



Growth and chemical constituents of cardoon plant in response to foliar application of various algal extracts



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ABSTRACT

Algal extracts play an important role in metabolic processes in cardoon plant (*Cynara cardunculus* L.). Cardoon contains phenolic compounds, fixed oil, sesquiterpenes, lactonens, flavones, sterols, coumarins, lignans, inulin, cynarin and silymarin. Also, it has medicinal properties, i.e. reduce the cholesterol, anti-diabetic, and improve the liver functions. The leaves of cardoon plant can inhibit the activity of several human pathogenic bacteria; possibly due to their specific phenolic components which have an antioxidant activity. The aim of this investigation was to evaluate the growth, seed yield and chemical constituents of cardoon due to spray with the algal extracts. Plants were treated with different doses (0, 1, 2 and 3 g/L) of four algal extracts (*Spirulina platensis*, *Chlorella vulgaris*, *Amphora coffeaeformis* and *Scenedesmus obliquus*). Morphological characters (plant height, leaf number, herb fresh weight, herb dry weight and seed yield) were recorded. Chemical contents (fixed oil, flavonoids, carbohydrates, free radical scavenging activity and phenolic compounds) were evaluated. The highest values of morphological characters were detected under 3 g/L of *Spirulina platensis* extract. Morphological characters increased by 70.9, 77.1, 45.6, 158.4 and 106.1% in plant height (cm); leaf number/plant, total fresh and dry weights (g/plant) and seed weight (g/plant) respectively, compared to the control. The *Scenedesmus obliquus* extract at 1 and 3 g/L resulted in the greatest amounts of fixed oil and total carbohydrates, the increase yielded 166.7 and 43.7% than control. *Chlorella vulgaris* extract in 1 and 2 g/L produced the highest increments in total flavonoids and inhibition of free radical scavenging activity, the increments were 58.8 and 105.5% than control. The highest value of total phenolic compounds was recorded due to the application of *Amphora coffeaeformis* extract at 3 g/L that produced 311.1% than control. Chlorogenic, caffeic and vanillic were detected as the major fractions of phenolic compounds. Algal extracts caused highly significant changes in major and minor fractions of phenolic compounds. Doses of 2, 3 g/L of *Chlorella vulgaris* and 2 g/L of *Amphora coffeaeformis* resulted in the greatest amounts of vanillic, chlorogenic and caffeic acids, these doses yielded 61.1, 31 and 66.7% respectively than control. It can be concluded that highly significant changes were reported in growth, yield and various chemical constituents of cardoon in response to the algal extracts applications.

1. Introduction

Algae are ecologically and economically important and have been used as food and medicines for centuries. Nowadays, Various species of algae provide not only food but also produce extracts are used in numerous foods, dairy, pharmaceutical, cosmetic, and other industrial applications (Raja et al., 2013). *Spirulina platensis* belongs to family Oscillatoriaceae and it has gained a significant position in recent years as a source of proteins, pigments and vitamins in healthy food, pharmaceutical and cosmetic industries (Usharani et al., 2012). *Chlorella*

vulgaris, *Amphora coffeaeformis* and *Scenedesmus obliquus* belong to families Chlorellaceae, Catenulaceae and Scenedesmaceae respectively, they have a great potential as a resource for biodiesel production due to faster growth and easier cultivation (Mandal and Mallick, 2009; Al-lwayzy et al., 2014; Chtourou et al., 2015).

Plant nutrition is one of the most factors that affects growth, yield and chemical constituents of medicinal plants (Yassen and Khalid, 2009; Khalid, 2012, 2013). Increasing restrictions in agrochemical law regulations results in a need for new ways in plant nutrition (Ronga et al., 2019). Currently, a great attention is paid to biologically active

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constituents of natural origin, including biomass in particular, defined as plant growth bio-regulators or bio-stimulant (Ronga et al., 2019). Pervious investigators indicated that the constituents of such activities occur commonly in an alga which is the source of some bio-stimulant (La Torre et al., 2016; Mulbry et al., 2017; Ronga et al., 2019). Algae contain different components such as hormones, vitamins, amino acids and various elements which can modify and improve the productivity of agricultural crops. The beneficial effects of algae were confirmed by Ronga et al. (2019). The role of biostimulants is control and accelerates the physiological processes of various crops such as increase the resistance to stress and stimulate their development (Calvo et al., 2014; Du Jardin, 2015). Their applications help to reduce the amount of chemicals used in agriculture and plant protection (Radkowski and Radkowska, 2013).

Exploring the pharmacological propensities of natural products has attracted much interest in the past decades. Such bio-resources have been found which possess broad spectrum of biological effects including antioxidant, antimicrobial, anticancer or antiviral. In addition, public concerns have emerged against synthetics (Alvarez-Rivera et al., 2019; Awortwe et al., 2019). For instance, synthetic antioxidants have been reported as carcinogenic and hepatotoxic (Da Cruz et al., 2019). From these perspectives, the search of novel bioactive compounds from natural products of medicinal plants to replace synthetic ones is one of the most attractive avenues in an endeavor to develop new drug regimen (Ally et al., 2019). Such plants need to be scrutinized scientifically for the presence of bioactive compounds (Sadeer et al., 2019). Cardoon (*Cynara cardunculus* L.) is a perennial herb belongs to family Compositae (Fratianni et al., 2007). The leaves, seeds, flowers and roots of cardoon contain various medical constituents such as fixed oil, sesquiterpenes, lactonens, flavones, sterols, coumarins, lignans, inulin, cynarin, silymarin and polyphenols (Curt et al., 2002; Raccuia and Melilli, 2004; Koubaa and Damak, 2003; Pinelli et al., 2007). It has medical and pharmacological properties such as serve to lower blood cholesterol, anti-diabetic, antimicrobial, choleric, cholagogue, diuretic, improve the function of liver and gallbladder (Krizkova et al., 2004; Fratianni et al., 2007; Grammelis et al., 2008).

The application of algal extracts is one method of scientific work that has the potential to increase the productivity of medicinal plants. So, the aim of this investigation was to evaluate the growth characters, yield and chemical constituents (carbohydrates, fixed oil, flavonoids, phenolic compounds and free radical scavenging) of cardoon plants that treated with various algal extracts (*Spirulina platensis*, *Chlorella vulgaris*, *Amphora coffeaeformis* and *Scenedesmus obliquus*).

2. Materials and methods

2.1. Experimental

Two field Experiments were conducted at the Experimental Farm of Faculty of Agriculture, Cairo University located at Giza region, Egypt, during two successive seasons, 2016/2017 and 2017/2018. The soil type of the Experimental Farm was clay loam (Ibrahim et al., 2014). Propagation: During the third week of September in both seasons, the seeds were sown in plastic bags (23 × 18 cm) in a mixture of sand and clay soil (1:1) under a sun screen. The uniform seedlings (60 days old) were transplanted into the open field according to a Complete Randomized Block Design (CRBD) with three replicates in the first week of November in both seasons, with a spacing of 100 cm between plants in 6 m² (3 × 2) plots. Each plot contained 2 rows, with 6 plants. All agriculture practices operations other than experimental treatments were performed according to the recommendations of the Ministry of Agriculture, Egypt. All plots were divided into five groups: the first, second, third and fourth groups were subjected to four algal extracts (*Spirulina platensis*, *Chlorella vulgaris*, *Amphora coffeaeformis* and *Scenedesmus obliquus*) (Fig. 1) with various doses of 1, 2 and 3 g/L; the fifth group was exposed to distilled water (as control). The algal extracts were applied as

foliar spray to run-off to foliage at the end of January and the first week of February in both seasons.

The algae were chosen for evaluating their biostimulants activity in this study according to previous investigators (Bhosle et al., 1993; Karen et al., 2000; Faheed and Abd-El Fattah, 2008; Elarroussia et al., 2016); they reported that *Spirulina platensis*, *Chlorella vulgaris*, *Amphora coffeaeformis* and *Scenedesmus obliquus* contain some biostimulants which improved the growth, yield and chemical content of some crops.

The various algae used in this study were prepared in the Food Toxicology and Contaminants Department, National Research Centre (NRC). Three culture medium were prepared for cultivation of microalgae species include Zarrouk's media (Zarrouk, 1966) for cultivation of *Spirulina platensis*, BG-11 medium (Rippka et al., 1979) for cultivation of *Scenedesmus obliquus* and *Chlorella vulgaris* and f/2 medium for cultivation of the marine diatom *Amphora coffeaeformis* (Guillard, 1975).

Zarrouk's medium is composed of 18.0 g NaHCO₃; 2.5 g NaNO₃; 0.5 g K₂HPO₄; 1.0 g K₂SO₄; 1.0 g NaCl; 0.04 g CaCl₂; 0.08 g Na₂EDTA; 0.2 g MgSO₄·7H₂O; 0.01 g FeSO₄·7H₂O and 1.0 ml trace elements (TE) in 1000 ml distilled water. TE (g/L) is a combined of H₃BO₃ 2.86; (NH₄)₆Mo₇O₂₄ 0.02; MnCl₂·4H₂O 1.8; CuSO₄ 0.08 and ZnSO₄·7H₂O 0.22. After autoclaving and cooling, the culture medium pH was adjusted to 8.2 using 1 M NaOH solution.

BG-11 medium is composed of 1.5 g NaNO₃; 0.004 g K₂HPO₄; 0.075 g MgSO₄·7H₂O; 0.036 g CaCl₂·2H₂O; 0.006 g citric acid; 0.02 mg Na₂CO₃; 0.001 g Na₂EDTA; 0.63 g ferric ammonium citrate and 1.0 ml trace elements (TE) in 1000 ml distilled water. TE (g/L) is combined of 2.86 g H₃BO₃; 1.81 g MnCl₂·4H₂O; 0.222 g ZnSO₄·7H₂O; 0.39 g Na₂MoO₄·2H₂O; 0.079 g CuSO₄·5H₂O and 0.0494 g Co(NO₃)₂·6H₂O. After autoclaving and cooling, pH was adjusted to 7.1. Modified BG-11 media is similar to BG-11 media in its composition except using 0.53 g urea (46.5%N) instead of 1.5 g NaNO₃.

The f/2 medium is composed of 75 mg NaNO₃; 5 mg NaH₂PO₄·H₂O; 15 mg NaSiO₃·9H₂O; trace elements TE (mg/L) 4.36 mg Na₂EDTA; 3.15 mg FeCl₃·6H₂O; 0.01 mg; CuSO₄·5H₂O; 0.022 mg CoCl₂·5H₂O; 0.01 mg ZnSO₄·5H₂O; 0.18 mg MnCl₂·4H₂O; 0.006 mg Na₂MoO₄·2H₂O.

Microalgae were cultivated in 0.5 L Erlenmeyer flasks using Environ-shaker incubator (MP-7552, cv-cc power supply, his Hefer, San-francisco), with photoperiod of 12 h light provided by fluorescent lamps at a light intensity of light intensity 440 w/m² and temperature 30 ± 2 °C. Experiments were initiated with 10% (v/v) inoculums of each species.

The algal solutions (1, 2 and 3 g/L) were prepared by adding 1, 2 or 3 g of crude algae to a 1000 ml volumetric flask and then adding enough distilled water to bring the total solution volume to 1000 mL.

2.2. Harvesting

At the end of July (250 days from transplanting) in both seasons, all plants (Fig. 2) were harvested by cutting the plants 5 cm above the soil surface. Plant height, PLHE (cm); leaf number, LENO/Plant; herb fresh weight, HFRWE (g/Plant); herb dry weight, HDRWE (g/Plant) and seed yield, SEYI (g/Plant) were recorded (Wahba et al., 2016).

2.3. Determination of total carbohydrates (TOCA)

The TOCA contents were determined in leaves collected at the fruiting stage of each treatment with the method of Dubois et al. (1956).

2.4. Determination of total fixed oil (TFIOI)

In order to extract TFIOI, 20 g of seeds were crushed to coarse powered and extracted with petroleum ether (40–60 °C) in a Soxhlet apparatus (AOAC, Association of Official Analytical Chemists (Anonymous, 2016).

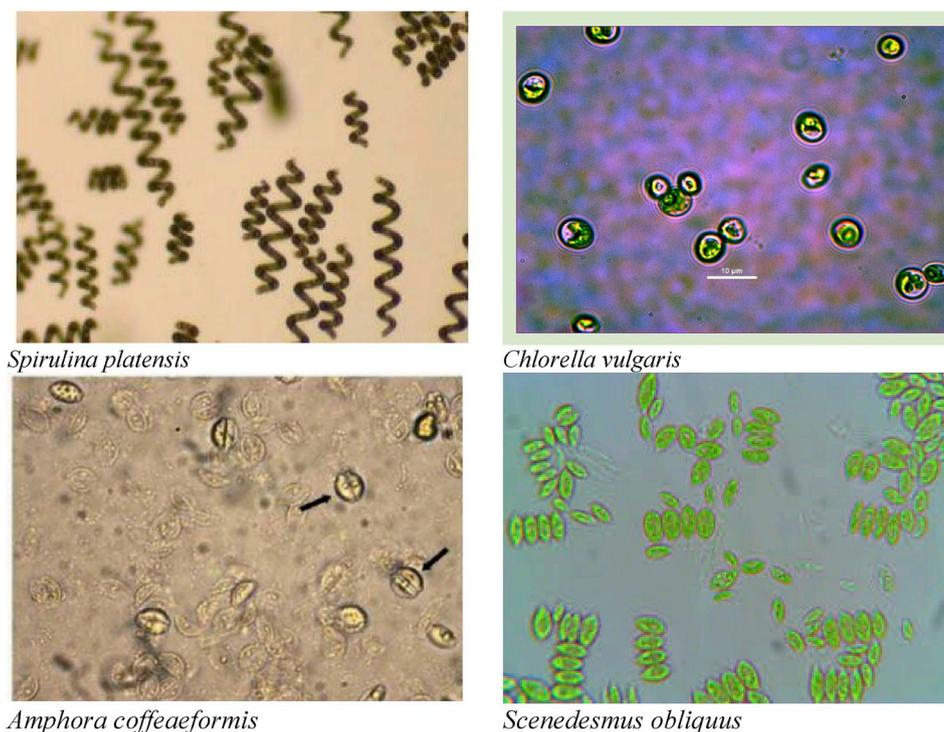


Fig. 1. Microscopic images of alga used in study.



Fig. 2. Cardoon (*Cynara cardunculus* L.).

2.5. Determination of total flavonoids (TOFLA)

The aluminum chloride colorimetric method was used to determine TOFLA in leaves according to Pourmorad et al. (2006).

2.6. Determination of free radical scavenging activity (FRSA)

The FRSA was determined in leaves according to the method described by Middha and Purohit (2011).

2.7. Determination of total phenolic compounds (TOPC)

Total phenolic contents were determined in leaf extracts by the method of Singleton and Rossi (1965) and then expressed as gallic acid equivalents.

2.8. Identification of phenolic compounds

Reverse phase high performance liquid chromatography (HPLC) was used to analyze phenolic compounds present in the 50% EE sample, using the separation module (LC-20 AT, Shimadzu Corporation, Japan) equipped with a C₁₈ column (Vydac, 218 TP, 250 × 4.6 mm, 5 µm particle size, Sigma–Aldrich, St. Louis, MO, USA) and a diode array detector (Rheodyne, USA). The samples were eluted with a gradient system consisting of solvent A (2% acetic acid, v/v) and solvent B (acetonitrile: methanol, 10:15, v/v), used as the mobile phase, with a flow rate of 1 ml/min. The temperature of the column was maintained at 25 °C and the injection volume was 10 µl. The gradient system started from 90% A at 0 min, to 80% A at 10 min, 70% A at 15 min, 60% A at 25 min, 50% A at 30–40 min, 75% A at 42 min, and 90% A at 44 min. The peaks of the phenolic compounds were monitored at 270 nm. UV–V is absorption spectra were re-recorded on-line from 200 to 600 nm during the HPLC analysis.

Electrospray ionization mass spectroscopic (ESI–MS) analysis of phenolic compounds in 50% EE sample was performed using an Applied Biosystems (API2000 LC/MS/MS System, ABI, Foster city, CA). Mass spectra were achieved by electrospray ionization in both positive and negative modes. The capillaries 4500 V (negative) and 5500 V (positive) were used in this study. The electrospray probe flow was adjusted to 20 ml/min. Continuous mass spectra were obtained by scanning from 100 to 800 m/z. Identification of the phenolic compounds of the 50% EE sample from leaves was achieved by comparison with retention times of standards and their UV–V is absorption spectra and ESI–MS spectra comparisons with literature reports or with reference standards available.

2.9. Statistical analysis

In this study, one factor was considered: algal extracts (13 treatments). For each treatment there were 3 replicates, the experimental design followed a CRBD. The averages data of both seasons were statistically analyzed using one way analysis of variance (ANOVA-1) (Statsoft, 2007). Significant values determined according to P values ($P < 0.05$ = significant, $P < 0.01$ = moderate significant and $P < 0.001$ = highly significant). The applications of that technique were according to the STAT-ITCF program version 10 (De-Smith, 2015).

3. Results

3.1. Effect of algal extracts on morphological characters

Morphological characters such as PLHE (cm), LENO/Plant, HFRWE (g/Plant), HDRWE (g/Plant) and SEYI (g/Plant) were affected by the application of algal extracts (Table 1). Plants treated with various doses of algal extracts resulted in highly significant increases ($P < 0.001$) in all morphological characters than those untreated control. The greatest morphological characters were recorded with the treatment of 3 g/L of *Spirulina platensis* that recorded the values of 145.3 cm, 24.8 branch/Plant, 2615.0 g/Plant, 941.4 g/Plant and 53.8 g/Plant; these values increased by 70.9, 77.1, 45.6, 158.4 and 106.1% of PLHE, LENO, HFRWE, HDRWE and SEYI, respectively, compared to the control.

3.2. Effect of algal extracts on TFIOI

Data in Table (2) revealed that adding the algal extracts with various levels had different highly significant increments ($P < 0.001$) in TFIOI

compared with control. The treatment of 1 g/L of *Scenedesmus obliquus* produced the highest amounts of TFIOI with the values of 2.4% which represented a 166.7% increase over the control.

3.3. Effect of algal extracts on TOCA

The effects of different doses of algal extracts on TOCA are presented in Table (2). Highly significant increases ($P < 0.001$) were observed in TOCA (%) under the application of algal extracts compared with control. The highest percentage of TOCA (22.7%) was recorded with the treatment of 3 g/L of *Scenedesmus obliquus* extract; the increase was 43.7% than control.

3.4. Effect of algal extracts on TOPC

The TOPC were affected by different doses of algal extracts (Table 2). All doses of algal extracts caused highly significant increases ($P < 0.001$) in TOPC. The highest value of TOPC (3.7%) was recorded due to the application of *Amphora coffeaeformis* extract at 3 g/L (increased by 311.1% than control).

3.5. Effect of algal extracts on TOFLA

Responses of TOFLA to the application of algal extracts are presented in Table (2). Different levels of algal extracts caused various changes (increased or decreased) in the amounts of TOFLA compared with control. The *Chlorella vulgaris* extract at 1 g/L resulted in the greatest amount of TOFLA with the value of 2.7 mg/g which considered a 58.8% increase than control. The changes in TOFLA were highly significant ($P < 0.001$) for all doses of algal extracts.

3.6. Effect of algal extracts on FRSA

The changes in FRSA under different doses of algal extracts are presented in Table (2). Plants exposed to different levels of algal extracts produced various increases in the inhibition of FRSA compared with untreated plants. The greatest inhibition in FRSA (41.3%; 105.5% over control) was recorded with the treatment of 2 g/L of *Chlorella vulgaris* extract. Different increases in the inhibitions of FRSA were highly significant ($P < 0.001$) for various doses of algal extracts.

Table 1
Effect of algal extracts on the morphological characters.

Doses of algae extract		PLHE (cm)	LENO	TFRWE g/Plant	TDRWE	SEYI
Control	0 g/L	85.0 ± 2.2	14.0 ± 0.0	1821.7 ± 2.1	364.3 ± 1.6	26.1 ± 0.8
<i>Spirulina platensis</i>	1 g/L	123.5 ± 1.3	18.0 ± 0.0	2148.3 ± 18.1	644.5 ± 2.1	35.0 ± 0.6
	2 g/L	134.5 ± 0.7	20.0 ± 0.9	2378.3 ± 11.1	808.6 ± 3.1	42.5 ± 0.5
	3 g/L	145.3 ± 2.5	24.8 ± 0.7	2615.0 ± 15.2	941.4 ± 9.8	53.8 ± 1.1
<i>Chlorella vulgaris</i>	1 g/L	116.3 ± 1.7	16.0 ± 0.2	2123.3 ± 7.6	594.5 ± 2.1	33.8 ± 0.7
	2 g/L	125.2 ± 2.3	20.5 ± 0.5	2295.0 ± 14.7	688.5 ± 7.8	32.2 ± 0.7
	3 g/L	116.3 ± 2.3	20.0 ± 0.5	2425.0 ± 13.7	818.8 ± 9.7	38.6 ± 0.7
<i>Amphora coffeaeformis</i>	1 g/L	99.8 ± 1.2	15.2 ± 0.7	1898.3 ± 7.6	417.6 ± 1.6	43.5 ± 0.9
	2 g/L	106.2 ± 2.1	16.2 ± 0.2	2014.2 ± 13.6	523.7 ± 9.5	37.3 ± 0.2
	3 g/L	113.8 ± 1.3	18.3 ± 0.6	2117.5 ± 11.1	586.4 ± 8.5	35.9 ± 0.1
<i>Scenedesmus obliquus</i>	1 g/L	101.8 ± 1.5	18.0 ± 0.5	1972.5 ± 21.1	477.4 ± 12.3	30.8 ± 0.3
	2 g/L	110.5 ± 1.1	19.3 ± 1.1	2210.8 ± 22.3	574.8 ± 10.2	47.4 ± 0.5
	3 g/L	121.0 ± 0.5	21.3 ± 0.5	2316.7 ± 28.1	695.0 ± 11.5	48.9 ± 0.2
F values		277.5***	76.9***	95.7***	450.6***	440.4***

Note: PLHE, plant height; LENO, leaf number; HFRWE, herb fresh weight; HDRWE, herb dry weight; SEYI, seed yield; ***, $P < 0.001$; values are given as Mean ± SD.

Table 2
Effect of algal extracts on the chemical contents.

Doses of algae extract		TFIOI	TOCA	TOPC	FRSA	TOFLA
		(%)				.mg/g
Control	0 g/L	0.9 ± 0.1	15.8 ± 0.2	0.9 ± 0.0	20.1 ± 0.8	1.7 ± 0.0
<i>Spirulina platensis</i>	1 g/L	1.6 ± 0.0	16.7 ± 0.6	1.1 ± 0.0	27.2 ± 0.4	2.2 ± 0.0
	2 g/L	1.4 ± 0.2	18.0 ± 0.0	1.1 ± 0.1	22.1 ± 0.6	1.9 ± 0.0
	3 g/L	1.5 ± 0.1	21.7 ± 0.5	1.3 ± 0.0	27.4 ± 0.2	1.9 ± 0.0
<i>Chlorella vulgaris</i>	1 g/L	1.8 ± 0.0	18.7 ± 0.6	1.2 ± 0.0	30.5 ± 0.2	2.7 ± 0.0
	2 g/L	1.3 ± 0.0	19.7 ± 0.6	2.1 ± 0.0	41.3 ± 0.2	2.5 ± 0.1
	3 g/L	1.4 ± 0.1	21.3 ± 0.5	1.5 ± 0.1	37.2 ± 0.6	1.3 ± 0.0
<i>Amphora coffeaeformis</i>	1 g/L	1.8 ± 0.0	17.0 ± 0.0	1.1 ± 0.0	23.5 ± 0.5	1.9 ± 0.0
	2 g/L	1.2 ± 0.0	18.3 ± 0.6	1.6 ± 0.0	28.6 ± 0.1	1.8 ± 0.0
	3 g/L	1.3 ± 0.0	19.0 ± 1.1	3.7 ± 0.0	23.0 ± 0.0	1.8 ± 0.0
<i>Scenedesmus obliquus</i>	1 g/L	2.4 ± 0.0	17.3 ± 0.5	1.1 ± 0.1	22.1 ± 0.4	1.4 ± 0.1
	2 g/L	1.6 ± 0.1	19.7 ± 0.5	1.0 ± 0.0	22.4 ± 0.5	1.4 ± 0.1
	3 g/L	1.3 ± 0.0	22.7 ± 0.6	1.0 ± 0.0	24.0 ± 0.4	1.6 ± 0.0
F values		62.3***	40.7***	1061.7***	317.6***	403.6***

Note: TFIOI, total fixed oil; TOCA, total carbohydrates; TOPC, total phenolic compounds; FRSA, free radical scavenging activity; TOFLA, total flavonoids; ***, $P < 0.001$; values are given as Mean ± SD.

3.7. Effect of algal extracts on the phenolic fractionations

The HPLC analysis indicated the presence of 16 phenolic fractions from cardoon leaves (Table 3). Chlorogenic, caffeic and vanillic were observed as major fractions of leaves collected from untreated plants. Different changes (increase, decrease or absent) were found in all identified fractions under algal extracts doses compared with control. The treatments of 2, 3 g/L of *Chlorella vulgaris* extract resulted in the highest percentage of vanillic and chlorogenic with the values of 8.7 and 5.5 mg/plant, respectively. The highest value of caffeic (10.5 mg/plant) was recorded with treatment of 2 g/L of *Amphora coffeaeformis* extract. When comparing the highest values of vanillic, chlorogenic and caffeic acids with control, it was observed that they produced increases 61.1, 31 and 66.7% than control respectively. All changes in various phenolic fractions (major or minor) were highly significant ($P < 0.001$) for various levels of algal extracts (Table 3).

4. Discussion

In this trial, the application of algal extracts with different doses caused significant enhancements in plant morphology and chemical constituents; these effects may be due to the attribution to auxin in algal extracts that has basic roles in cell divisions and cell enlargement which led to an increase in dry matter, various morphological characters, seed yields and various chemical compositions (Gollan and Wright, 2006). In this concern Chu et al. (2010) reported that auxin resulted in significant increases of morphological characters and TOCA of *Aechmea blanchetiana*. Algal extracts is a rich source of various elements (macro and micro) such N, P, K, Ca, Cu, Fe, Mg, Mn and Zn which have different roles in physiological processes of various crops inducing the photosynthesis, cell divisions and cell elongation that reflect good plant growth, dry matter contents, yield and different chemical constituents (Lopez et al., 2008; Marrez et al., 2014). In this respect, Khalid (2012) indicted that macro and micro element produced various increases in PLHE, LENO, HFRWE, HDRW, SEYI, TOCA and FIOI contents of anise, coriander and sweet fennel plants. On the other hand algal extracts contain various bio-stimulants (hormones, amino acids and vitamins) fulfill various roles against stress conditions, increase the nutrient uptakes and assimilation, improve plant enzymes required for protein synthesis and stimulate different growth characters, yield, crop productivity and chemical composition (Panda et al., 2012). In this concern Khattab et al. (2017) revealed that bio-stimulant resulted in different

stimulating of the accumulation of phenols, flavonoids, and consequently the FRSA of *Ammi visnaga* L. The algal extracts contain mannitol and alginic acid: Mannitol that stimulates the synthesis of the polyamide and facilitates the transport of nutrients. However, alginic acid increases the intake of substances by foliar application of algal extracts. Phytoalexins in the algal extracts increase plant resistance to threats from pathogens and improve plant tolerance to heat stress and also play an important role in the transportation of amino acids and nutrients as well as in the synthesis of proteins (Khan et al., 2009; Craigie, 2011; Panda et al., 2012). Algal extract can increase the utilization of solar energy that reflect an increase in the chlorophyll activity and improve the crop productivity (Khan et al., 2009).

Significant increases in morphological characters and chemical composition may be due to the types of algal extracts and its doses, besides the effect of other factors, including plant species, growing, environmental conditions and soil properties (Dean and Ritchie, 1987; Piccaglia and Marottu, 1993). In this concern, environmental and genetic factors resulted in significant changes in the yield and chemical composition of *Salvia* species (Rowshan et al., 2010). Metrological conditions such as humidity, temperature caused significant changes in Indian borage (Khalid and El-Gohary, 2014). It was found that significant relationship between soil properties and plant productivity for example; soil pH has an impact on plant production (Robson, 1989).

Obtained results were confirmed by previous literature on some crops. Blue green algae treatments (*Cylindrospermum muscicola* and *Anabaena oryzae*) caused significant increases in growth measurements (fresh and dry mass) and TOCA of *Lupinus termis* (Haroun and Hussein, 2003). Various significant increases were occurred in root and stem length as well as fresh and dry mass of stem during the maize, wheat, bean and lettuce crops were exposed to *Chlorella vulgaris* treatments (Gonzales and Bashan, 2000; Faheed and El-Fattah, 2008). Different increments were observed in the growth, yield and chemical constituents of some vegetable crops (potato, tomato, peas and garlic) with the application of algal extract (seaweed, *Spirulina platensis* and *Arthrospira fusiformis*) (Awad et al., 2006; Nour et al., 2010; Nawar and Ibraheem, 2014; Shalaby and El-Ramady, 2014). The effects of foliar spray of algal extract (*Spirulina platensis*) on the growth, yield oil contents of fennel (*Foeniculum vulgare* Mill) were investigated by Abd El-Aleem et al. (2017); obtained results showed that plants treated with algae extract at 0.5% resulted in the highest values of growth characters, fruit yield and oil contents. Dill plants (*Anethum graveolens* L.) were subjected to different doses of blue-green algae extracts, *Spirulina platensis* (0, 0.25,

Table 3
Effect of algal extracts on the phenolic fractions.

No.	Phenolic compounds (mg/Plant)	Doses of algae extract												F values			
		0 g/L			Spirulina platensis			Chlorella vulgaris			Amphora coffeaeformis				Scenedesmus obliquus		
		1 g/L	2 g/L	3 g/L	1 g/L	2 g/L	3 g/L	1 g/L	2 g/L	3 g/L	1 g/L	2 g/L	3 g/L		1 g/L	2 g/L	3 g/L
1	Protocatechuic	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	4882.1***
2	p-hydroxybenzoic	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	1056.3***
3	Gentisic	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	2048.5***
4	Catechin	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	5492.0***
5	Chlorogenic	4.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	5.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2565.5***
6	Caffeic	6.3 ± 0.1	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	3764.7***
7	Syringic	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	2426.2***
8	Vanillic	5.4 ± 0.3	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	4854.7***
9	Rutin	0.6 ± 0.0	0.9 ± 0.3	0.7 ± 0.0	1.1 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	680.6***
10	Apigenin-7-glucoside	1.0 ± 0.0	1.5 ± 0.3	0.7 ± 0.0	1.5 ± 0.1	1.5 ± 0.1	2.3 ± 0.1	2.8 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	1.4 ± 0.2	1.9 ± 0.2	1.1 ± 0.3	0.7 ± 0.1	0.8 ± 0.2	0.8 ± 0.2	2394.7***
11	Rosmarinic	0.2 ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	1211.1***
12	Cinnamic	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	1056.4***
13	Quercetin	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	1590.6***
14	Apigenin	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	822.8***
15	Kaempferol	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	2274.9***
16	Chrysin	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	.tr. ± 0.0	1627.9***

Note: ***, P < 0.001; tr., traces (less than 0.05); values are given as Mean ± SD.

0.5 and 0.75%) (Toaima et al., 2017), the dose of 0.75% resulted in the highest values of fruit yield and some oil constituents compared with other doses. The treatment of algal extract (seaweed) at 2% as foliar spray caused significant increases in growth, fruit yield, fruit quality and nutrient contents of mango (*Mangifera indica* L.) (El-Sharony et al., 2015). Mixture application of algal extracts (*Laurencia obtusa* and *Corallina elongata*) produced various significant increments in plant length, number of leaves, fresh mass, dry mass and various nutrient contents of maize (*Zea mays* L.) (Safinaz and Ragaa, 2013). Peanut and maize plants were subjected to algal extract (seaweed) at 1.5 g/L as foliar application (Nofal et al., 2016); obtained results indicated that the plants treated with algal extract produced higher growth, yield and protein and nutrient contents than those treated with distilled water (control). Adding 4 ton/ha of dried algae enhanced the yield of amaranthus (*Amaranthus cruentus*) (Azu et al., 2017).

Previously, there was a few scientific researches were found on the response of medicinal plants to algal extracts in Egypt. So these trials will lead the producers to use algal extracts as an attractive and cheap method to increase productivity of medicinal plants.

5. Conclusion

In this study, the effects of different doses (0, 1, 2 and 3 g/L) of some algal extracts (*Spirulina platensis*, *Chlorella vulgaris*, *Amphora coffeaeformis* and *Scenedesmus musobliquus*) on the growth, seed yield and chemical composition of caroon plants were evaluated. The conclusion of obtained results indicated that plants treated with algal extracted produced higher values of morphological characters, seed yield and chemical constituents than those untreated control. The application of algal extracts considered as biological fertilizers. They are promising alternative agrochemicals in order to avoid the pollution and enhance the growth, yield and the active ingredients of caroon plant. On the other hand this investigation will assist the producers and pharmaceutical companies to improve the yield and active constituents as natural source in food and drug industries.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bcab.2019.101336>.

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