Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(2): 173 – 193 (2023)
www.ejabf.journals.ekb.eg

Phytochemical Screening of Bioactive Components of the Brown Seaweed *Sargassum swartzii* and its Stimulatory Effect on Seed Germination of Fenugreek and Barely

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ARTICLE INFO
Article History:
Received: Dec. 2, 2022
Accepted: Dec. 27, 2022
Online: March 17, 2023

Keywords:
Seaweeds,
Sargassum swartzii,
Antioxidants,
Phenolics,
GC-MS profile,
Seed germination

ABSTRACT
Algae are an important raw material for the bio-based economy due to their unique content of bioactive compounds. Therefore, this study aimed to evaluate simple applicable extraction methods of the brown seaweed *Sargassum swarttzii* and identify some of its bioactive phytocomponents using a Gas Chromatography-Mass Spectrometry of the most effective extract. Serial concentrations from the three different *S. swartzii* extracts; autoclaved water extract (SAWE), ethanolic extract (SEE) and cold water extract (SCWE) were compared for their effect on seed germination of two economic crops in Egypt; fenugreek (*Trigonella foenum-graecum* L.) and barley (*Hordeum vulgare* L.). The total carbohydrates, protein and lipid contents were estimated for the algal biomass; in addition, the total phenolics, total flavonoids and total antioxidant activity were evaluated. The results proved that the SCWE enhanced seed germination of both plants by 5.9% in fenugreek and barely, respectively. The SCWE improved the seedling length by 59.3% at 20% SCWE and 41.6% at the concentration of 40% SCWE in fenugreek and barely, respectively. Moreover, the bioactive compounds and the total antioxidant activity increased significantly in the SCWE over the SAWE and SEE. On top of the 32 bioactive phytocomponents detected by the GC-MS of SCWE were palmitic acid, alpha-ionone, oleic acid, triacetin and lucenin-2. Thus, SCWE could be a promising alternative to harmful synthetic antioxidants and biofertilizers.

INTRODUCTION

Biomass biorefning for the production of diverse products is associated with sustainable development goals. Seaweeds, which play a key ecological role in coastal ecosystems can address problems associated with climate change, bioenergy production, sustainable agriculture, animal and human health, valuable chemicals, and bioactive compounds. In addition, seaweeds could offer a sustainable circular bioeconomy approach if used appropriately **(Barbier *et al.*, 2020)**. In biorefineries, seaweeds have many medical applications including foods and food supplements, animal feed, cosmetics, nutraceuticals, pharmaceuticals and biofertilizers/plant enhancers **(FAO,**

2018). Recent studies reported different medical and pharmaceutical applications of seaweeds including antioxidant and antitumor activities **(El-sheekh *et al*., 2022)**. Besides, macroalgal biomass can be utilized in different biofuels production in parallel with wastewater treatment **(Abomohra *et al*., 2021)**.

There is a wide variety of algae species. A wide diversity of species, including macroalgae, microalgae and cyanobacteria are used as feedstock for the creation of algal extracts **(Izabela & Katarzyna, 2014)**. "*Sargassum*" is a genus of brown macroalgae (seaweed). *Sargassum* is predominantly cold-water organisms that benefit from nutrients upwelling **(Hogan *et al.*, 2011)**. Although natural extracts have a wide range of applications, one issue with natural products is that their composition might vary depending on the source material (season, location) and the extraction method **(Alves *et al.*, 2013)**. Different studies on the development of a variety of extraction methods to obtain valuable algal extracts have been reported **(Izabela & Katarzyna, 2014)**.

Seaweed extracts have been used as agricultural biostimulants to enhance plant growth **(EBIC 2012)**. This is because seaweeds produce a broad spectrum of secondary metabolites as they live in a non-friendly environment but photodynamically they are not damaged, owing to their capability to synthesize several protective compounds and develop different protecting mechanisms **(Gupta *et al.*, 2012)**.

Secondary metabolites from macroalgae, especially marine species may be potentially bioactive compounds that can be incorporated into human food products for safety and preservation as they are edible, non-toxic and cheap in animal food and modern agriculture in addition to food, cosmetic and pharmaceutical industries **(Izabela & Chojnacka, 2014; Hayes *et al.* 2015)**. These compounds render the macroalgae as biological weapons used for killing or incapacitating the targeted host **(Barzkar *et al.*, 2019)**.

Higher productivity in modern agriculture ought to go hand in hand with a less negative environmental effect and greater sustainability. These biostimulants meet the requirements for regular fertilizing efficiency improvement (increased nutrient absorption efficiency), crop production and quality improvement, enhanced tolerance to environmental stress and antioxidant characteristics **(Rathore *et al.*, 2009)**. Natural compounds known as "biostimulants" help plants grow, absorb nutrients and adapt to a variety of environmental stresses and climatic conditions **(Spinelli *et al.*, 2010)**.

Fenugreek (*Trigonella foenum-graecum* L.) belongs to the family Leguminosae (Fabace) and subfamily Papilionacea. It is widely used as spice, condiment **(Dwivedi *et al.*, 2006)** and medicinal plant since both seeds & leaves are a rich source of a wide diversity of medicinal phytochemicals **(Zandi *et al.*, 2017)**. The antioxidant activity of fenugreek prevents the oxidative damage occuring by reactive oxygen species (ROS) via acting as ROS scavengers and may also prevent the occurrence of diseases such as cancer and aging **(Lee *et al*., 2004)**. Moreover, continuous feeding of 1% fenugreek seed

powder prevents colon cancer and slows down the development of cancer cell lines in the breast, pancreas and prostate **(Shabbeer *et al*., 2009)**.

Barley (*Hordeum vulgare* L.) is a member of the grass family. It is ranked among the top three cereal crops in the world following maize, wheat and rice. It is one of the oldest food crops that have been cultivated since the dawn of civilization. Due to its extremely resistant character, barley is effectively grown in a variety of topographical environments, including plains and hilly locations, under rained and irrigated conditions and severe agro environments such as drought, salt, alkalinity, etc... Because of its input requirements and the superior capacity to withstand severe environments, barley is typically regarded as a poor man's crop **(Kumar *et al*., 2012)**. Moreover, there has been a resurgence of interest and use of barley as food, primarily in the developed world, due to an increasing focus on incorporating the diversity in consumption of whole grain in people's diets due to its health benefits **(Basudeb *et al.*, 2013)**. Nevertheless, barley has served as a crucial crop model for several genetic, biochemical and developmental biology investigations during the last century, especially for barley's near sibling, wheat (**Langridge, 2018)**.

The present study aimed to evaluate and assess which method of extraction of the examined algal extract is the most effective as a biostimulant for the germination of two socioeconomic crops "fenugreek and barely". In addition, this work was designed to determine the main qualitative and quantitative phytochemical composition of the most effective extract and illustrate the different responses of the two plant species to the concentration effect of the three extracts.

2. MATERIALS AND METHODS

2.1. Seaweeds collection and preparation of different algal extracts

The seaweed *S. swartzii* samples were collected in July 2020 from Jizan city's coastline region near the industrial area (about 16°49'20.8"N, 42°37'17.0"E), Red Sea, Kingdom of Saudi Arabia. The seaweeds were rinsed on-site with seawater, followed by tap three times washing with water, and finally with bottled drinking water; they were morphologically identified on genus level **(Guiry, 2010)**. The seaweeds were shade dried for 7 days at 40°C. The dry algal biomass was cut, milled and sieved using a 2mm mesh and stored for future work.

For the preparation of algal water extracts, 5g of milled algal dry biomass were mixed with 50ml of distilled water, kept in water bath at 35℃ for 6 hours for cold extract preparation and autoclaved for 20 minutes at 121°C for autoclaved extract preparation. The ethanolic extraction of *S. swartzii* was prepared by mixing 5g of algal powder with 50ml of absolute ethanol and kept at room temperature for 6 hours, then ethanol was evaporated and the residue was re-dissolved in 50ml of distilled water.

2.2. Plant materials

Healthy looking and uniform sized seeds of fenugreek (*T. foenum-graecum* L) and barely (*H. vulgare* L) were provided from the Agriculture Research Center (ARC), Giza, Egypt.

2.3. Bioassay of the effect of different algal extracts

Ten serial dilutions (10%:100%) of each algal extract were prepared using distilled water. Twenty seeds from each tested plant were surface sterilized using 0.01% $HgCl_2$ for one minute and rinsed gently with sterilized distilled water. The seeds from each tested plant were presoaked in each extract dilution for 6 hours. Simultaneously, another 20 seeds of each crop were treated with distilled water only as control samples, then all seeds were separately kept on filter paper (Whatman No. 1) inside sterilized Petri dishes (9 cm) at room temperature (28 °C ±1). Each filter paper was kept moist via the addition of regular tap water for both control and treatment seeds.

2.4. Germination parameters

Germination percentage (GP), as well as seedling length (SL; cm) were determined after 7 days from sowing for the 10 concentrations of each extract.

Germination (%) is the number of normal seedlings; it was counted according to the next formula:

$$\text{Germination (\%)} = \frac{\text{No. of germinated seeds}}{\text{Total No. of tested seeds}} \text{ x100}$$

2.5. Determination of the physio-biochemical indices of *Sargassum* cold water liquid extract (SCWE)

2.5.1. Determination of S. swartzii Primary Metabolites

2.5.1.1. Estimation of total carbohydrates, protein and lipids. The total carbohydrate content in macroalgal biomass was estimated by the phenol-sulfuric acid at 490nm **(DuBois *et al.*, 2002)**.The total protein in macroalgal biomass was estimated using the Lowry method at 750nm **(Lowry *et al.*, 1951)**. Lipid extraction was performed using the modified method of **Folch *et al.* (1957),** with the use of chloroform/methanol (2/1, v/v).

2.5.2. Determination of secondary metabolites, nonenzymatic and total antioxidant capacity

2.5.2.1. Estimation of total phenolics. Total phenolic content was estimated according to **Singleton and Rossi (1965)** using Folin-Ciocalteu reagent. The absorbance was

measured at 725nm. Using a standard curve, the total phenolics were calculated as µg gallic acid equivalent/ml of algal extract.

2.5.2. 2. Estimation of total flavonoids . Total flavonoid content was estimated via the aluminum chloride calorimetric assay **(Zhuang *et al*., 1992)**. The total flavonoids were calculated from the standard plot and expressed as µg catechin equivalent/ ml algal extract.

2. 5.2.3. Estimation of total antioxidant capacity. The total antioxidant capacity of the different algal extracts was performed using the phosphomolybdenum method **(Prieto *et al*. 1999)**. The antioxidant activity is expressed as the number of µg equivalent of ascorbic acid/ml of algal extract.

2.6. Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical composition of the SCWE was performed using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C/ min to 250°C, and held for 2 min, increased to the final temperature of 300°C by 30°C/ min and held for 2min. The injector and MS transfer line temperatures were kept at 270 and 260°C, respectively; helium was used as a carrier gas at a constant flow rate of 1ml/ min. The solvent delay was 4min and diluted samples of 1.0µl were automatically injected using auto-sampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200°C. The components were identified by comparing their mass spectra with those of WILEY 09 and NIST 14 mass spectral database **(Mamoun & Abd El-Kareem, 2016)**.

2.6.1. Determination of phytochemical constituents of the GC- analysis for S. swartzii cold water extract (SCWE)

Wiley Registry, NIST, RepLib, Hit Spectrum and MainLib libraries were used to identify the phytochemical constituents by comparing the recognized mass spectra for each compound with the data stored in the previous libraries. The percentage amount for each component was calculated using by retention time index (R.T) and comparing its average peak area with total peak areas **(Ali *et al*., 2017, 2018; Abeed *et al*., 2021)**.

2.7. Statistical analysis

Each experiment was conducted three times, and the results were calculated as the mean ± standard deviation (SD). SPSS software (IBM, v25) was used to run the one-way analysis of variance (ANOVA) followed by post hoc Duncan's test that was used to determine the difference in growth and metabolic parameters between the target treatment groups and the control group at a probability level ($P \leq 0.05$).

3. RESULTS

3.1. Seed germination and seedling length

S. swartzii as a marine macroalga is rich in bioactive phytochemicals (Fig. 1). The different *Sargassum* extracts employed in this investigation; *S. swartzii* autoclaved water extract (SAWE), *S. swartzii* ethanolic extract (SEE), and *S. swartzii* cold water extract (SCWE) exhibited varied implications on fenugreek and barely seeds not only in the issue of the extract type but also in the tolerance of the seeds to the varied concentration of each extract.

Fig. 1. Fresh part of *S. swartzii*

Figs. (2, 3) illustrate that the seed germination pattern of fenugreek under the different concentrations of the SAWE, SEE, and SCWE varies widely. The data illustrated in Figs. (2, 3) reveal that all the extracts enhanced seed germination percent by 5.9% over the control; however, this enhancement took place at different concentration of each type of the extracts.

The SAWE had a positive effect on both tested seeds germination to varying degrees. Its stimulating effect was recorded on concentrations ranging between 60-90% on fenugreek, while this effect was clear on barley at concentrations of 40-70%. With respect to the SEE, the induction of the maximum seed germination percentage started at 30% till 70% in fenugreek and from 30% till 60% in barely, and then fell down to the control level at 80-100% in fenugreek and 70% in barely, and finally suppressed the germination of barely at the concentration of 80-100% in barely. On the other hand, The SCWE initiated the induction of the highest germination percentage at 10% till 50% in fenugreek and from 30% till 50% in barely, and then dropped off to the control level at 90% and 80% in fenugreek and barely, and finally inhibited the germination of both crops at the concentrations 100% and 90-100% in fenugreek and barely, respectively.

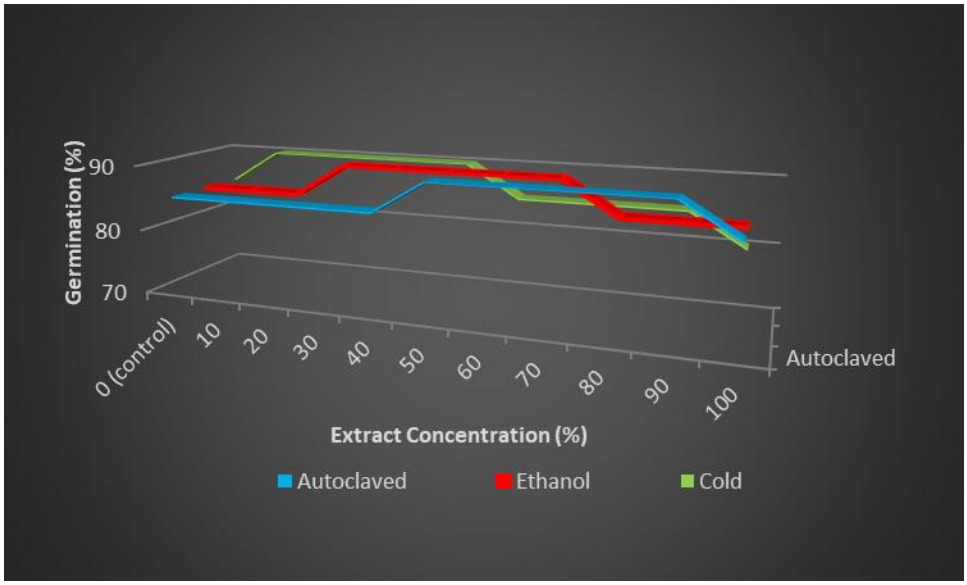

Fig. 2. Illustration of seed germination pattern of fenugreek under the different serial dilutions of the autoclaved water *S. swartzii* extract (SAWE), ethanol *S. swartzii* extract (SEE) and cold water *S. swartzii* extract (SCWE)

Compared to terrestrial plants, the chemical structure of seaweeds is less well understood. They are a rich source of carbohydrates, alginate, fucoidan and laminarin, proteins, minerals (K, Ca, Mg, Na, Zn, Cu, Co, I, B), vitamins (B12, K, C, E, A, D), oils, fats and polyunsaturated fatty acids (e.g. omega-3 and omega-6 fatty acids. Algae also include bioactive components including pigments (carotenoids [carotene xanthophyll]), antioxidants (polyphenols, tocopherols [vitamin E]), chlorophylls and phycobilins. It is important to note that, seaweeds have the ability to synthesize special unique chemicals that are not synthesized by terrestrial plants **(Pulz & Gross, 2004; Plaza *et al.*, 2008; Iba˜nez *et al.*, 2012)**.

In the current investigation, the influence of applying the SAWE, SEE and SCWE on seed germination of fenugreek and barely (Figs. 2, 3) were somewhat similar. This comparable proficient effect might be adjudicated by the relation between the different extracts of phytochemicals and fenugreek and barely metabolism (donor-receiver relation) **(Abeed *et al.*, 2021)**.

The data enumerated in Tables (1, 2) expose the influence of the different extracts on the seedling length that was more pronounced. The results indicate that the SCWE improved the seedling length by 59.3% at the concentration 20% SCWE and by 41.6% at the concentration of 40% SCWE in fenugreek and barely one to-one.

In the second order came the SAWE (8.38 and 9.64 cm seedling length) at concentrations of 80% and 60%, followed by the SEE (8.09 and 9.61cm seedling length at concentrations 50% and 40% in fenugreek and barely in that order.

Table 1. The influence of different *S. swartzii* extracts concentrations on fenugreek seeds shoot length (cm)

Algal extract concentration (%)	Autoclaved (121℃)	Ethanol	Cold (35℃)
0 (control)		6.02±3.16^{b}	
10	6.03 ± 3.15^{b}	6.01 ±2.19^{b}	8.24 ±3.35^{ab}
20	6.84 ± 3.48^{b}	6.44 ±2.78^{b}	9.59 ±1.46^{a}
30	6.98 ± 2.77^{b}	6.73 ±2.33^{b}	8.36 ±3.03^{ab}
40	7.03 ± 3.29^{b}	8.00 ±3.05^{ab}	8.02 ±3.61^{ab}
50	6.88 ± 2.74^{b}	8.09 ±2.32^{ab}	8.08 ±2.88^{ab}
60	6.96 ± 2.9^{b}	7.61 ±3.22^{ab}	7.84 ±3.97^{ab}
70	7.55 ± 2.91^{ab}	7.43 ±2.47^{ab}	7.46 ±4.1^{ab}
80	8.38 ± 3.48^{ab}	7.16 ±2.58^{ab}	6.74 ±3.22^{b}
90	7.58 ± 2.77^{ab}	7.14 ±2.7^{ab}	6.51 ±2.99^{b}
100	6.78 ± 3.36^{b}	6.29 ±2.68^{b}	5.84 ±4.22^{b}

Data are presented as mean ± SD (n = 20). Data with different letters are significantly different according to DMRTs at 0.05 level.

Table 2. Influence of different *S. swartzii* extracts concentrations on barely shoot length (cm)

Algal extract concentration (%)	Autoclaved (121℃)	Ethanol	Cold (35℃)
0 (Control)		7.07± 3.5^{b}	
10	7.34±3.7^{ab}	7.37±3.16^{ab}	7.67±3.54^{ab}
20	7.27±2.13^{ab}	7.95±4.1^{ab}	7.59±3.66^{ab}
30	7.96±3.8^{ab}	9.22±1.64^{ab}	8.32±2.48^{ab}
40	8.76±2.74^{ab}	9.61±2.88^{ab}	10.01±3.22^{a}
50	9.27±2.83^{ab}	8.80±2.84^{ab}	9.25±1.22^{ab}
60	9.64±1.2^{ab}	8.23±2.85^{ab}	8.28±3.98^{ab}
70	9.52±2.73^{ab}	8.07±2.97^{ab}	7.86±2.27^{ab}
80	9.28±3.08^{ab}	7.95±3.64^{ab}	7.70±3.56^{ab}
90	8.82±3.64^{ab}	7.86±2.86^{ab}	6.87±3.93^{ab}
100	8.31±3.04^{ab}	7.03±4.48^{b}	7.39±3.97^{b}

Data are presented as mean ± SD (n = 20). Data with different letters are significantly different according to DMRTs at 0.05 level.

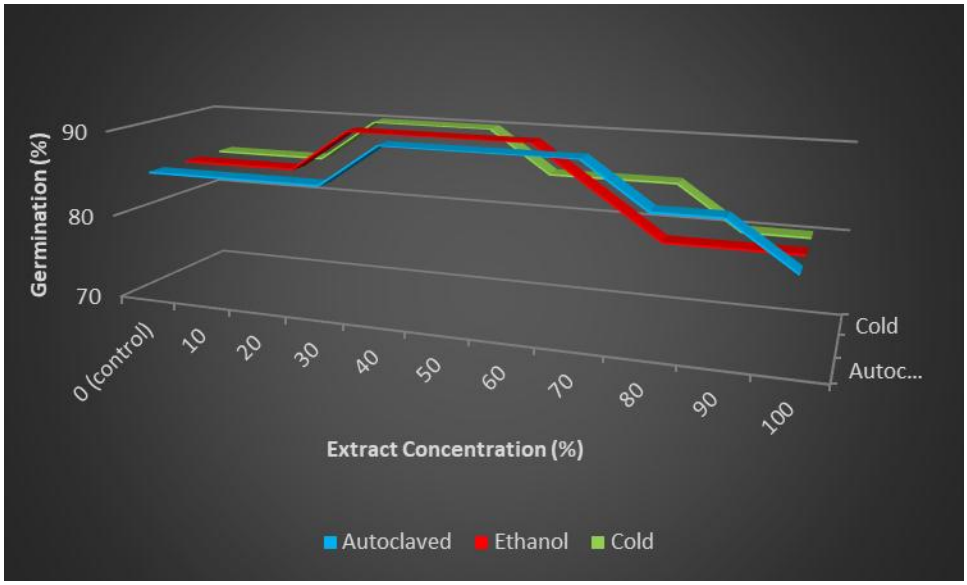

Fig. 3. Illustration of seed germination pattern of barely under the different serial dilutions of the autoclaved water *S. swartzii* extract (AWSE), ethanol *S. swartzii* extract (ESE) and cold water *S. swartzii* extract (SCWE)

Seaweed extracts are beneficial for plant cultivation since they can improve a variety of physiological processes, including crop production, growth and resistance to cold. Moreover, they serve as bio-fungicides and bio-insecticides and enhance soil nutrient absorption since they include plant growth regulators **(Stirk & Van Staden, 1997)**.

Given that the active ingredients are effective even in tiny amounts, the extracts are utilized in low dosages **(Stirk & Van Staden, 1997)**. Extracts that are important for plant growth are cytokinins, auxins, abscisic acid, vitamins, amino acids and nutrients. According to **Rathore *et al.* (2009)**, the outcome is the consequence of the interaction between a number of substances found in algal extracts, including phytohormones, betaines (organic osmolytes), polymers, minerals and alginic acid (a soil conditioner that promotes soil structure) **(Spinelli *et al.*, 2010; Jannin *et al.*, 2013)**. Hence, the aforementioned results signpost the cold water extract to be the best application to produce the highest seedling lengths.

3.2. Algal primary metabolites

Regarding the physio-biochemical indices, the primary metabolites were estimated in the algal (*S. swartzii*) biomass. Whereas, the secondary metabolites were determined in the SCWE because it was found to be the most efficient extract on fenugreek and barley seedling growth.

3.21. Algal carbohydrates content

Microalgal polysaccharides are components of the algal cell walls (fucoidan, alginate, laminarin). These saccharides have several roles as they provide strength and flexibility, maintain ionic equilibrium and prevent from desiccation **(Spolaore *et al.*, 2006; Kindleysides *et al.*, 2012; Balboa *et al.*, 2013; Onofrejova *et al.*, 2010)**.

The total carbohydrate content of the examined alga in this investigation is 54.33% Dwt **(Table 3)**. This carbohydrate enhances plant growth in different ways of which are the probable degradation of the algal polysaccharide into simple sugars and the considerable conversion of the carbon skeleton of algal nitrogenous compounds to carbohydrates in the plant tissues **(Opik, 1966)**.

3.2.2. Algal proteins content

The results displayed in Table (3) reveal that the total protein content of *S. swartzii* is relatively low (9.67% Dwt). This outcome may be attributed to the anti-oxidant role of the microalgal protein, peptides and essential amino acids, Proteins known as phytochelatins, which are produced in reaction to exposure to hazardous metal ions, are found in algal cells. However, the attempt to extract and utilize these proteins is not documented in the literature that is now available **(Volland, 2013)**.

3.2.3. Algal lipids content

As a macro alga, *S. swartzii* has a very low lipid content (1.08% Dwt) compared to the green microalgae (Table 3) even though the microalgal lipids are mainly polyunsaturated fatty acids (PUFA) (ω-3 and ω-6) and are found in higher level compared to terrestrial plants. They act as structural membrane lipids being important in human and livestock diet. They serve as structural membrane lipids and are crucial to the diets of both humans and animals. They are composed of glycerol, sugars, bases and saturated or unsaturated fatty acids. Additionally, the fatty acid compounds in brown seaweed have started to gain researchers attention as it proved to exhibit antibacterial properties **(Zheng *et al.*, 2005)**. The key components of antibacterial properties that inhibit the growth of unnecessary microorganisms are fatty acids **(Freese *et al.*, 1973; Zheng *et al.*, 2005)**. Synthesis of fatty acids in microbes is crucial for developing several lipid-containing elements such as the cell membrane.

A previous study proposed that unsaturated fatty acids such as linoleic acid exhibited antibacterial properties by selectively inhibiting bacterial enoyl-acyl carrier protein reductase, a crucial component of the synthesis of bacterial fatty acid **(Zheng *et al.*, 2005)**. This was supported by further studies, which showed that fatty acid has important phytopharmaceutical potentials, which contribute to its antibacterial properties **(Desbois & Smith, 2010)**.

Table 3. Primary metabolites, total carbohydrate, total protein and lipid contents of *S. swartzii*

Moisture content %	Carbohydrates %	Protein %	Lipid %
82.76	54.33	9.67	1.08

3.3. Algal secondary metabolites

The three examined extracts, SCWE, SAWE and SEE, were compared for their non-enzymatic substances, the total phenolics and flavonoids and for their total antioxidant capacities (Table 4). The obtained results revealed that, SCWE have the highest levels of phenolics (5749.04 µg/ml) and flavonoids (352.22µg/ ml) and exceeds significantly the SAWE (4559.09 µg/ml) phenolics and flavonoids (317.00µg/ ml), and greatly proceeds the SEE, which shows the lowest values of phenolics (662.21µg/ ml) and flavonoids (39.00µg/ ml). Consequently, the total antioxidant activity was the maximum value in SCWE as the maximum total antioxidant activity (8688.39 µg/ml) over the SAWE (7535.35 µg/ml) and the SEE with its the lowest activity (297.21 µg/ml) (Table 4).

Algae exist in the surroundings that are continually changing in terms of salinity, temperature, light availability, ebbs and flows. Most algae have developed defense mechanisms and created a wide range of secondary metabolites, with potential biological activity to live in these severe and unfavorable environments **(Iba˜nez *et al*., 2012)**.

Table 4. SCWE contents of secondary metabolites, nonenzymatic (total phenolics and flavonoids) and total antioxidant capacity

Treatment	Total phenolics (µg/ml)	Total Flavonoids (µg/ml)	Total antioxidant capacity (µg/ml)
SCWE	5749.04 ±118.2^{a}	352.22 ±7.7^{a}	8688.39 ± 510.^{5a}
SAWE	4559.09 ±79.8^{b}	317.00 ±30.3^{a}	7535.35 ± 26.4^{b}
SEE	662.21 ±26.3^{c}	39.00 ±1.7^{b}	297.21 ± 65.1^{c}

Data are presented as mean ± SD (n = 20). Data with different letters are significantly different according to DMRTs at 0.05 level.

3.3.1. Phenolics and flavonoids

The promotion of nourishing-promoting secondary metabolites by SCWE was closely correlated to the elevated levels of phenolics (5749.04µg/ ml) and flavonoids (352.22µg/ ml) in the SCWE, which significantly exceeds the SAWE (4559.09µg/ ml) phenolic and flavonoid (317.00 µg/ml), and greatly proceeds the SEE which shows the lowest values of phenolics (662.21 µg/ml) & flavonoids (39.00 µg/ml) (Table 4).

For the microalgal phenolics and phlorotannins, the latters are not found in higher terrestrial plants. The phenol rings in polyphenols serve as electron traps for radicals scavenging. This characteristic lends this phenolic antimicrobial, antioxidant and antiviral properties to protect the algae from abiotic and several biotic stress conditions; for example, phlorotannins which are formed from oligomeric structures and phloroglucinol. **(Spolaore *et al.*, 2006; Onofrejova *et al*., 2010; Kindleysides *et al.*, 2012; Balboa *et al.*, 2013)**.

Various studies have shown that a high dietary intake of natural phenols combined with the presence of different types of antioxidants such as flavonoids has health benefits **(Moraes-de-Souza *et al.*, 2008)**. Antioxidants, which are frequently present in plants and seaweeds lower the risk of several chronic diseases and different forms of cancer, making them a rich source of possible new medications, with reduced toxicity that significantly impact life expectancy **(Hodgson & Croft 2006; Halliwell 2007; Yan & Asmah 2010)**.

3.3.2. Total antioxidant activity

The data tabulated in Table (4) show that the SCWE has the maximum total antioxidant activity (8688.39µ/ ml), followed by the SAWE (7535.35µ/ ml) and the SEE, which has the lowest activity (297.21µ/ ml). In brown algae, there are compounds, which have a broad range of biological characteristics such as antioxidant and anticoagulation **(Barrow & Shahidi, 2007)**. One of the brown seaweed compounds extensively studied is fucoidan (fucosecontaining-sulfated polysaccharide), which exhibits many biological and pharmacological properties, viz. anticoagulant/antithrombotic, antitumor, antivirus and anti-inflammatory effects **(Li *et al.*, 2008)**.

4.4. GC- MS analysis of the SCWE

Besides the estimation of the primary and secondary constituents of the SCWE, and in an attempt to reveal the mechanism of phytochemical components action and mapping out how receiver plants could respond to them, a GC-MS was performed for the SCWE. We tried to comprehend the various putatively biological activities of each component identified in the used extracts via comparing against various databases, such as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, Yeast Metabolome Database (YMDB), LIPID MAPS Database, PathWhiz and Chemical Entities of Biological Interest (ChEBI).

Table 5. GC- MS profile of *Sargassum* cold water extract (SCWE).

No.	R.T	Compound name	Molecular formula	Molecular weight	CAS	Peak Area %
1	13.1	Triacetin	$C_9H_{14}O_6$	218.2039	102-76-1	3.24
2	16.41	alpha-Ionone	$C_{13}H_{20}O$	192.2973	14901-07-6	1.87
3	26.19	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	112-39-0	5.62
4	27.68	Ethylene brassylate	$C_{15}H_{26}O_4$	270.3645	105-95-3	1.31
5	28.51	1,2-Dipalmitoyl-rac-glycerol	$C_{35}H_{68}O_5$	568.9	761-35-3	1.27
6	29.06	Epoxyoleic acid	$C_{18}H_{34}O_3$	298.4608	24560-98-3	2.05
7	29.32	Methyl linolelaidate	$C_{19}H_{34}O_2$	294.4721	2566-97-4	5.12
8	29.46	Methyl elaidate	$C_{19}H_{36}O_2$	296.4879	1937-62-8	9.89
9	29.57	Oleic Acid	$C_{18}H_{34}O_2$	282.4614	112-80-1	2.36
10	29.97	Methyl stearate	$C_{19}H_{38}O_2$	298.5038	112-61-8	3.7
11	30.87	Glycerol α-palmitate	$C_{19}H_{38}O_4$	330.5026	542-44-9	0.82
12	31.06	Linolein, 2-mono-	$C_{21}H_{38}O_4$	354.524	3443-82-1	0.84
13	31.55	Ethyl Iso-Allocholate	$C_{26}H_{44}O_5$	436.6	NA	0.71
14	31.98	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.4986	544-35-4	2
15	32.11	Glycidyl Oleate	$C_{21}H_{38}O_3$	338.52	5431-33-4	5.03
16	32.6	Oleinic acid	$C_{18}H_{34}O_2$	282.4614	112-80-1	1.57
17	32.87	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568.9	NA	3.64
18	33.45	Tetraneurin - A -Diol	$C_{15}H_{20}O_5$	280.3163	NA	0.91
19	33.8	2-Oleoylglycerol	$C_{21}H_{40}O_4$	356.547	3443-84-3	2.24
20	33.9	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	280.48854	NA	4.4
21	34.37	2-Bromooctadecanal	$C_{18}H_{35}BrO$	347.4	56599-95-2	0.84
22	34.89	Glyceryl monolinoleate	$C_{21}H_{38}O_4$	354.5	2277-28-3	3.46
23	34.89	Monoelaidin	$C_{21}H_{40}O_4$	356.5	18465-99-1	3.46
24	34.99	Triolein	$C_{57}H_{104}O_6$	885.4321	537-39-3	5.45
25	35.44	Tego-oleic	$C_{18}H_{34}O_2$	282.4614	112-80-1	1.19
26	35.64	Cis-2-Phenyl-1,3-Dioxolane-4-Methyloctadec-9, 12, 15-Trienoate	$C_{28}H_{40}O_4$	440.6		7.99
27	35.74	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356.5	3443-84-3	10.67
28	36.08	2-Hydroxy-3-[(9e)-9-Octadec Enoyloxy]Propyl	$C_{39}H_{72}O_5$	620	2465-32-9	1.62
29	36.18	Glyceryl 1,3-distearate	$C_{39}H_{76}O_5$	625	504-40-5	3.96
30	36.37	Elaidic acid	$C_{18}H_{34}O_2$	282.4614	112-79-8	1.88
31	36.61	lucenin-2	$C_{27}H_{30}O_{16}$	610.5175	29428-58-8	3.69
32	36.92	Oelsauere	$C_{18}H_{34}O_2$	282.4614	112-80-1	0.68

According to GCMS analysis, 32 bioactive phytochemical compounds were identified in SCWE crude extract as shown in Table (5) and Fig. (4). The identified phytochemical compounds are listed based on the retention time, compound formula, compound molecular mass, CAS registry number and the percentage of peak area (Table 5).

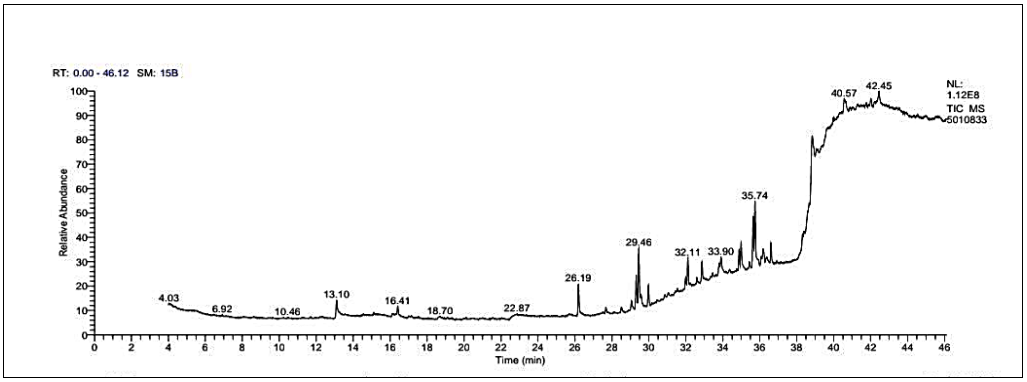

Fig. 4. GC-MS spectra for *S. swartzii* cold water extract

The analysis of SCWE by GC-MS has shown the existence of 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (10.67%) as a primary compound, followed by methyl elaidate (9.89%), cis-2-phenyl-1,3-dioxolane-4-methyloctadec-9, 12, 15-trienoate (7.99%), palmitic acid, methyl ester (5.62%), triolein (5.45%), methyl linolelaidate (5.12 %), glycidyl oleate (5.03%), 12-methyl-E,E-2,13-octadecadien-1-ol (4.4%), glyceryl 1,3-distearate (3.96%), methyl stearate (3.7%), lucenin-2 (3.69%), hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (3.64%), glyceryl monolinoleate (3.46 %), monoelaidin (3.46 %), triacetin (3.24%), oleic acid (2.36%), 2-oleoylglycerol (2.24%), epoxyoleic acid (2.05%), linoleic acid ethyl ester (2%), elaidic acid (1.88%), alpha-Ionone (1.87%), 2-hydroxy-3-[(9e)-9-octadec enoyloxy]propyl (1.62%), oleinic acid (1.57%), ethylene brassylate (1.31%), 1,2-dipalmitoyl-rac-glycerol (1.27%), tego-oleic (1.19%), tetraneurin-A-diol (0.91%), linolein, 2-mono-(0.84%), glycerol α-palmitate (0.82%), ethyl iso-allocholate, (0.71%), and finally oelsauere (0.68%).

On top of the phytochemicals in *Sargassum* cold water extract (SCWE) elucidated by GC-MS analysis are the following different groups of fatty acids and flavonoids (Table 5). These compounds are of great importance in enhancing the biological activity of the SCWE on the seed germination and seedling length of both studied plant strains, fenugreek and barely (Tables 1, 2).

4.4.1. Palmitic acid (PA; C00249)

Palmitic acid, also known as C16 or hexadecanoate acid, is one of the fatty acid compounds with a 16-carbon chain and belongs to the class of saturated fatty acid that found in plants, microorganisms, and animals. Various palmitic acid compounds such as (glycerol α-palmitate; palmitoyl glycerol; 1,3 dipalmitin trimethylsilyl ether; palmitic acid, methyl ester, and 1, 2- dipalmitoyl- sn- glycerol- palmitin, 1, 2- di) (KEGG: https://www.kegg.jp/entry/C00249) are a major component in the fruit of oil palms (*Elaeis guineensis*). Palmitic acid (PA) compounds plays important roles in various plant pathways, for-example; fatty acid biosynthesis; map00061, fatty acid elongation;

map00062, fatty acid degradation; map00071, cutin, suberine and wax biosynthesis; map00073, biosynthesis of unsaturated fatty acids; map01040, biosynthesis of plant secondary metabolites; map01060, metabolic pathways; map01100 and fatty acid metabolism; map01212. They are associated with phosphatidyl inositols and have a key role in the structure and functions of plant cell membranes **(Zhukov *et al.*, 2015).**

4.4.2. Alpha-ionone (C12286)

Alpha-Ionone, also known as alpha-cyclocitrylideneacetone or (E)-α-ionone which found in a variety of plants and essential oils such as; ginkgo nuts, wild celery, wild carrot, tea and rose oil (FooDB : https://foodb.ca/compounds/FDB014484). The ionone components are derived from the degradation of carotenoids or can be metabolized from all of the carotenoid derivatives such as; xanthophyll β-cryptoxanthin, carotenes α-carotene, γ-carotene and β-carotene (Kegg: https://www.kegg.jp/pathway/map=map00906&keyword=carotenoid). Plant apocarotenoids, including β-ionone have various roles in plants as repellants or insect attractants, antibacterial, fungicidal properties, and can accumulated when plants are exposed to photo-oxidative stress **(Paparella *et al.*, 2021)**. Moreover, certain ionones cause regulation of the activity or expression of cell cycle regulatory proteins **(Aloum *et al.*, 2020).**

4.4.3. Oleic acid (C00712)

Oleic acid is a type of Unsaturated Fatty Acids that occurs naturally in various oil plants e.g. olive, almond, roasted peanuts, soybean, palm oil tree, corn, safflower and canola **(Choi *et al.*, 2010)**. In chemical terms, oleic acid is classified as a monounsaturated omega-nine fatty acid and has a various isomer (e.g. epoxyoleic acid, oleinic acid, tego-oleic, elaidic acid and oelsauere). Moreover, oleic acid and their isomers are involved in various pathways such as (Fatty acid biosynthesis, map00061; cutin, suberine and wax biosynthesis, map00073; biosynthesis of unsaturated fatty acids, map01040; biosynthesis of plant secondary metabolites, map01060 and longevity regulating pathway-worm, map04212). Furthermore, oleic acid and their simple isomers compounds plays an important roles in plant such as; act as a precursors for various bioactive molecules (e.g., nitroalkenes and jasmonates), regulators of stress signaling, ingredients and modulators of cellular membranes in glycerolipids, stocks of extracellular barrier constituents, reserve of carbon and energy in triacylglycerol (TAG), plant defense against various biotic and abiotic stresses **(He and Ding, 2020).**

4.4.4. Triacetin (D00384)

Triacetin or enzactin is a class of triacylglycerols compounds **(Kong *et al.*, 2016)**. Triacylglycerol is a key lipid compound play a central role within the cell by engaged in different processes such as energy production, plant stress response and cellular transport

(Lu *et al.*, 2020). Recent researches suggest that triacylglycerols (TAGs) are more than source of carbon and energy in seeds and other storage tissues. TAGs have different physiological functions in various tissues during plant development under varying and optimal environmental conditions. For example, TAG metabolism is involved in cell expansion and division, membrane lipid remodeling, organ formation, successful pollination, and stomatal opening **(Yang and Benning, 2018)**.

4.4.5. Lucenin-2 (C10102)

Lucenin-2 or Luteolin 6,8-di-C-glucoside is a class of flavonoids compounds that found in blood orange (*Citrus sinensis* L. Osbeck), Kumquat (Fortunella japonica Swingle) and seaweeds (*Ulva lactuca*) **(Alam *et al.*, 2022; Anjali *et al.*, 2019; Barreca *et al.*, 2014).** Flavonoids are secondary metabolites which widely distributed in various plants with different groups, such as isoflavonoids, flavonols, flavanonols, anthocyanidins, flavanones, flavones, chalcones, and flavan-3-ols **(Darwish et al., 2022; Farias et al., 2021)**. Furthermore, these flavonoids groups may also act as plant pigments and floral pigmentation, UV filtration, symbiotic nitrogen fixation, chemical messengers, cell cycle inhibitors, physiological regulators, formation of a root nodule, and cellular responses such as ion fluxes **(Galeotti *et al.*, 2008)**. As we know, the Angiosperm seeds consist of three parts embryo, endosperm and seed coat, and each one having a unique genetic composition that is related to seed development. Also, the final size of seed depending on the integration of signals and complex cross talk between these different regions. Therefore, recent researchers found that flavonoids might also play a key role in regulating communication between the endosperm and the seed coat and have a role in seed dormancy through auxin transport regulators **(Doughty *et al.*, 2014)**.

4. CONCLUSION

This study presents state of the art in the extraction of compounds from macroalgal biomass (*S. swartzii*) using different traditional extraction techniques; cold water, autoclaved water, and ethanolic extraction approach. The obtained results proved that the cold-water extraction method was the best among the three tested methods in terms of the extracted content of phenolics and flavonoids as well as antioxidant activity. In addition, SCWE has also the highest stimulatory effect on the germination of the seeds of the two plants, fenugreek, and barley, at concentrations of 20% and 40% respectively. Thus, the water extraction technique can be approved as a safe, cheap, and eco-friendly biofertilizer that helps achieve global food security, sustainability, and quality.

REFERENCES

Abd El-Kareem, M. S.; Rabbih, M. A. E. F.; Selim, E. T. M.; Elsherbiny, E. A. E. M. and El-Khateeb, A. Y. (2016). Application of GC/EIMS in combination with semi-empirical calculations for identification and investigation of some volatile components in basil essential oil. Int. J. Anal .l- Mass Spectrom. Chromatog., 4(1): 14-25. doi: 10.4236/ijamsc.2016.41002

Abeed, A.H.A.; Ali, M.; Ali, E.F.; Majrashi, A. and Eissa, M.A. (2021). Induction of Catharanthus roseus Secondary Metabolites When *Calotropis procera* Was Used as BioStimulant. Plants,10: 1623. https://doi.org/10.3390/plants10081623

Abomohra, A. E. F. ; El-Hefnawy, M. E.; Wang, Q.; Huang, J.; Li, L.; Tang, J. and Mohammed, S. (2021). Sequential bioethanol and biogas production coupled with heavy metal removal using dry seaweeds: Towards enhanced economic feasibility. J. Cleaner Prod., 316: 128341.

Alam, F.; Mohammadin, K.; Shafique, Z.; Amjad, S. T. and Asad, M. (2022).Citrus flavonoids as potential therapeutic agents: A review. Phytother. Res. : PTR, 36(4): 1417–1441. https://doi.org/10.1002/ptr.7261

Ali, M.; Penghui, L.; Guangbiao, S.; Daopu, C.; Xiaochun, W. and Jian, Z. (2017). Transcriptome and metabolite analyses reveal the complex metabolic genes involved in volatile terpenoid biosynthesis in garden sage (*Salvia officinalis*). Sci. Rep., 7. doi:10.1038/s41598- 017- 15478- 3. 19.

Ali, M.; Reem, M.H.; Naveed, U.R.; Guangbiao, S.; Penghui, L.; Xiaochun, W.; Liang, G. and Jian, Z. (2018). De novo transcriptome sequencing and metabolite profiling analyses reveal the complex metabolic genes involved in the terpenoid biosynthesis in Blue Anise Sage (*Salvia guaranitica* L.). DNA Res., 25: 597–617. doi:10.1093/dnares/dsy028.

Aloum, L.; Alefishat, E.; Adem, A., and Petroianu, G.(2020). Ionone Is More than a Violet's Fragrance: A Review. Molecules (Basel, Switzerland), 25(24): 5822. https://doi.org/10.3390/molecules25245822

Alves, A.; Sousa, R. A. and Reis, R. L. (2013). A practical perspective on ulvan extracted from green algae. J. of App. Phycol., 25(2): 407-424. https://doi.org/10.1007/s10811-012-9875-4

Anjali, K. P.; Sangeetha, B. M.; Devi, G.; Raghunathan, R.; and Dutta, S. (2019). Bioprospecting of seaweeds (*Ulva lactuca* and *Stoechospermum marginatum*): The compound characterization and functional applications in medicine-a comparative study. J. photochem. photobiol. B, Biology, 200: 111622. doi.org/10.1016/j. jphotobiol.2019.111622

Balboa, E.M.; Conde, E.; Moure, A.; Falqué, E.; and Domínguez, H. (2013) In vitro antioxidant properties of crude extracts and compounds from brown algae. Food Chem., 138: 1764–1785.

Barbier, M.; Araújo, R.; Rebours, C.; Jacquemin, B.; Holdt, S. L. and Charrier, B. (2020). Development and objectives of the PHYCOMORPH European Guidelines for the Sustainable Aquaculture of Seaweeds (PEGASUS). Botanica marina, 63(1): 5-16.

Barreca, D.; Bellocco, E.; Leuzzi, U.; and Gattuso, G. (2014). First evidence of C- and O-glycosyl flavone in blood orange (*Citrus sinensis* (L.) Osbeck) juice and their

influence on antioxidant properties. Food chem., 149: 244–252. https://doi.org/10.1016/j.foodchem.2013.10.096

Barrow, C. and Shahidi, F. (2007). Marine Nutraceuticals and Functional Foods. CRC Press, 7: 494. https://doi.org/10.1201/9781420015812

Barzkar, N.; Jahromi, S. T.; Poorsaheli, H. B. and Vianello, F. (2019). Metabolites from marine microorganisms, micro, and macroalgae: Immense scope for pharmacology. Marine drugs, 17(8): 464, https://doi.org/10.3390/md17080464

Basudeb S.; Bidhan R.; Kharub AS.; Selvakumar R.; Dinesh K.; Verma RPS. and Indu S. (2013). In book: breeding, biotechnology and seed production of cereals , Chapter: 14 (Barley (*Hordeum vulgare* L.) Edition: First, Publisher: New India Publishing Agency, Vikas Surya Plaza, CU Block, LSC Market, Pitam Pura, New Delhi, pp.419-479. https://www.researchgate.net/publication/297538956.

Darwish, D. B. E.; Ali, M.; Abdelkawy, A. M.; Zayed, M.; Alatawy, M. and Nagah, A. (2022).Constitutive overexpression of GsIMaT2 gene from wild soybean enhances rhizobia interaction and increase nodulation in soybean (Glycine max). BMC plant boil., *22*(1): 1-18. https://doi.org/10.1186/s12870-022-03811-6

de Souza Farias, S. A.; da Costa, K. S. and Martins, J. (2021). Analysis of Conformational, Structural, Magnetic, and Electronic Properties Related to Antioxidant Activity: Revisiting Flavan, Anthocyanidin, Flavanone, Flavonol, Isoflavone, Flavone, and Flavan-3-ol. ACS omega, 6(13): 8908–8918. https://doi.org/10.1021/acsomega.0c06156

Desbois, A. P. and Smith, V. J. (2010). Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl. Microbiol. Biotechnol., 85(6): 1629–1671. https://link.springer.com/article/10.1007/s00253-009-2355-3

Doughty, J. (2014). Aljabri, M., and Scott, R. J.; Flavonoids and the regulation of seed size in Arabidopsis. Biochem. Soc. trans., 42(2): 364–369. https://doi.org/10.1042/BST20140040

DuBois, M.; Gilles, K.A.E.; Hamilton, J.K.; Rebers, A.J.P. and Smith, F. (1965).Calorimetric Dubois method for determination of sugar and related substances. Anal. Chem. 28: 350–356. https://pubs.acs.org/doi/pdf/10.1021/ac60111a017

Dwivedi A.K.; Singh, S. and Ranjan, R. (2006). Suitable varieties of fenugreek for Jharkhand spices and medicinal and Aromatic plants in eastern region.65, pp. 95-101.

EBIC. European Biostimulant Industry Council. 2012. Available online at: http://www.biostimulants.eu/ (acessed on January 7, 2019).

El-Sheekh, M. M.; Nassef, M.; Bases, E.; Shafay, S. E. and El-Shenody, R. (2022). Antitumor immunity and therapeutic properties of marine seaweeds-derived extracts in the treatment of cancer. Cancer Cell Int., 22(1): 1-19.

FAO (2018). The global status of seaweed production, trade and utilization—volume 124. https://www.fao.org/in-action/globefsh/publications/details-publication/en/c/1154074/

Folch, J.; Lees, M. and Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. boil. Chem., 226(1): 497-509. https://pubmed.ncbi.nlm.nih.gov/13428781.

Freese, E.; Sheu, C. W. and Galliers, E. (1973). Function 304 of lipophilic acids as antimicrobial food additives. Nature, 305(5388): 321–321. https://www.nature.com/articles/241321a0.

Galeotti, F.; Barile, E.; Curir, P.; Dolci, M. and Lanzotti V. (2008)."Flavonoids from carnation (*Dianthus caryophyllus*) and their antifungal activity". Phytochem. Lett. 1: 44–48. doi:10.1016/j.phytol.2007.10.001

Guiry, M. D. (2010).AlgaeBase version 4.2. World-wide electronic publication. http://www. algaebase. org.

Gupta, S.; Cox, S.; Rajauria, G.; Jaiswal, A. K. and Abu-Ghannam, N. (2012). Growth inhibition of common food spoilage and pathogenic microorganisms in the presence of brown seaweed extracts. Food Bioprocess Technol., 5(5): 1907-1916. https://doi.org/10.1007/s11947-010-0502-6

Halliwell, B. (2007). Oxidative stress and cancer: have we moved forward?. Biochem. J., 401(1): 1-11. https://doi.org/10.1042/BJ20061131ratho

Hayes, M. (2015). Seaweeds: A nutraceutical and health food. In Seaweed Sustainability. 1st ed.; Tiwari, B.K., Troy, D.J., Eds.; Academic Press: London, UK, pp. 365–387

He M. and Ding N-Z. (2020). Plant Unsaturated Fatty Acids: Multiple Roles in Stress Response. Front. Plant Sci., 11: 562785. doi: 10.3389/fpls.2020.562785

Hodgson, J. M., and Croft, K. D. (2006). Dietary flavonoids: effects on endothelial function and blood pressure. J. Sci. of Food Agric., 86(15): 2492-2498. https://doi.org/10.1002/jsfa.2675

Hogan, M. C.; Monosson, E. and Cleveland C.J. (2011). Brown algae Encyclopedia of Earth. Washington DC: National Council for Science and the Environment.

Iba˜nez, E.; Herrero, M.; Mendiola, J. A. and Castro-Puyana, M. (2012). Extraction and characterization of bioactive compounds with health benefits frommarine resources:Macro and micro algae, cyanobacteria, and invertebrates, in: Hayes, M. (Ed.), Marine Bioactive Compounds: Sources, Characterization and Applications, Springer Science+Business Media, LLC, New York. https://link.springer.com/chapter/10.1007/978-1-4614-1247-2_2

Izabela, M. and Katarzyna, C. (2014). Algal extracts: Technology and advances, A review, Eng. Life Sci., 14: 581–591. https://doi.org/10.1002/elsc.201400139

Jannin, L.; Arkoun, M.; Etienne, P.; Laîne, P.; Goux, D.; Garnica, M.; Fuentes, M.; San Francisco, S.; Baigorri, R.; Cruz, F.; Houdusse, F.; Garcia-Mina, J.-M.; Yvin, J.-C. and Ourry, A. (2013). Brassica napus growth is promoted by Ascophyllum nodosum (L.) Le Jol. Seaweed extract: microarray analysis and physiological characterization of N, C, and S metabolisms. J. Plant Growth Regul., 32: 31–52. https://doi.org/10.1007/s00344-012-9273-9

Kindleysides, S.; Quek, S.-Y. and Miller, M.R. (2012). Inhibition of fish oil oxidation and the radical scavenging activity of New Zealand seaweed extracts. Food Chem., 133: 1624–1631. https://doi.org/10.1016/j.foodchem.2012.02.068

Kumar, S.; Mishra, C.; Sarkar, B. and Singh, S. (2012). In book: Breeding Field Crops Edition: First, Chapter 12: Barley (*Hordeum vulgare* L.) Publisher: AGROBIOS India, pp.99-112. https://www.researchgate.net/publication/297539975

Langridge, P. (2018). Economic and academic importance of barley. In The barley genome pp. 1-10. Springer, Cham. https://link.springer.com/chapter/10.1007/978-3-319-92528-8_1.

Lee, J.; Koo, N. and Min, D. B. (2004). Reactive oxygen species, aging, and antioxidative nutraceuticals. Comprehensive rev. food sci. safety, 3(1): 21-33. https://doi.org/10.1111/j.1541-4337.2004.tb00058.

Li, B.; Lu, F.; Wei, X. and Zhao, R. (2008). Fucoidan: structure and bioactivity. Molecules, 13:1671–95. https://doi.org/10.3390/molecules13081671

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem.,193: 265–275.

Lu, J.; Xu, Y.; Wang, J.; Singer, S. D. and Chen, G. (2020).The Role of Triacylglycerol in Plant Stress Response. Plants (Basel, Switzerland), 9(4): 472. https://doi.org/10.3390/plants9040472

Moraes-de-Souza, R. A.; Oldoni, T. L. C.; Regitano-d'Arce, M. A. B., and Alencar, S. M. (2008). Antioxidant activity and phenolic composition of herbal infusions consumed in brazil actividad antioxidante y compuestos fenólicos en infusiones herbarias consumid as en Brasil. CYTA j. Food, 6(1): 41-47. https://doi.org/10.1080/11358120809487626

Onofrejova, L.; Vasickova, J.; Klejdus, B.; Stratil, P.; Misurcova, L.; Kracmar, S.; Kopecky, J. and Vacek, J. (2010).Bioactive phenols in algae: the application of pressurized-liquid and solid-phase extraction techniques. J. Pharm. Biomed. Anal., 51: 464–470. https://doi.org/10.1016/j.jpba.2009.03.027

Opik H. (1966).Changes in the fine structure of cotyledons of *Phaseolus vulgaris* L. during seed germination .J. Exp. Bot., 240: pp.1675-80. https://doi.org/10.1093/jxb/17.3.427

Paparella, A.; Shaltiel-Harpaza, L.; and Ibdah, M. (2021). β-Ionone: Its Occurrence and Biological Function and Metabolic Engineering. Plants, 10: 754. https://doi.org/10.3390/plants10040754

Perlikowski, D.; Lechowicz, K.; Skirycz, A.; Michaelis, Ä.; Pawłowicz, I. and Kosmala, A. (2022). The Role of Triacylglycerol in the Protection of Cells against Lipotoxicity under Drought in Lolium multiflorum/Festucaarundinacea Introgression Forms. Plant cell physiol., 63(3): 353–368. https://doi.org/10.1093/pcp/pcac003

Plaza, M.; Cifuentes, A. and Iba˜nez, E. (2008). In the search of new functional food ingredients from algae. Trends Food Sci. Technol., 19: 31–39. https://doi.org/10.1016/j.tifs.2007.07.012

Prieto, P.; Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem., 269(2): 337-341.

Pulz, O. and Gross, W. (2004).Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol., 65: 635–648. https://link.springer.com/article/10.1007/s00253-004-1647-x

Rathore, S. S.; Chaudhary, D. R.; Boricha, G. N.; Ghosh, A.; Bhatt, B. P.; Zodape, S. T. and Patolia, J. S. (2009). Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (Glycine max) under rainfed conditions. South African J. Bot., *75*(2): 351-355. https://doi.org/10.1016/j.sajb.2008.10.009

San Kong, P.; Aroua, M. K.; Daud, W. M. A. W.; Lee, H. V.; Cognet, P. and Pérès, Y. (2016).Catalytic role of solid acid catalysts in glycerol acetylation for the

production of bio-additives: a review. RSC advances, 6 (73): 68885-68905. https://doi.org/10.1039/C6RA10686B.

Shabbeer, S.; Sobolewski, M.; Anchoori, R. K.; Kachhap, S.; Hidalgo, M.; Jimeno, A.; ... and Khan, S. R. (2009).Fenugreek: a naturally occurring edible spice as an anticancer agent. Cancer boil. ther. 8(3): 272-278, https://doi.org/10.4161/cbt.8.3.7443

Shin-Geon C.; Se-Ra W. and Hae-Ik R. (2010). Olives and Olive Oil in Health and Disease Prevention. Chapter 153 - Oleic Acid and Inhibition of Glucosyltransferase, Academic Press, PP: 1375-1383. https://doi.org/10.1016/B978-0-12-374420-3.00153-4

Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. of Enol. Vitic., 16(3): 144-158.https://www.ajevonline.org/content/16/3/144

Spinelli, F.; Fiori, G.; Noferini, M.; Sprocatti, M. and Costa, G. (2010). A novel type of seaweed extract as a natural alternative to the use of iron chelates in strawberry production. Sci. hortic., 125(3): 263-269, https://doi.org/10.1016/j.scienta.2010. 03. 011

Spolaore, P.; Joannis-Cassan, C.; Duran, E. and Isambert, A. (2006). Commercial applications of microalgae. J. biosci. bioeng., 101(2): 87-96. https://doi.org/10.1263/jbb.101.87

Stirk, W.A. and Van Staden, J. (1997). Comparison of cytokinin- and auxin like activity in some commercially used seaweed extracts. J. Appl. Phycol., 8: 503–508. https://doi.org/10.1007/BF02186328

Volland, S.; Schaumlöffel, D.; Dobritzsch, D.; Krauss, G.-J. and Lütz-Meindl, U. (2013). Identification of phytochelatins in the cadmium-stressed conjugating green alga *Micrasterias denticulata*. Chemosphere, 91: 448–454. https://doi.org/10.1016/j.chemosphere.2012.11.064

Yan S. and Asmah R. (2010). Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf and torch ginger flower. Int. Food Res. J., 17: pp. 417-23.

Yang, Y. and Benning, C. (2018). Functions of triacylglycerols during plant development and stress. Curr. Opin. biotechnol., 49: 191–198. https://doi.org/10.1016/j.copbio.2017.09.003

Zandi, P.; Basu, S. K.; Cetzal-Ix, W.; Kordrostami, M.; Chalaras, S. K., and Khatibai, L. B. (2017). Fenugreek (*Trigonella foenum*-graecum L.): an important medicinal and aromatic crop. Active Ingredients from aromatic and medicinal plants, 207-224..https://www.intechopen.com/chapters/53418

Zheng, C. J.; Yoo, J. S.; Lee, T. G.; Cho, Y. H.; Kim, Y. H. and Kim, Y. G. (2005). Fatty acid synthesis is target for antibacterial activity of unsaturated fatty acids. FEBS Lett., 579(23):5157–5162. 2710.https://doi.org/10.1016/j.febslet.2005.08.028

Zhuang, X. P.; Lu, Y. Y., and Yang, G. S. (1992). Extraction and determination of flavonoid in ginkgo. Chi. Herb. Med., 23: 122-124.

Zhukov, A.V. (2015).Palmitic Acid and Its Role in the Structure and Functions of Plant Cell Membranes. Russ. J. Plant Physiol. 62: 706–713.https://doi:10.1134/s10214 437150 50192