derivative of 1,25-dihydroxyvitamin D2. of the detected compounds, stigmasterol is a hormonal compound that promotes plant growth (Kaur et al., 2011; Sohn et al., 2021). It has antimicrobial activity (Duke & Bogenschutz, 1994). Moreover, 9- octadecenoic acid (Z)-methyl ester is a linoleic acid ester compound that has been reported to have anti-gastric and breast cancer due to antioxidant properties (Eluvakkal et al., 2010). Palmitic acid, TMS derivative, is antifungal, anti-tumor, and antibacterial (Duke & Bogenschutz,

1994). Our results revealed the presence of phytol, TMS derivative, in both extracts. This diterpene has antioxidant activity and represents a unique and promising class of medicines for the treatment of chronic inflammatory diseases (Adnan et al., 2019; Dulara et al., 2019).

Besides the medicinal properties of seaweed extracts, they contain many nutritional compounds such as proteins, polysaccharides, vitamins, hormones, fatty acids, sterols, carotenoids, oxylipins, minerals, peptides, and amino acids (Pise & Sabale, 2010; Du Jardin, 2015). These substances vary greatly in seaweed extracts based on the class and species of seaweed as well as the type of extraction method utilized. Among many different algal polysaccharides, the most important types are galactans, fucoidan, laminarin, and alginates. Most of these are proportionally represented in seaweed extracts (Ali et al., 2021a). The analysis of soluble and total proteins in the present study revealed that brown seaweeds were a rich source of proteins, and red seaweeds were considered rich sources of carbohydrates. For example, *E.bicyslis*, *S.horneri*, and *S. japonica* had high soluble and total proteins content. Moreover, the total protein content of brown seaweeds was double fold compared with the content of red seaweeds. Carbohydrates content of brown seaweeds was lower than those detected in red seaweeds. In detail, total carbohydrates were higher in green seaweed *Monostroma nitidum* (171.08 mg/g DW) and red seaweed *Compylaephora hypnaeoides* (137.85), while brown seaweeds recorded almost half the amount of total carbohydrate, such as *U. pinnatifida* (86.11 mg/g DW). Seaweed extracts contain stimulatory components, such as amino acids, proteins, and carbohydrates. Thus, seaweeds have been commonly used as nutritive and biostimulator for plant growth, as well as for the protection of plants against various biotic and/or abiotic stresses (Pise & Sabale, 2010; Du Jardin, 2015).

Seaweed extracts were shown to positively affect seed germination and plant growth at all stages up to harvest and even post-harvest (Ali et al., 2019; Ali et al., 2021b). They were used as treatment against several kinds of stressors (Vera et al., 2011). In this work, a bioassay was carried out by using fenugreek seeds to study the effect of seaweed extracts on germination and growth of seedlings and using seaweed extracts against Al stress. Results revealed the enhancement of germination and growth of fenugreek seedlings in response to seaweed extracts. Oppositely, a reduction of germination and growth of seedlings was noticed in response to Al stress. This reduction can be alleviated by the addition of seaweed extracts, especially extracts of *S. horneri* and *E. bicyclis*. Seedlings subjected to a certain concentration of Al mixed with seaweed extracts showed growth parameters like untreated seedlings. The enhancement and priming effects of seaweed extracts on the plant's defences against both abiotic and biotic stresses can be attributed to the chemical composition of the extracts as well as their eliciting properties (Yakhin et al., 2017). Seaweed products have been shown to promote increased germination rates and cause significant increases in seedling vigour by enhancing root size and density (Rayorath et al., 2008). Seaweeds contain bioactive substances that elicit and directly promote plant growth and defence reactions (Khan et al., 2009). It is well known that Al stress accumulates reactive oxygen species, causing oxidative stress in plants (Ameri et al., 2020, Liang et al., 2022). Moreover, Al toxicity of roots by disruption of cell membranes, crystallizing cell walls, and restricts water and nutrient flow (Kochian et al., 2015, Ofoe et al., 2022). Due to their biosorption characteristics, seaweeds can effectively be used against heavy metal pollution in contaminated soils (Ortiz-Calderon et al., 2017). The promotional effect of seaweeds on the growth of seedlings might be due to their content of nutrients, hormones and other bio-stimulants. Extracts can be used against different kinds of stressors including Al stress due to their contents of bioactive compounds. Briefly, the impact of utilizing promising seaweed extracts in mitigating aluminium toxicity is multifaceted, encompassing antioxidant defense, reduced aluminum accumulation, stimulation of plant growth, regulation of ion homeostasis, activation of stress response mechanisms, and improvement in chlorophyll content. The incorporation of seaweed extracts as a natural and sustainable solution holds great promise for enhancing the adaptive capacity of plants exposed to aluminium stress, thereby contributing to the sustainability of agricultural systems. From the presented study, seaweeds possess a wealth of bioactive compounds that can efficiently be used in many purposes such as medicinal, nutritional, and agricultural.

In conclusion, Brown seaweeds had higher contents of polyphenols, flavonoids and higher

total antioxidant activities than other seaweeds. Extracts of seaweeds stimulated the growth of fenugreek seedlings. Also, they help to protect seedlings from Al toxicity consequences at certain concentrations. Moreover, extracts of *E. bicyclis* and *S. horneri* showed antimicrobial effects against four of the tested pathogens. Thus, using algal extracts for agricultural purposes and pharmacological activities is very promising in the future.

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Declaration:

No relevant financial or non-financial interests to disclose.

Conflict of interest:

No conflict of interest.

Data Availability Statement:

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

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إستكشاف المركبات النشطة بيولوجيًا ومضادات الأكسدة والخصائص المضادة للميكروبات لمستخلصات الأعشاب البحرية لتخفيف إجهاد الألومنيوم في إنبات بذور نبات الحلبة

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تهتم هذه الدراسة بتحليل الخصائص المضادة للأكسدة والمضادة للميكروبات بالإضافة إلى محتوى المركبات النشطة بيولوجيًا في إثني عشر نوعًا من الأعشاب البحرية الصالحة للأكل وتسليط الضوء على إستخدامها ضد سمية الألومنيوم (*Eisenia bicyclis* & *Sargassum horneri*) في بادرات نبات الحلبة. من بين الأعشاب البحرية التي تم اختبارها، كان لدى الأنواع البُنية أعلى إجمالي أنشطة مضادة للأكسدة حيث أظهرا إمكانية التخلص من 74% و77%علي (DPPH).التوالي من الجذور الحرة وحمض البالمتيك والتي تعتبرذات أهمية طبية، وأيضا كمحفزات لنمو النبات وكانت كميات البوليفينول والفلافونويد أعلى في الأعشاب البحرية البنية عنها في الأعشاب البحرية الخضراء أو الحمراء. مستخلصات *E. bicyclis* & *S. horneri* أظهرت تأثيرات مضادة للميكروبات ضد أربعة من أصل ستة كائنات دقيقة تم إختبارهم. أيضا تم إجراء إختبار حيوي بإستخدام بادرات نبات الحلبة المعرضة لإجهاد الألومنيوم و/أو مستخلصات الأعشاب البحرية حيث أدي إستخدام الألومنيوم إلي تثبيط نمو البادرات بينما تعزز نموها عند إنباتها في مستخلصات نباتى *S. horneri* & *E. bicyclis* مع تجنب سمية الألومنيوم. ألقت نتائج دراستنا الضوء على إستخدام مستخلصات الأعشاب البحرية كمصادر للمركبات النشطة بيولوجياً ضد إجهاد الألومنيوم في النباتات، وأيضا كعوامل طبيعية مضادة للميكروبات في حفظ الأغذية والأدوية وغيرها من الصناعات، ويعتبر إستخدامها آمن ومنخفض التكلفة وصديق للبيئة.