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PHYTOCHEMICAL CONSTITUENTS, CYTOTOXIC POTENTIALS AND EFFECTS ON WHEAT GROWTH PARAMETERS POSSESSED BY EXTRACTS OF SOME SEAWEED COLLECTED FROM THE WESTERN LIBYAN COAST

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ABSTRACT

Various inhabitants of the water such as, animals, and microbes produce compounds that have potential as pharmaceuticals. They produce "secondary metabolites" chemicals which are not vital for primary metabolic processes of these organisms, yet are believed to confer some evolutionary advantage. Many of these organisms are non-motile and have developed chemical compounds due to living in densely populated habitats. The natural products drug discovery research aimed its investigations at the marine environment recently due to its unraveled biodiversity compared to other environments. The pioneering work of Bergmann in the 1950s introduced the potential of the marine natural products as pharmaceuticals. A total of 5 ethanol and dichloromethane extracts of macroalgal species (2 green, 2 brown, 1 red) collected from Libyan coast were screened for their major phytochemical groups. The 5 algal species also subjected to be evaluated against brine shrimp lethality bioassay. The effect of seaweeds liquid fertilizer on growth parameters of *Traticumsativum* were also examined using in vitro seed germination in petri dishes bioassay. Ethanolic and dichloromethane extracts of the five algae samples represented the presence of several chemical constituents. All extracts exhibited LC50 >1000 ppm. There are different effects on growth of wheat seeds, in our study all algal extracts are non toxic according to Brine shrimp leathality assay so they may be considered as edible seaweeds. Different effects on growth parameters of *Traticumsativum* suggested the presence of micro elements, macro elements and different concentrations of plant growth hormones.

KEYWORDS: Marine Algae, Artemia, Fertilizer, Wheat

INTRODUCTION

Countless unique organisms roam the vast oceans many of which possess a novel structure and biological activities, this marvelous source of potential discoveries is not yet harvested so it could be the womb of countless bioactive natural products[1]. Various inhabitants of the water such as animals and microbes produce secondary metabolites which are not vital for primary metabolic processes and many of them have potential as pharmaceuticals[2]. Marine macroalgae are multicellular[3]. They are members of the kingdom Protista[4] and they can be found everywhere as long as there is a light to carry out photosynthesis[5]. They are classified according to color to 3 different groups: red green and brown. Extracts derived from algae contain components such as

Polysaccharides, Proteins, Polyunsaturated fatty acids (PUFAs), Pigments, Polyphenols, minerals, and plant growth hormones[6]. Marine macroalgae have many uses in different fields such as Pharmaceuticals[7], cosmetics[8], bioremediationand industrial chemicals[9]. Chemical fertilizers are the most important component to increase an agriculture production as they have excellent effects on plant growth and health, today there is an increasing demand which represent hazardous effects either directly or indirectly on environment and human health in particular nitrogen based fertilizers so many farmers began to use natural organic based fertilizers instead of chemical ones[10]. The objective of this study is to carry out qualitative phytochemical screening, cytotoxic activities and the effect on growth parameters of wheat seeds using algae species collected from Libyan coast.

MATERIALS AND METHODS

2.1 Collection and processing of algal samples

Ulva lactuca, *Cystoceracompressa*, *Sargassumhornschuchii*, *Gelidiumpusillum and Enteromarpha intestinalis* were collected randomly by hand picking from western coast of Libya (Tajuraelhmidia and marine biology research center) between January and April 2017, The algal samples were taxonomically identified at marine biology research center, Tajura (east of Tripoli), Libya. Algae samples were cleaned with fresh seawater and then in distilled water to remove epiphytes, suspended matter and sand particles. The materials were dried completely in shade at room temperature, then blended to fine powder in an electronic grinder.

2.2 Extraction of algal samples

Each of the powdered algal samples were subjected to Soxhlet apparatus for continuous hot extraction with 97% ethanol and dichloromethane respectively for 24 hours. Afterwards, the ethanolic and dichloromethane extracts were filtered through filter paper - grade 42 - and the resultant filtrates were concentrated to dryness under reduced pressure using rotary evaporator. Finally, the dried extracts were stored is small jars at 2^{o}C until use.

2.3 Phytochemical screening

The algal extracts were subjected to phytochemical screening to detect different chemical groups of compounds[11].

2.3.1 Tests for alkaloids

Mayer's Test (Potassium mercuric iodide solution): each of individual algal extract was dissolved in dilute HCL then few drops of Mayer's reagent were added. Cream colour precipitate indicates the presence of alkaloids.

Dragendorf's Test (Potassium bismuth iodide solution): each of individual algal extract was dissolved in dilute HCL then few drops of Dragendorf's reagent were added. Reddish brown precipitate indicates the presence of alkaloids.

Wagner's Test (Solution of Iodine in Potassium Iodide): each of individual algal extract was dissolved in dilute HCL then few drops of Wagner's reagent were added. Reddish brown precipitate indicates the presence of alkaloids.

2.3.2 Tests for flavonoids

Shinoda test (magnesium hydrochloride reduction test): few fragments of magnesium ribbon were added to each of individual algal extract then concentrated hydrochloric acid was added drop wise, pink scarlet colour indicate the presence of flavonoids.

Alkaline reagent test: Few drops of sodium hydroxide solution was added to extract solution, formation of an intense yellow colour which turns to colorless by the addition of few drops of dilute acetic acid indicate the presence of flavonoids.

2.3.3 Tests for phenolic compounds

Ferric chloride test: few drops of neutral 5% ferric chloride solution were added to each of individual extract, a dark green colour indicates the presence of phenolic compounds.

Lead acetate test: few drops of 10% lead acetate solution were added to extracts. White precipitate indicates the presence of phenolic compounds.

Gelatin test: few drops of 10% gelatin solution were added to test extract solution, white precipitate indicates the presence of phenolic compounds.

2.3.4 Tests for tannins

Ferric chloride test: few drops of ferric chloride test reagent were added to extract solution, an intense green, purple, blue or black colour developed was taken as an evidence for the presence of tannins.

2.3.5 Tests for amino acids

Ninhydrin test: few drops of 5% ninhydrin solution were added to extracts and boiled, violet colour indicates the presence of amino acids.

2.3.6 Test for protein

Biuret test: 4%NaOH solution and few drops of 1% $CuSO_4$ solution were added to extract solution, violet colour indicate the presence of protein.

2.3.7 Tests for sterols and triterpenoids

Libermann_Burchard test: extracts were treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid was added along the side of test tube, shows brown ring at the junction of two layers and the upper layer turns green which shows the presence of sterols and formation of deep red colour indicates the presence of triterpenoids.

Salkowski's test: extract was treated in chloroform with few drops of concentrated sulphuric acid, shaken well and allow to stand for some time, red colour appears in the lower layer indicate the presence of sterols and formation of yellow coloured lower layer indicate the presence of triterpenoids.

2.3.8 Tests for carbohydrates

Fehling's test: equal volume of fehling A (copper sulphate in distilled water) and fehling B (potassium tartarate and sodium hydroxide in distilled water) reagents are mixed and few drops of sample was added and boiled, reducing sugars forming a brike red precipitate of cuprous oxide.

2.3.9 Tests for oils and fats

A small quantity of extract was pressed in between the two filter papers. Oil stain on the filter papers indicates the presence of oils and fats.

2.3.10 Tests for saponins

Froth test:a pinch of the dried powdered plant was added to 2_3ml of distilled water. The mixture was shaken vigorously. Formation of foam indicates the presence of saponins.

2.3.11 Tests for organic acids

Oxalic acid: When few drops of 1% $KMnO_4$ and dilute H_2SO_4 added to extract solutions, colour disappears.

Malic acid: Three drops of 40% $FeCl_3$ solution were added to extracs test solutions, appearance of yellowish colour indicates the presence of malic acid.

2.3.12 Tests inorganic acids

Sulphate test: Lead acetate reagent was added to extract test solutions; white precipitate appears which is soluble in NaOH.

Carbonate test: To extract test solution, dilute HCl was added, liberate CO_2 gas indicate the presence of carbonate.

2.3.13 Tests for cumarine

To 2ml of test solution, a few drops of alcoholic sodium hydroxide were added. Appearance of yellow colour indicates the presence of coumarine.

2.4 Brine shrimp lethality assay

2.4.1 Hatching of brine shrimp

About 1.6g of *Artemia Salina* (Linnaeus) cysts were aerated in 1 L capacity cylinder containing seawater. In the bottom of the cylinder the air stone was placed to ensure the complete aeration of the cysts. Newly hatched free-swimming pink-colourednauplii was harvested from the bottom After 24 hours' incubation at room temperature[12].

2.4.2 Preparation of test solution positive and negative controls

40mg of each of the test samples were taken and dissolved in 200µl of pure dimethyl sulfoxide (DMSO) and finally, the volume was made to 20 ml with sea water. Thus, the concentration of the stock solution was 2000µg/ml. Then the solution was serial diluted to 250, 500, 1000 µg/ml with sea water. Then 2.5 ml of plant extract solution was added to 2.5 ml of sea water containing 10 nauplii. Potassium dichromate was used as a positive control as it is strong oxidizing agent, it was evaluated at very low concentration (1200, 600, 300, 150, 75, 37.5, 18.75 µg/ml).50µl of DMSO was added to each of three pre-marked dish containing 4.95ml of sea water and 10 shrimp nauplii to be used as negative control[13].

2.4.3 Counting of nauplii

The number of survived nauplii in each tube was counted After 24 hours using a magnifying glass against a black background, Larvae were considered dead if they did not exhibit any internal or external movement during the observation period[14]. The concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC50) which represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure time.

2.5 In vitro seed germination in Petri dishes bioassay

2.5.1 Preparation of seaweed liquid fertilizer

Fifty gram of finely powdered material was extracted for 60 min with 500 mL boiling water and then filtered. The resulting extract was cooled and taken as 100% concentration of the SLF, then the SLF refrigerated between 0 and 4 °C. [15]

2.5.2 Experimental design and treatments

The seeds of Wheat (*TriticumSativum*) with uniform size, color and weight were surface sterilized with 5% sodium hypochlorite. The treatments were 2.5%, 5%, 7.5%, 10% and 20% aqueous extracts of seaweeds (each concentration had 5 treatments 20 seeds for each treatment), Five Petri plates were watered with 10ml of distilled water and considered as the control, the remainders of them were treated with 10 ml of 2.5%, 5%, 7.5%, 10% and 20% of aqueous seaweed extract at the first and three days later. All Petri plates were 130 and they were taken on 7th day after sowing.

2.5.3 Growth analysis

The growth parameters including germination percentage, fresh and dry weight, shoot length and root length were calculated.

RESULTS

3.1 The Percentage yield of algal extracts

Table 1: % yield of algal extracts by Soxhlet apparatus

Algae species	% Yield of Ethanolic Extract (W/W)	%Yield of DCM Extract (W/W)
Cystosieracompressa	11.7%	0.89%
Sargassumhornschuchii	4.7%	0.87%
Enteromarpha intestinalis	5.75%	3.25%
Ulva lactuca	20.30%	0.2%
Gelidiumpusillum	7.7%	2.3%

These results presented that the % of yield of Ethanolic extracts is higher than that of DCM extracts.

3.2 Phytochemical Screening

Phytochemical screening of the ethanolic extracts of all algae species showed the presence of alkaloids, phenolic compounds, tannins, amino acids, organic acids, inorganic acids, cumarines, saponins, fats, carbohydrates, steroids and triterpenoids but protiens were absent. Dichloromethane extracts represented the presence of oils, fats, saponins, carbohydrates (except one brown algae which belongs to Sargassumspecies), steroids and terpenoids while alkaloids, phenolics, tannins, amino acids, protiens, organic acids, inorganic acids and cumarines were absent.

3.3 Brine shrimp lethality assay

For this study the crude ethanol and dichloromethane extracts of the five tested species are considered to be nontoxic as they exhibit LC50 values above 1000 ppm[16].

3.4 In vitro seed germination in Petri dishes bioassay

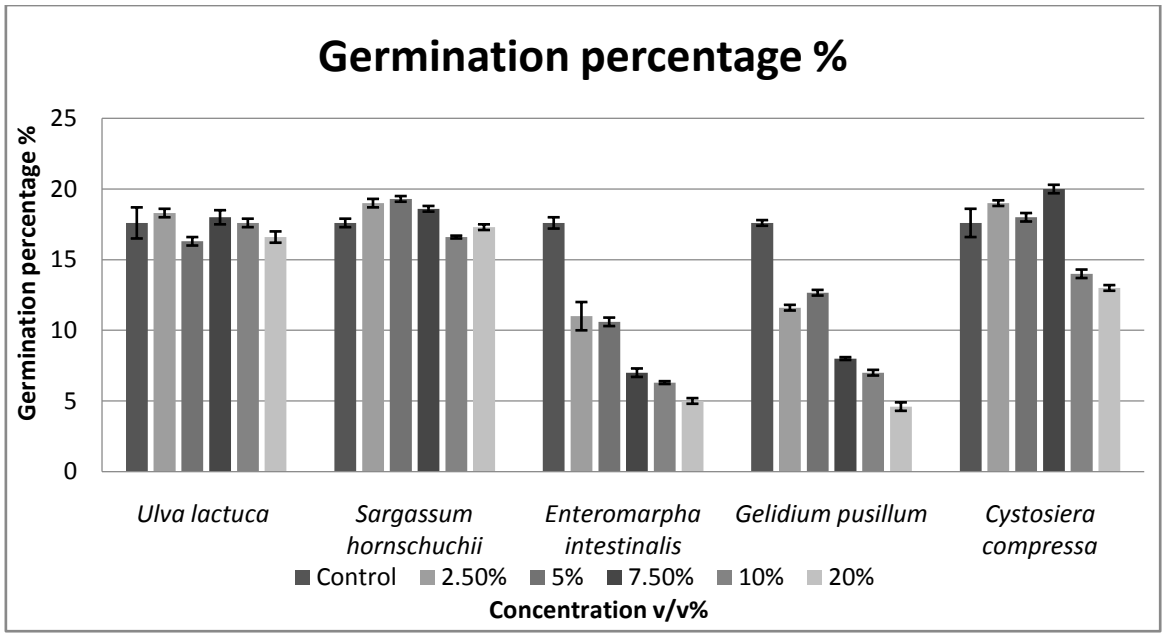

Fig. 1: Effect of the five seaweed liquid fertilizer on germination percentage
The highest germination percentage (Fig. 1) was obtained by *Cystosieracompressa* while the lowest one was by *Gelidiumpusillum.*

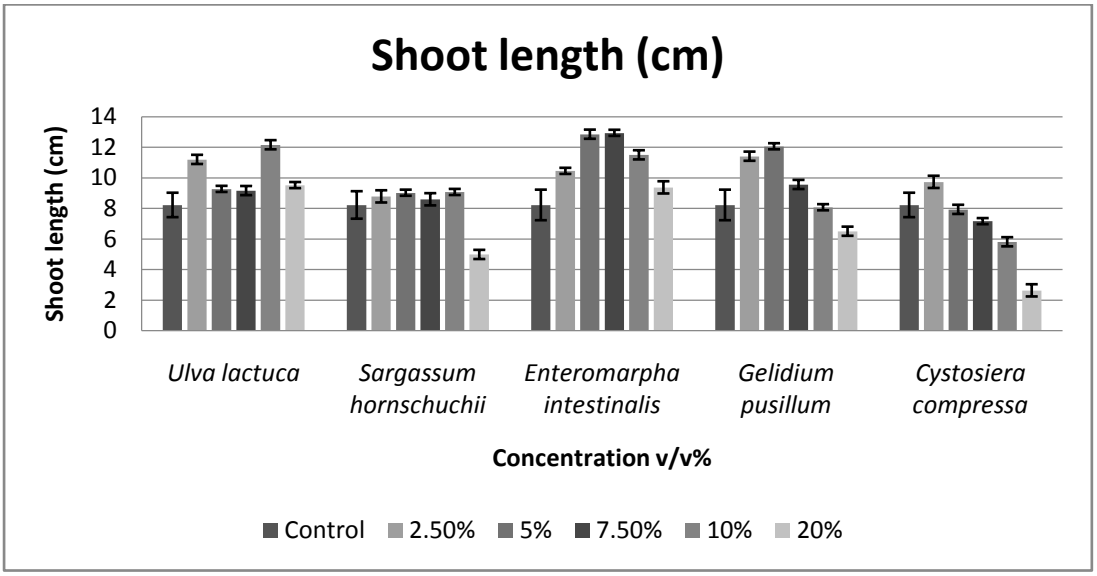

Fig. 2: Effect of the five seaweed liquid fertilizer on shoot length *Enteromarpha intestinalis* produce the highest shoot length, whereas *Sargassumhornschuchii*produce the lowest one (Fig. 2)

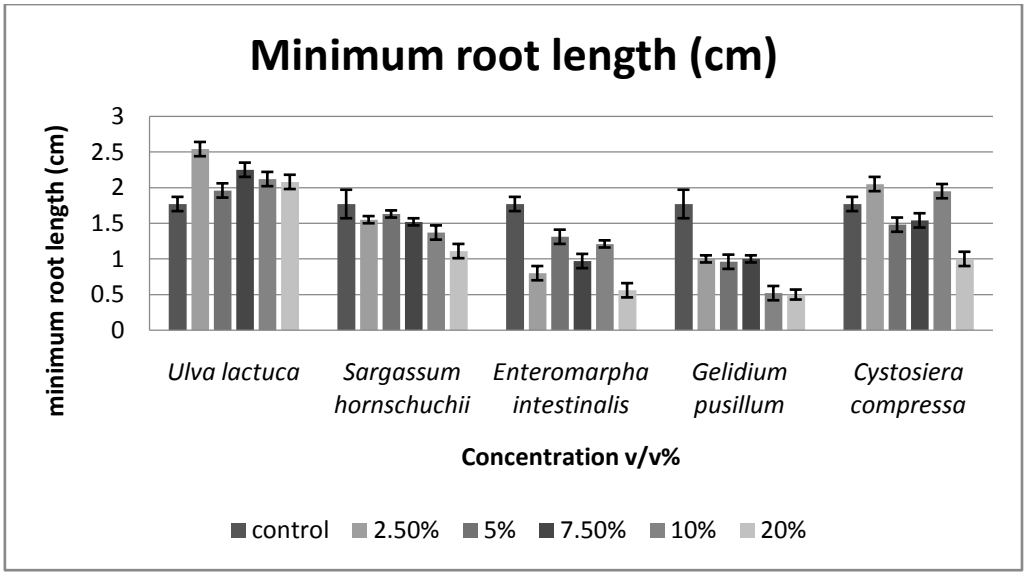

Fig. 3: Effect of the five seaweed liquid fertilizer on Maximum root length

Maximum root length (Fig. 3) is increased by *Ulva lactuca* but *Gelidiumpusillum* reduces it.

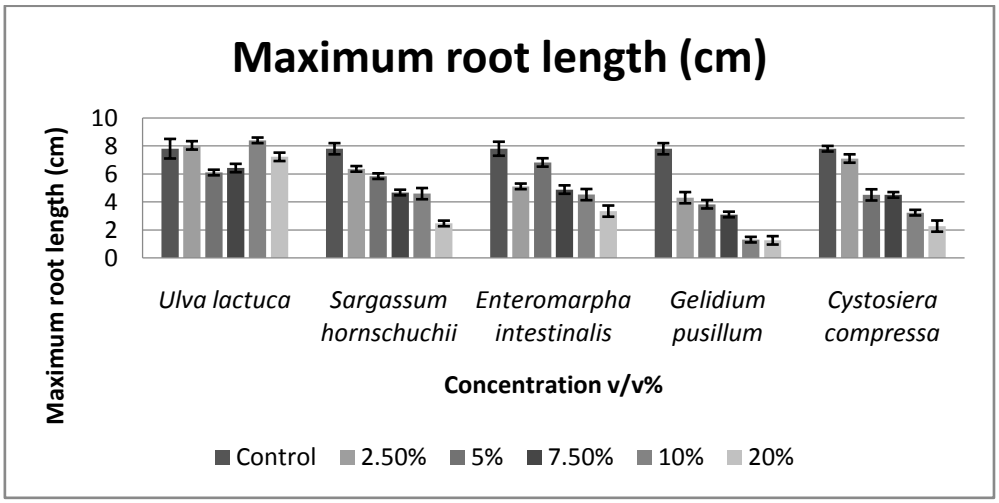

Fig. 4: Effect of the five seaweed liquid fertilizer on minimum root length

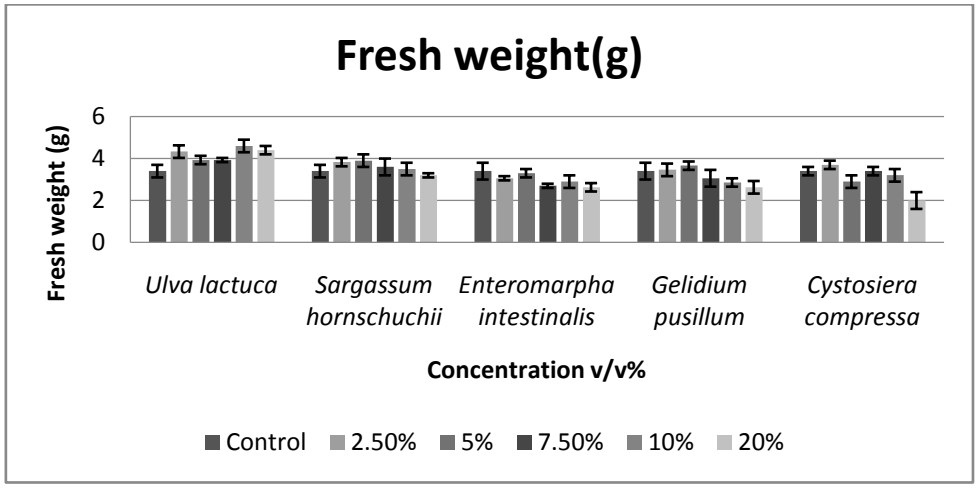

Fig. 5: Effects of the five seaweed liquid fertilizer on fresh weight of *Traticumsativum*

***Ulva lactuca* increases the fresh weight (Fig. 5) while *Cystosieracompressa* lowers it.**

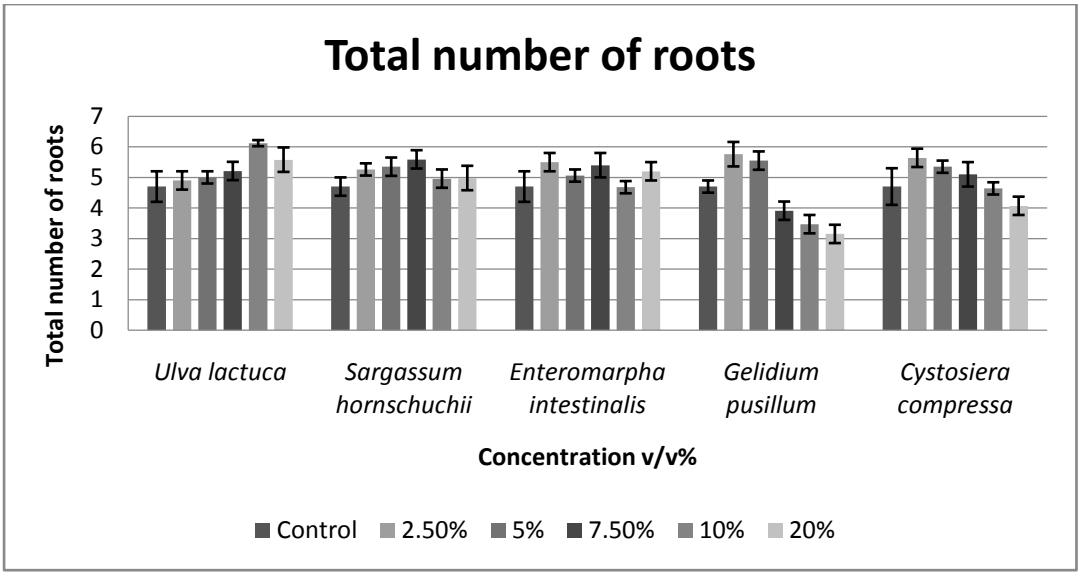

Fig. 6: effects of seaweed liquid fertilizer of the five algae species on the total number of roots *Ulva lactuca* increases the total number of roots while *Gelidiumpusillum* reduces it. all of observations mentioned above are compared with control.(Fig. 6)

DISCUSSION

Phytochemical constituents of *Ulva lactuca and Enteromarpha*intestinalis demonstrated in this study contract with the results obtained byAlshalmani, the presence of phenols and flavonoids in all tested algae is interesting because of their possible use as natural antioxidant, antimicrobials, antifungal, antiviral activities, reduction of cardiovascular disease risk by lowering serum cholesterol and blood pressure, also they have anticarcinogenic, antidiabetic and antiplatelet aggregation effects[17].

To achieve applied meaning, bioassays must be incorporated in natural product chemistry especially benchtop bioassays because they are rapid, inexpensive, simple(requiring little technical training),so specific bioassays are performed on the active ones and negative ones are thrown out[18] one of these benchtop bioassays is Brine shrimp lethality assay,the % of Brine shrimp lethality assay of *Ulvalactuca* demonstrated in this study contract with the results obtained by Jehan [19], while the results of the rest algal samples shows in this first study no toxicity as they produce LC50 above 1000ppm.

The different effects on growth parameters may be due to the presence of different concentrations of plant growth hormones plus the amount of macro and microelements that are found in algae.

Conflict of interest statement

We declare that we have no conflict of interest.

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