Effect of licorice root and cabbage leaf extracts as a natural fertilizer on growth and productivity of *Cynara cardunculus* L

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Background
There is an ongoing need to find safe natural sources of plant nutrients. Licorice root and cabbage leaf extracts are being used as sources that can be used for growth and yield of crops to substitute inorganic fertilizers.
Objective
To study the effect of extracts of cabbage leaves (waste) and licorice roots on *Cynara cardunculus L*.
Materials and methods
This experiment was carried out during two seasons (2017/2018 and 2018/2019) at Aladlya Field, Sekem, Sharkia Governorate, Egypt, to study the influence of some plant extracts, that is cabbage leaves' extract at 0, 1, 2, and 3 g/l as well as licorice root's extract at 0, 5, 10, and 15 g/l, on growth, yield, and chemical constituents (NPK, total phenolic content, and phenolic compounds) of *C. cardunculus* L. plants.
Results and conclusion
Both licorice root and cabbage leaf extracts had positive effects compared with control. However, licorice root extract had more effect on *C. cardunculus* L. plants compared with cabbage leaves' extract. The main phenolic compounds were apigeni-7-glucoside (50.9272–161.8283 μg/g), rutin (79.8306–152.3828 μg/g), chlorogenic (4.5107–25.7202 μg/g).

Keywords:
cabbage, *Cynara cardunculus* L, growth, licorice, phenolic compounds

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Introduction

Cynara cardunculus L. belongs to the family Asteraceae. The leaves of *C. cardunculus* are particularly known in folk tradition for their therapeutic potential as choleretic, diuretic, antidiabetic, antimicrobial, and cholagogue as mentioned [1,2]. The seeds are used as a source of protein and edible oil, and also as a source of fixed oil, which produces biodiesel [3]. After oil extraction from seeds, the residual flour could be used for animal feed, both for the quantity and quality of its proteins [4,5]. Previous investigations had shown the presence of saponins, sesquiterpene, lactones, flavones, sterols, coumarins, and lignans in leaves and seeds [6,7]. Moreover, the inulin extracted from the roots and cynarin extracted from leaves of cardoon plant are pharmacological active compounds [8,9]. Cynarin found in the leaves improves liver and gall bladder function, stimulates the secretion of digestive juices especially bile, and lowers blood cholesterol levels [10]. In traditional European medicine, it is clear that the leaves of this plant are rich in polyphenols compounds which has pharmacological properties [11,12].

Organic extracts are useful for many agriculture purposes [13]. There is an ongoing need to find safe natural sources as plant nutrients. Licorice root and cabbage leaves are being used as sources for studying their effect on growth and yield of the crops as substitution for the inorganic fertilizers. Many studies have been carried out to study the effect of these extracts on crops but very rare on medicinal and aromatic plants. In this connection, some authors [14,15] showed that spraying onion plant with licorice root extract caused significant increment of vegetative growth and bulb production. Moreover, this extract had favorable effect on fresh and dry weight of plants, flowering, total yield, flower production, and fruit quality of plants such as onion, cucumber, and snap bean [16–20]. The enhanced effect of this extract may be owing to its richness in amino acids, vitamins, and growth-stimulating photo-hormones that increase the activity of apical meristem tissue, which causes cell division and elongation [21]. The outer leaves of cabbage (waste) that are peeled off before cabbages are distributed in the

market were used as the raw material to produce bio-extract. Cabbage is a sulfur-rich plant because the glucosinolate accumulated in cabbage is able to breakdown to produce elemental sulfur, so, cabbage is a sulfur-rich plant. In this respect, cabbage waste contains minerals (N, P, K, Ca, Mg, and S.), vitamins, and amino acids (aspartic, tryptophan, glycine, etc.) [22].

Materials and methods

Field experiment

This experiment was carried out at Aladlyal Farm, Sekem, Belbis El-Sharkia Governorate, Egypt, to study the influence of some plant extracts on growth, yield, and chemical constituents of *C. cardunculus* L. plants. The groups of applied treatments were as follows:

T1=sprayed with distilled water.
T2=sprayed with cabbage leaves' extract at 1 g/l.
T3=sprayed with cabbage leaves' extract at 2 g/l.
T4=sprayed with cabbage leaves; extract at 3 g/l.
T5=sprayed with licorice root's extract at 5 g/l.
T6=sprayed with licorice root's extract at 10 g/l.
T7=sprayed with licorice root's extract at 15 g/l.

Preparation of plants extracts

Ethanolic extraction of cabbage leaves

The powdered samples were soaked in ethanol 80% and shaken on a shaker at room temperature for 48 h. Extracts were filtered using Whatman No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator [23,24].

Aqueous licorice root's extract

The aqueous extract of licorice roots (*Glycyrrhiza glabra*) was prepared by grinding plant roots which were well dried, and the powdered sample was soaked in water and the mixture was put on a rotary shaker. The extract was purified by filtering twice through Whatman No. 1 filter paper. After purification, each extract was diluted to the required volume for each concentration [24].

Seeds of *C. cardunculus* L. were kindly provided by 'SEKEM' company (3 Cairo-Belbeis Desert Road El Salam City, Cairo, Egypt) and were sown directly in the field on October 21, 2017, and 2018. After 45 days, the plants were sprayed with materials extracts monthly. The plants were thinned twice, leaving one plant per hill.

The plants were harvested at February 21 during both seasons 2018 and 2019. Vegetative growth parameters were recorded, including number of leaves and fresh and dry weight of leaves (g/plant). Chemical analyses were carried out during the second season as follows.

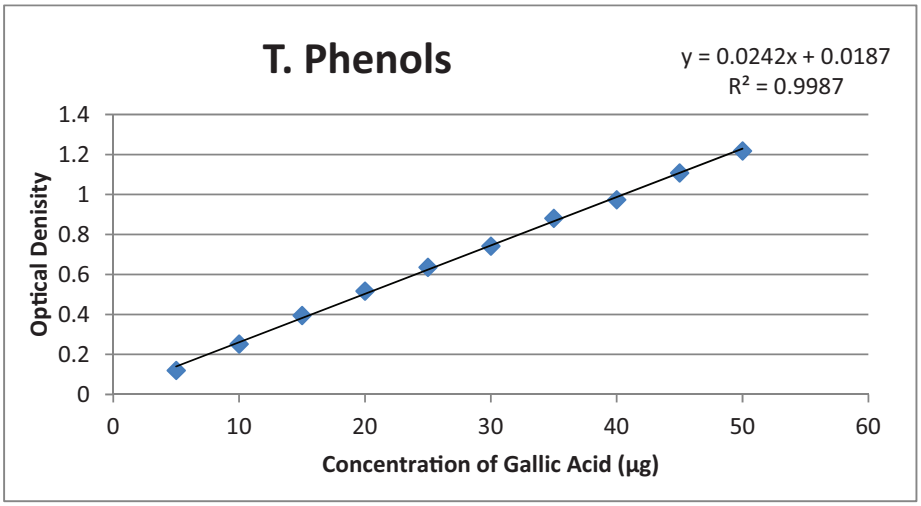
Nutrient determination

Nutrient contents such as total nitrogen (%) were estimated by using the method of Kjeldahl [25], and phosphors and potassium percentages were determined according to Cottenie *et al.* [26].

Determination of total phenolic content

The total phenolic content was determined according to the Folin-Ciocalteu procedure [27]. The total phenolic content was determined by means of a calibration curve prepared with gallic acid (Fig. 1)

Figure 1



Calibration curve prepared with gallic acid.

and expressed as μg of gallic acid equivalent per gram of sample.

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, 14th Floor, Hankyu Terminal Bldg., 1-1-4 Shibata, Kita-ku, Osaka 530-0012, Japan) equipped with a C_{18} column (Vydac, 218 TP, 250 ×4.6 mm, 5 μm particle size; Sigma-Aldrich, St Louis, Missouri, USA) and a diode array detector (Rheodyne, 2809-10th, Berkeley, California 94710, 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. Ultraviolet–visible absorption spectra were recorded on-line from 200 to 600 nm during the HPLC analysis.

Electrospray ionization mass spectroscopic analysis of phenolic compounds in 50% EE sample was performed using an Applied Biosystem (API2000 LC/MS/MS System; ABI, Foster city, California, USA). 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 ultraviolet–visible absorption spectra and electrospray ionization mass spectroscopic spectra comparisons with literature reports or with reference standards available.

Statistical analysis

The experiment design was completed randomized with three replicates, and each replicate contained 10 plants. The statistical analysis of obtained data was carried out according to Snedecor and Cochran [28]. Differences between means were compared by using Duncan's multiple range tests at 0.05 [29].

Results and discussion

Variance analysis

Results of variance analysis (Table 1) for growth parameters under study showed that they were significantly affected by different treatments.

Vegetative growth and characteristics

Results of Tables 2 and 3 revealed that number of leaves and leaves' fresh weight were significantly affected by spraying materials. Spraying with licorice root extract at 15 g/l resulted in the highest values of leaves number (42 and 36 for first and second seasons, respectively) and leaves' fresh weight (1558.7 and 1525 g/plant for first and second seasons, respectively), followed by spraying with licorice root extract at 10 g/l on the number of leaves (30 and 31 for first and second seasons, respectively). Application of cabbage leaves' extract at 3 g/l gave the highest mean values for leaves' fresh weight (1440.7 g/plant) in the first season, whereas licorice root extract at 10 g/l gave leaves' fresh weight of 1425 g/plant during the second one. On the contrary, the treatment of spraying with water gave the lowest mean values for the number of leaves (20 and 16) and leaves' fresh weight (384.75 and 655 g/plant, for the first and second season, respectively).

Table 1 Analysis of variances summary between treatments' studied traits (number of leaves, fresh weights, and dry weights during 2017/2018 and 2018/2019)

		First season		
	DF	Number of leaves	Leaves' fresh weight (g/plant)	Leaves' dry weight (g/plant)
Replications	2	0.571^{NS}	100^{NS}	1.286^{NS}
Treatments	6	136^{**}	485195.9^{***}	29266.6^{***}
Error	12	1.238	75	4.286
CV%		4.121	0.741	0.790
Second season				
Replications	2	1.286^{NS}	1128.6^{NS}	39^{NS}
Treatments	6	131^{***}	243042.9^{***}	15856.5^{***}
Error	12	0.786	1528.57	14
CV%		3.409	3.182	1.288

CV, coefficient of variation; NS, not significant. ***High significant.

Table 2 Influence of cabbage and licorice extracts on number of leaves and leaves' fresh weight (g/plant) of *Cynara cardunculus* L. plants for first season

Treatments (g/l)	Number of leaves/plant	Leaves' fresh weight (g/plant)	Leaves' dry weight (g/plant)
Control	20^{e}	384.7^{g}	80.79^{g}
Cab 1	21^{e}	1046.2^{f}	219.66^{f}
Cab 2	24^{d}	1180.0^{e}	342.20^{b}
Cab 3	27^{c}	1440.7^{b}	273.73^{d}
Lic 5	27^{c}	1205.0^{d}	241.0^{e}
Lic 10	30^{b}	1405.0^{c}	286.20^{e}
Lic 15	42^{a}	1558.7^{a}	389.7^{a}

Cab, cabbage extract; Lic, licorice extract. Means with the same letters in each column indicate no significant difference between treatments at 5% level of probability.

Table 3 Influence of cabbage and licorice extracts on number of leaves and leaves' fresh weight (g/plant) of *Cynara cardunculus* L. plants for second season

Treatments (g/l)	Number of leaves/plant	Leaves' fresh weight (g/plant)	Leaves' dry weight (g/plant)
Control	16^{e}	655^{f}	167.03^{g}
Cab 1	20^{d}	1125^{e}	244.01^{f}
Cab 2	26^{c}	1220^{d}	257.85^{e}
Cab 3	26^{c}	1350^{c}	341.66^{b}
Lic 5	27^{c}	1300^{c}	307.52^{d}
Lic 10	31^{b}	1425^{b}	331.84^{c}
Lic 15	36^{a}	1525^{a}	383.37^{a}

Cab, cabbage extract; Lic, licorice extract.

Generally, leaves' dry weight gave the same trend as mentioned by leaves' fresh weight, with some exceptions.

N, P, and K content (%)

The effect of different extract treatments on nutrients content such as N, P, and K is shown in Table 4. It can be noticed that extract treatments had a pronounced effect on N and P % compared with control. Cabbage extract at 3 g/l gave the highest values of N (1.29%) and P (0.18%). These treatments had slight effect on K %, where cabbage extract at 3 g/l gave the maximum value of P (3.4%).

Table 4 Influence of cabbage and licorice extracts on total phenolic compounds (mg/g), total carbohydrate (%), and some minerals content (%) of *Cynara cardunculus* L. plants during the second season

Treatments (g/l)	Total phenolic compounds (mg/g)	N (%)	P (%)	K (%)
Control	8.95	1.20	0.12	3.2
Cab 1	8.93	1.26	0.15	3.2
Cab 2	10.88	1.26	0.16	3.2
Cab 3	14.27	1.29	0.18	3.4
Lic 5	14.22	1.21	0.13	3.2
Lic 10	16.70	1.21	0.13	3.2
Lic 15	12.72	1.26	0.17	3.3

Cab, cabbage extract; Lic, licorice extract.

Total phenolic content (mg/g)

Data tabulated in Table 4 clears that total phenolic compounds ranged from 8.93 to 16.70 mg/g. Most extract treatments increased total phenolic compounds, except cabbage at 1 g/l, compared with control. Licorice extract at 10 g/l gave the maximum value of total phenolic compounds (16.70 mg/g) followed by cabbage extract at 3 g/l, which was recorded at 14.27 mg/g.

Phenolic compounds (μg/g)

As shown in Table 4, nine main phenolic compounds were determined using HPLC detection and were identified in *C. cardunculus* L. samples under different treatments (Table 5). The main phenolic compounds were apigeni-7-glucoside (50.9272–161.8283 μg/g), rutin (79.8306–152.3828 μ/g), and chlorogenic (7.878–25.7202 μg/g). The results showed great differences between the different extract treatments. The highest values of apigeni-7-glucoside and chlorogenic were obtained from plants treated with cabbage extract at 1 g/l, whereas cabbage extract at 2 g/l gave the maximum value of rutin compound.

Generally, it can be noticed that licorice root extract gave the best results compared with cabbage extract. In this connection, the licorice extract contains about 100 various compounds where some of these compounds are accumulated in large amounts, and most vital of them are triterpene saponins (including glycyrrhizin), phenolic as well as flavonoid (liquiritin, isoliquiritin, and others which cause the yellow color) compounds [30,31]. Moreover, hispaglabridins A and B as well as isoflavones glabridin which have a significant antioxidant activity are present. Additionally, licorice extract contains protein, amino acid (Asparagine), monosaccharide, tannins, lignins, starch, phytosterols, choline, different types of vitamins (i.e. B1, B2, B3, B6, C, and E), biotin, folic acid, pantothenic acid, many minerals (Al, Ca, Fe, Mg, Co, Zn, P, Na, Si, K, and Sn), as well as bitter principles [32–35]. Moreover, many investigators [36–38] found that licorice root extract contains some compounds that have similar effect to that of growth promoters, a wide range of minerals, amino acids, vitamins, and in addition carbohydrate and nitrogen. It also contains mevalonic acid utilized in gibberellin synthesis [39]. Different studies were done on two strawberry varieties, to examine the effect of three concentrations of licorice root extracts (0, 2, and 4 g/l) as a foliar spray on the vegetative and flowering parameters [40], the treatments with licorice extract at 2 g/l gave a significant increment in average leaf area and foliage dry weight, but 4 g/l caused a significant increase in total chlorophyll content. Cabbage waste contains minerals (N, P, K, Ca, Mg,

Table 5 Influence of cabbage and licorice extracts on phenolic fractions (μg/g) of *Cynara cardunculus* L. plants for the second season

Compounds	Control	Cab 1 (g/l)	Cab 2 (g/l)	Cab 3 (g/l)	Lic 5 (g/l)	Lic 10 (g/l)	Lic 15 (g/l)
Protocatechuic	1.2527	1.7277	2.3747	2.4159	0.0000	1.7182	0.6156
p-hydroxybenzoic	0.0000	0.3161	0.1118	0.1016	0.0000	0.0000	0.0000
Chlorogenic	12.0324	25.7202	18.4457	20.0469	4.5107	14.3585	7.8780
Vanillic	0.0710	0.3957	0.3331	0.0000	0.0000	0.2089	0.0860
p-coumaric	1.5443	1.6025	1.8913	1.3173	0.8512	1.5113	0.8498
Rutin	130.5149	105.5496	152.3828	106.6557	81.7401	107.6681	79.8306
Apigeni-7-glucoside	100.0594	161.8283	156.9914	124.3520	32.3616	98.1446	50.9272
Rosmarinic	15.1318	15.3581	15.8840	12.3068	10.4541	10.2929	10.4823
Kaempferol	0.1655	0.0739	0.2222	0.1307	0.1054	0.1039	0.1060

Cab, cabbage extract; Lic, licorice extract.

and S), vitamins, and amino acids (aspartic, tryptophan, glycine, etc.) [22].

From the aforementioned, it can be concluded that the extracts of licorice roots and leaves of cabbage have high nutritive value, where they are high in vitamins, minerals, etc., which influence every phase of plant development and growth.

Conclusion

It can be concluded that both licorice root extract and cabbage leaf extract had a positive effect as compared with the control, but the licorice root extract was more effective on *C. cardunculus* L. plants as compared with cabbage leaf extract.

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Conflicts of interest

There are no conflicts of interest.

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