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PHYTOCHEMICAL STUDIES ON CELERY AND GARDEN ROCKET SEEDS

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ABSTRACT

From ancient time celery (*Apium graveolens*) and garden rocket (*Eruca Sativa, Mill*) are used in traditional medicine. The seeds of these plants were conducted to separate and qualitative identify their active components. (i.e., alkaloids, flavonoids, terpenes, saponins, polyphenols, ... etc.,). The seeds were extracted with methanol which considered the mother liquor. The later solvent extract was subjected to successive extraction with solvents increased in their polarity started with pet. Ether followed by chloroform and ethyl acetate. Effects of three celery seeds extracts as antimicrobial activity against different strains of pathogenic fungi were studied. The resulted rocket and celery seeds extracts were used also as natural antioxidants. The antioxidant power of these extracts was measured by different methods. The ethyl acetate extract of celery seed when used as antioxidant was more effective than ethyl acetate extract of rocket seed and artificial antioxidant BHT.

The phenolic compounds were extracted and identified by HPLC in both rocket and celery seeds. The phenolic compounds in rocket were: phenol, gallic acid, dadzin, genstin and p-OH benzoic acid. Celery seed were distinguished with phenolic compounds of salicylic acid, phenol, quercetin, catechine and rutin. Caffeic acid, quercetin and vanillin disappeared in rocket seed, while cinnamic acid, euganol and pyrogallic disappeared in celery seed. Generally, these phenolic compounds in both two plants have important effects as natural antioxidants.

Keywords: *Apium graveolens* - *Eruca Sativa* – Antibacterial – Antifungal – Flavonoids.

INTRODUCTION

Many authors indicated that celery has been cultivated for the last 300 years, notably in pharonic Egypt, and was known in china in the 5th century, BC. Celery has been used as a food and at various times both the whole plant and the seeds have been consumed as a medicine (Chevalier, 1998). The same author also reported that celery seeds are implicated in arthritic pain relief for treating rheumatic condition and gout.

Rocket (*E. Sativa*) is widely distributed all over the world and is usually consumed fresh (leafs or sprouts) for its typical spicy taste. Nevertheless, it is mentioned in traditional pharmacopoeia and ancient literature for several therapeutic properties, and it does contain a number of healthy promoting agents including carotenoids, vitamin C, Fibers, Falvonoids, and glucosinolates (GLS). The major GL found in rocket seeds is gluoerucin, GER that represents 95% of total GLS. Unlike other GLS (e.g., glicorophanin, the bio-precursor of sulforaphane), GER possesses good direct as well as indirect antioxidant activity. GER (and its metabolite erucin, ERN) effectively decomposes hydrogen peroxide and alkyl hydroperoxides. (Barillari et al, 2005).

El-Missiry and El-Gindy (2000) tried to use the oil of Eruca sativa seeds (ESS) for prevention and treatment of diabetes mellitus (DM) induced experimentally by alloxan injection. They found that a single dose of alloxan (100 mg/kg) produce a decrease in insulin level, hyperglycemia, elevated total lipids, triglycerides cholesterol, decrease high density lipoprotein and hepatic glycogen contents and elevated hepatic glucose-6-phospahate activity. They suggested that essential oil (ESS) oil could be used as antidiabetic complement in case of DM. This may be related to its antioxidant properties and its effect in increasing hepatic glutathione (GSH).

Mahran et al., (1991) mentioned that seeds of Eruca sativa contain volatile oil, sterols, triterpenes, carbohydrates, glycosides, tannins, flavonoids and gluconsinolates. They added that the volatile oil increased Na^+, K^+ and Cl^- excretion in urine and used as diuretic.

Some amino acids of E. sativa such as arginine, cysteine and tryptophan had antioxidant and hypoglycemic effect (Larson, 1987).

Eruca sativa meal extracts were used to detect their antimicrobial activity against different strains of bacteria, yeast and fungi. The chemical analysis of these extracts indicated the presence of high total soluble sugars and free phenols. Methanolic E. sativa meal extracts had no antimicrobial activity against tested Bacillus strains. No antiyeast activity was obtained by ethanolic and methanolic of E. sativa against all tested yeast strains. The minimum inhibitory concentration (MIC) being 8.8 mg/100 ml was recorded by ethanolic *E. sativa* extraction against *A. tumefaciens, S. aureus* 26 and *M. roseus* (Azza et al., 2006).

Phytochemical investigations of celery seeds revealed the presence of apigenin as a major constituent (Perry, 1980).

From the crude methanol extract of celery seeds, sixteen compounds were isolated: five sesquiterpenoids glucosides (celerioside), three phthalide glycosides, six aromatic compounds glycosides and two norcarotenoid glucosides. Their structures were identified by spectral investigation (Junicni et al., 2003).

The present work aims to separate and identify some active components from both rocket and celery seeds extracts. Their effect as antimicrobial and natural antioxidant was also studied.

MATERIALS AND METHODS

Plant materials:-

1. The seeds of garden rocket and celery were purchased from local market.

2. Fungal strains:- Three fungal strains i.e. *B. cinerea, S. cepivorum* and *F. solani* were obtained from plant pathology department, Faculty of Agriculture, Mansoura University, Egypt.

3. Preparation of seed samples: Both celery and rocket seed samples were free from foreign materials, washed, air dried and ground to fine powder.

   3.1 Chemical analysis: The powdered seed of each sample was subjected to following analysis: Moisture, crude protein, ach content, crude lipids, crude fibers and total soluble sugars according to the methods of A.O.A.C (1990). The

determination of free amino acids as lysine was carried out according to the method mentioned by Jayaraman (1985) .

3.2 Preparation of methanol extract: The powdered seed of each sample was soaked in pet. ether (40-60 °C) for 24 hours to remove fats. The defatted powder was extracted with 80% aqueous methanol several times. The extracts were filtered and kept as the mother liquor .

3.2.1 Preliminary phytochemical screening for methanolic extracts of rocket and celery seeds : The presence or absence of tannins, terpenes, falvonoids, saponins, glucosides, phenols and alkaloids were detected by the method described by Balbaa (1981), Romo (1966), Fieser and Fieser (1959), Kiang (1961), Trease (1961) and Claus (1967) .

3.2.2 Extraction and identification for phenolic compounds of methanolic extracts of rocket and celery seeds by HPLC: The method outlines by Ben-Hammouda et al., (1995). Identification of individual phenolic compounds of the plant samples was performed on JASCO HPLC, using a hypersil C_{18} reversed-phase column (250×4.6 mm) with 5 run particle size. Injection by means of a Rheodyne injection valve (MODEL 7125) with 50 PJ fixed loop was used. A constant flow rate of 0.7 ml/min was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.65; and solvent (B) 0.5% acetic acid in 99.5 acetonitrile. The elution gradient was linear starting with (A) and ending with (B) over 35 min, using an UV detector set at wavelength 254 nm. Phenolic compounds of each sample were identified by comparing their relative retention times with those of the standard mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements, then converted to μg phenolic g^{-1} dry weight. All chemicals and solvents used were HPLC spectral grade. Sixteen standard phenolic compounds were obtained from sigma (st. Lousi, USA) and rom Merck-Shcuchrdt (Munich, Germany chemical companies).

3.2.3 Successive extraction: Methanol extracts (mother liquor) was subjected to successive extraction with three solvents

graduated in their polarity started with pet. ether followed by chloroform and ethyl acetate.

3.2.3a.Antifungal activity: The antifungal activity of pet. ether, chloroform and ethyl acetate extracts were tried as described by Chkhikvishvili and Gogiya (1995). Active plant extracts were dissolved in dimethyl formamide (DMF) and put into pores in Petri dishes in three concentrations of 100, 300 and 500 ppm. The dishes were incubated at 37°C for 10 days. Finally, the radius of growth inhibit was measured (cm) in comparison with control.

3.2.3b.The antioxidant activity:This parameter was determined according to the Egyptian standard (1975).

3.2.3c.Antioxidant Power: The antioxidant power of the resulting natural extracts (produced by successive extraction) was evaluated also by different methods (acid value and iodine value) against artificial antioxidant BHT according to A.O.A.C (1975).

RESULTS AND DISCUSSION

1.The chemical composition of rocket and celery seeds:

From Table (1) it is clear that , moisture, crude protein, ash, crude lipids, crude fiber, total soluble sugars and free amino acids of these seeds are (6.5, 12%), (16.46, 15%), (4, 11%), (27.5, 7%), (6.2,14.2%), (21.4, 26.2%) and (2.25, 2.40) respectively.

These results indicated that rocket seeds had high contents of crude protein and crude lipids (16.46 and 27.5%, respectively), while celery seeds contain high contents of ash, crude fiber and total soluble sugars (11, 14.2 and 26.2%, respectively) in comparison with rocket seeds sample . These results are similar to those obtained by Kanya and Kantharaj (1989) who stated that rocket meal was rich in protein contents.

Table (1). The chemical composition of rocket and celery seeds.

Plant	Moisture	Crude protein	Ash	Crude lipids	Fibers	Total soluble sugars	Free amino acids as lysine
Rocket	6.5	16.46	4.0	27.5	6.2	21.4	2.25
Celery	12.0	15.0	11.0	7.0	14.2	26.2	2.40

Results are in gm/100gm seed samples.

2.Preliminary phytochemical screening of methanol extracts :

The preliminary phytochemical tests for methanolic extract of rocket seeds, were tabulated in table (2). Results showed the presence of tannins, flavonoids, saponins, phenols and glucosides. While the methanol extract of celery seeds sample showed to contain terpenes beside the above mentioned components except saponins. Alkaloids were not detected in both two seeds of methanolic extracts. These results agreed with those mentioned by Mahran *et al.,* (1991), who reported that seeds of rocket contain volatile oil, sterols, triterpenes, carbohydrate, glucosides, tannins, flavonoids and glucosinolates. They added that the volatile oil of rocket seeds increased Na^+, K^+ and Cl^- excretion in urine and used as a diuretic agent. Some amino acids of rocket seeds such as arginine, cysteine and tryptophan had antioxidant and hypoglycemic effect (Larson, 1987).

Table (2). Preliminary phytochemical screening of rocket and celery seeds methanol extracts.

Plant	Tannins	Terpens	Flavonoids	Saponins	Glucosides	Alkaloids	Phenols
Rocket	+	-	++	++	++	-	+
Celery	++	++	++	-	+	-	+

The different extracts of both rocket and celary seeds (pet. ether, chloroform and ethyl acetate) were tested as antifungal factors. The rocket seeds extracts showed an undetectable effect on the treated pathogenic fungi. So, results for celery seeds extracts were only undertaken.

Table (3) showed the effect of three celery extracts i.e. pet. ether, chloroform and ethyl acetate in three concentrations 100. 300 and 500 ppm for each on three pathogenic fungi (*B. cinerea, S. cepivorum* and *F. solani*).

The inhibitory effect of these extracts were studied using agar diffusion method. Data in the same Table showed that the concentration of 500 ppm are the most effective one for inhibition of the pathogenic fungi. The largest inhibition zone diameters of the three extracts were detected against *F. solani* with inhibition

diameters of 5, 4.5 and 5 cm respectively. The next inhibition was observed for *B. cinerea* with inhibition zone diameters of 3.5, 4 and 4 cm respectively. While *S. cepivorum* inhibited by the three extracts on level of 500 ppm at inhibition zone diameters of 3, 2.5 and 3 cm for pet. ether, chloroform and ethyl acetate respectively.

Table (3). Inhibition effect of three celery extracts on pathogenic fungi.

Extracts	*B. cinerea*			*S. cepivorum*			*F. solani*		
	100	300	500	100	300	500	100	300	500
Pet. ether	2	3	3.5	2	2.5	3	3	3.5	5
Chloroform	2	3	4	1.5	2	2.5	3.5	4	4.5
E. acetate	1.5	3	4	2	2	3	3.5	4	5

Results are in cm.

On the other hand, the lowest inhibition zone diameters were observed in the concentration of 100 ppm against *B. cinerea* that had the values of 2, 2 and 1.5 cm for the three mentioned extracts respectively.

The results in Table (3) showed also that the best extract was ethyl acetate followed by pet. ether and chloroform against *F. solani*. These results were confirmed as shown in figure (1) which declare the inhibition zone diameters (cm) for the ethyl acetate extract against *F. solani* at 100, 300 and 500 ppm .

Data also, revealed that the fungal strains of *F. solani* was more susceptible to the three celery extracts than other strains. The lowest susceptible was recorded by the fungal strains of *S. cepivorum*.

These results were drawn graphically in histograms as shown in figures 2, 3 and 4. These histograms revealed that the concentration of 500 ppm was the most effective one.

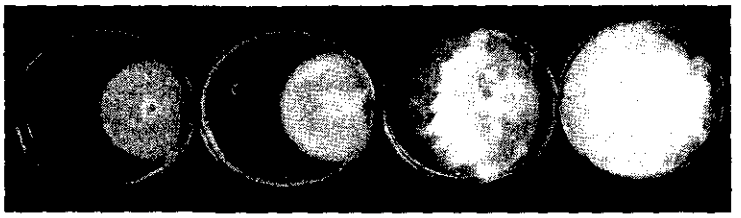

Fig. (1): Effect of ethyl acetate extract for celery seeds on *F. solani.*

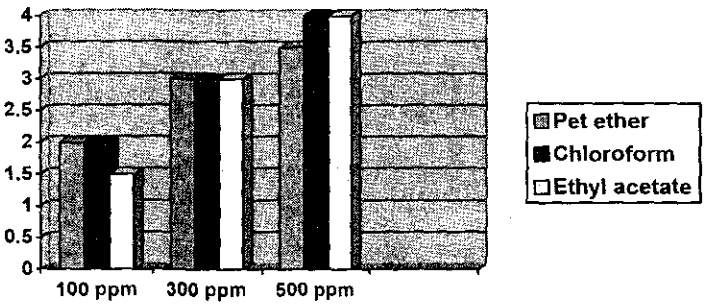

Fig (2) Effect of celery extracts on *B. cinerea*

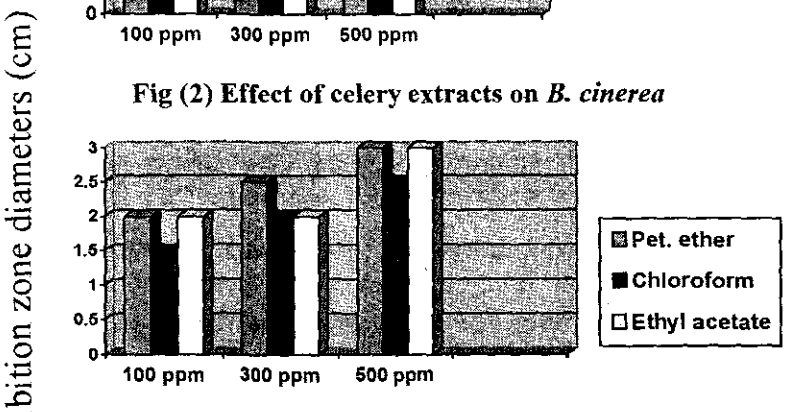

Fig (3) Effect of celery extracts on *S. cepivorum*

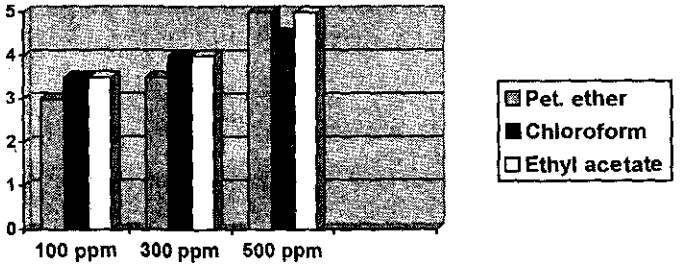

Fig (4) Effect of celery extracts on *F. solani*

The obtained results agreed with those mentioned by Kowalczyk and Krzyanowska (1999), they reported that the antifungal activity of some dipsacaceae family plants may be due to the presence of poly phenolic compounds especially Flavonoids. Similar results were obtained also by Curini et al., (2004) for anti yeast of alcoholic extracts of *Paronychia kapela* against *C. albicans* and *Trichophyton metegraphytes.*

3.HPLC for phenolic compounds for methanolic extracts of seeds samples under investigation :-

The phenolic content for methanolic extracts of samples were identified by high performance liquid chromatography (HPLC) against standard phenolic compounds and the results were recorded in Table (4). The common phenolic compounds (as ppm) of rocket seed samples which appeared in higher values were: phenol (530.87), gallic acid (220.31), dadazin (197.05), genstin (189.69), p-OH benzoic (120.86), p-coumaric acid (96.93), rutin (94.30) and pyrogallic acid (71.47). While the rest phenolic compounds were detected in lower values as follows: cinnamic acid (19.16), salicylic acid (10.39), Ferulic (8.60), dadazian (58.92) and gestein (6.49 ppm).

The same Table (4) revealed that the methanolic extracts of celery seeds contained higher amount of phenolic compounds i.e., salicylic acid (781.36), phenol (596.5), quercetin (544.53), catechine (347.9), p-coumaric acid (176.06), caffeic (155.8), ferulic (153.66), dadzin (150.9), rutin (138.92) and Kaempherol (83.08 ppm), while the rest compounds were appeared in lower values as follows: dadazian (14.31), vanillin (7.65), gallic acid (4.43), p-OH benzoic (7.18) and galangin (0.24 ppm).

It is quite clear that the high level of salicylic acid, catechine and ferulic in celery seed were decreased in rocket seeds. Caffeic, quercetin and kampherol were detected in celery seed while disappeared in rocket seed. The major of phenolic components in celery seeds are salicylic acid, phenol, quercetin, catechine and rutin, while the major phenolic components in rocket seeds are phenol, gallic acid, dadzin, genstin, p-OH benzoic and p-coumaric acids. These results agreed with those mentioned by Yanishlieva and Marinova (1995).

From Table (4) it is clear also that celery seeds contained higher amount of phenolic compounds (3167.06 ppm) than those of rocket seed (1626.15 ppm). It could be easily concluded that the higher level of phenolic compounds in both rocket and celery seeds are responsible for their antioxidant behavior.

Table (4). HPLC of Phenolic components (ppm) for methanolic extracts of rocket and celery seeds.

Components	Rocket	Celery
Galic	220.31	4.43
p-OH benzoic	120.86	7.18
Caffeic	0.00	155.80
Phenol	530.87	596.50
p-coumaric	96.93	176.06
Salicylic	10.39	781.36
Ferulic	8.60	153.66
Cinnamic	19.16	0.00
Quercetin	0.00	544.53
Euganol	0.08	0.00
Chrysin	0.00	0.08
Galangin	0.00	0.24
Pinostrobin	0.00	0.00
Vanillin	0.00	7.65
3, 5 di methoxy benzyl	0.67	0.89
Catechine	0.36	347.90
Dadzin	197.05	150.90
Genstin	189.69	1.61
Dadazian	58.92	14.31
Gestein	6.49	1.96
Pyrogallic	71.47	0.00
Rutin	94.30	138.92
Kaempherol	0.00	83.08
Total	1626.15	3167.06

4.Antioxidant activity:

The antioxidant activity of the ethyl acetate extract of rocket and celery seeds was carried out on sunflower oil free from antioxidant by using the method of activity test according to Egyptian standard (1975). The results in Table (5) show the effect of 0.01% ethyl acetate extracts of rocket and celery seeds on the stability of the oil, where the peroxide values for the drawing samples after 1, 2, 3, hours were 2.312, 2.321 and 2.350 meq. O_2/1000 gm oil for ethyl acetate extract of celery seed, while the results in the same Table for ethyl acetate extract of rocket seeds gave 2.313, 2.333 and 2.370 meq. O_2/1000 gm oil at the same time respectively.

Table (5). Peroxide values of oil samples treated with ethyl acetate extracts as antioxidant.

Treatment	Peroxide value		
	1 hour	2 hour	3 hour
Control	2.330	2.370	2.440
Ethyl acetate extract of rocket seed	2.313	2.333	2.370
Ethyl acetate extract of celery seed	2.312	2.321	2.350
BHT	2.320	2.350	2.400

The method of activity test revealed that, the raise of peroxide value in treated oil samples with antioxidant do not increase than the half raise of peroxide value in control sample (without antioxidant) under the same condition, this proved that the natural antioxidants used in this research is very active as mentioned in Egyptian standard (1975).

These results indicate that using of ethyl acetate extract of celery seed is more effective than the other one of rocket compared with BHT as artificial antioxidant. The peroxide value of the original crude oil samples was 2.30 meq.O_2/1000 gm oil. The peroxide value of original sample must not be increase more than 3.0 as mentioned in Egyptian standard (1975).

On the other hand, these results were evaluated also by measuring acid value and iodine value. Table (6) revealed the acid and iodine values of the oil samples treated with ethyl acetate extracts of rocket and celery seeds compared with control.

Table (6). Acid and iodine values of oil samples treated with ethyl acetate extracts as antioxidant.

Treatment	Acid value			Iodine value		
	1 hour	2 hour	3 hour	1 hour	2 hour	3 hour
Control	0.850	0.880	0.884	119.20	115.16	109.10
Ethyl acetate extract of rocket seed	0.589	0.683	0.774	147.76	140.10	98.82
Ethyl acetate extract of celery seed	0.33	0.45	0.55	119.22	115.67	110.35
BHT	0.34	0.50	1.00	114.68	106.77	105.58

The acid value of control sample after 1,2, 3 hours under the same condition were 0.850, 0.880, 0.884 respectively, while the sample treated with ethyl acetate extracts of rocket gave acid values of 0.589, 0.683 and 0.774 respectively. Also, the acid values for the ethyl acetate extracts of celery were 0.33, 0.45 and 0.55 respectively. The acid and iodine values of the original crude oil sample were 0.46 and 110.09, respectively.

From the obtained results in Table (6) it was clear that acid value of the treated samples with the two extracts were lower than the control, but the ethyl acetate extracts of celery seed was more effective than the other one of rocket seed. Finally, it can be deduced that the ethyl acetate extracts of rocket and celery are considered as antioxidants which have high ability to protect the oils from oxidation, may be due to their contents of phenolic compounds which are able to neutralize free radical and prevent unsaturated fatty acid oxidation as mentioned by Adel-Moein et al., (2006).

Conclusion: The present study proved that the examined extracts had positive inhibition on the growth of pathogenic fungi. Also, these extracts can be used as natural antioxidants to improve the stability of oils. Thus utilization of these plant extracts achieved health improvement.

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دراسات فيتو كيميائية علي بذور الكرفس والجرجير

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من قديم الزمان يستخدم الكرفس والجرجير في الطب التقليدي وقد تعرضت بذور هذه النباتات لعمليات فصل وتعريف وصفي للمركبات الفعالة مثل القلويدات والفلافونيدات والتربينات والصابونينات والفينولات الكلية حيث تم استخلاص للبذور بواسطة مذيبات عضوية مثل الميثانول والذي يعتبر المذيب الأم ثم أجري له بعد ذلك الاستخلاص المتتابع بمذيبات تزيد في قطبيتها بدءاً بالأثير البترولي متبوعاً بالكلوروفورم ثم خلات الإيثيل.

وقد اختبرت تأثيرات المستخلصات الثلاثة المفصولة لبذور الكرفس لبيان تأثيرها المضاد لنشاط الميكروبات الدقيقة من سلالات مختلفة للفطريات الممرضة وقد استخدمت المستخلصات الناتجة من بذور النباتات أيضا كمضادات أكسدة طبيعية وقدرت فاعليتها كمضاد أكسدة طبيعي بعديد من الطرق حيث ثبت أن مستخلص خلات الإيثيل لبذور الكرفس كانت أكثر فعالية في المحافظة علي خواص الزيت وحمايته من الأكسدة مقارنة ببذور الجرجير ومضاد الأكسدة الصناعي (BHT).

وقد تم استخلاص وتعريف وتقدير المركبات الفينولية في بذور كل من الجرجير والكرفس بتكنيك التحليل الكروماتوجرافي عالي الأداء. وأظهرت النتائج أن كلا من النباتين يحتويان علي مركبات فينولية هامة مثل الفينول وحمض الجاليك والدادزين والجنستين والبارا هيدروكسي حمض البنزويك في بذور الجرجير بينما تحتوي بذور الكرفس علي أهم المركبات الفينولية الأتية: حمض الساليسليك والفينول والكورستين والكاتشين والروتين. وقد اختفي حمض الكافييك والكورستين والفاتليين من بذور الجرجير بينما اختفي حمض السينامك والأيوجانول وحمض البيروجاليك من بذور الكرفس. وقد كان لهذه الفينولات الموجودة في كل من النباتين تأثيرات هامة كمضادات أكسدة طبيعية.