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Research Article
Study of Safflower Varieties Cultivated Under Southern Egypt Conditions for Seeds and Flowers

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Abstract

Background and Objective: Safflower has some valuable characteristics that have made the species throughout the centuries as a multi-purpose oilseed. Safflower is an oil crop with high fat content with a good amount of polyunsaturated fatty acids. Safflower seed oil has gained interest as an excellent health care product and also it is a rich source of vitamin E. Safflower flower is used as source for dyes or for medicinal purposes. **Materials and Methods:** In this study, three safflower (*Carthamus tinctorius*) varieties (Giza1, Kharega 1 and Kharega 2) cultivated at semi-arid regions in Egypt were studied for its chemical composition, oil content, fatty acid composition, lipid composition and sterol content. Moreover, safflower seed and flower extracts obtained by solvent extraction were evaluated regarding the content of total phenolic compounds and the antioxidant activity. **Results:** The varieties contained oil contents between 29.4 and 31.0% with considerable amounts of sterols (2326.0-2715.5 mg kg^{-1}) and high contents of unsaturated fatty acid (85-88%). In the varieties appreciable amounts of total phenolic compounds (3.17-4.89 mg gallic acid equivalents/g) were found and they showed a good DPPH scavenging activity, with IC_{50} values ranging between 0.8-1.31 mg and a high inhibition of linoleic acid peroxidation (AAC) ranging between 64.6 and 72.6. Extracts of safflower flowers also showed remarkable higher contents of total phenolic compounds (6.44-13 mg g^{-1}) than the seeds, with IC_{50} values ranging between 1.68 and 3.21 mg and AAC from 106.94-201.24. **Conclusion:** Safflower presents comparable high amounts of total phenolic compounds showing a good antioxidant activity.

Key words: Safflower varieties, Safflower seeds, safflower flower, phenolic compounds, antioxidant activity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Safflower (*Carthamus tinctorius*) is one of the most important oilseed crops with a long-term history going back until 3500 B.C. when the crop has been used for dyeing mummy wrappings and other robes. After soybean, groundnut, rapeseed, sunflower, sesame, linseed and castor safflower ranks eighth regarding the total harvested yield[1]. According to the 2014 FAO statistics, safflower production in the world was realized on an area of1, 010, 180 ha with a total world production reaching about 867, 659 tons[2]. Safflower is also classified as a moderately salt-tolerant plant[3]. Safflower can be grown successfully on soil with poor fertility and in areas with relatively low temperatures[4]. Safflower has attracted significant interest as an alternative oil seed due to its high adaptability for dry climatic conditions with little precipitation. Safflower growth as well as composition and quality of its seeds are influenced by many factors like genotype, environment and agronomic practices.

Consumers are increasingly demanding healthier oils, naturally low in saturated fats and high in bio-active components such as tocopherols and phenolic compounds with an antioxidative activity. Oilseeds contain lipophilic antioxidants, fundamentally tocopherols, which are extracted during oilseed processing. Oilseed cakes and extracted meals after the removal of nonpolar antioxidants, still contain antioxidants of medium to high polarity, such as phenolic acids, their esters, flavonoids and their glycosides, or lignan derivatives. They have moderate antioxidant activities, therewith, they could be applied successfully in food applications[5].

Safflower has received a lot of importance as a source of vegetable oil. Today this crop supplies oil, meal and bird seeds for the food and industrial products market. The phenolic compounds in safflower seeds include flavones, conjugated serotonins and lignans that have a good effect for bone protection[6]. Thus safflower seeds have recently received much attention due to their content of phenolic compounds that posse a therapeutic effect against several pathological disease.

In addition to the seeds, the brilliant colored flowers, which were used to extract yellow and orange dyes for food and fabrics is well known for safflower since ancient times. However, recently interest in safflower flowers as a source of color for use in food is gaining importance owing to a recent ban on the use of synthetic colors in food in the European countries and elsewhere. Safflower was cultivated in Egypt, Morocco, China and India for the production of the dye carthamin in ancient times as early as 4500 BC[7]. The flowers are also reported to have medicinal properties to cure several chronic diseases. Due to their non-allergic and non-carcinogenic properties natural food dyes will continue to be widely acceptable in food [8]. The two main problems hindering safflower (*Carthamus tinctorius* L.) planting in Egypt are the limitation of genetic materials and the genetic background information. For many years, Giza1 was the only safflower cultivar grown in Egypt. This motivate the Egyptian oil production sector to evaluate new safflower genotypes under different Egyptian environments. In this present study, among 14 safflower varieties and genotypes (3 local namely: Giza1 Kharega 1 and Kharega 2 and 11 exotic) cultivated at Shandaweel Research Station in Sohag Governorate (Egypt) Giza1 in addition to (Kharega 1 and Kharega 2) were the best for most investigated parameters such as plant height, weight seed yield/plant, 100-seed weight and seed yield/ha area[9]. However there is a lack of information about those local safflower cultivars and its oil characteristics. Thus, the objective of this work was to obtain information about the safflower seed, safflower oil as well as safflower flowers form those local safflower genotypes under hot and dry climate of upper Egypt.

MATERIALS AND METHODS

Materials: Three different safflower varieties namely Giza1, Kharega 1 and Kharega 2 were obtained from the National Centre for Agriculture Research, Cairo, Egypt. Safflower genotypes were conducted at the Experimental Farm of the Shandaweel Research Station at Sohag Governorate (at Upper Egypt) in 2012/2013. The trial was laid out in randomized complete block design with 3 replications, accommodating 5 rows, 60 cm apart, 4 m length with 15 cm plant to plant distance and seeding rate of 12 kg fad. Nitrogen fertilizer was applied at a rate of 60 kg in the form of urea (46.5%) as top dressing or broad casting in equal doses, 21 and 45 days after sowing, respectively. Phosphorus fertilizer was applied at a level of 150 kg as superphosphate (15.5%). Whole of phosphorus was applied basally before sowing in all treatments. Genotype seeds were sown by hand on 4th, November.

Methods: Chemical composition of seed samples. Content of moisture, crude protein, crude fat and ash were determined according to the AOCS methods[10]. Carbohydrate content was estimated by difference to the other ingredients.

Safflower seed oil extraction: At the beginning of the study, safflower seeds were cleaned and dried. The oil extraction was done according to the International Standard ISO 659:1998 - Oilseeds-Determination of hexane extract (or light petroleum extract), called "oil content", ISO[11].

In brief, 3.0 g of the dried seeds were ground in a ball mill and extracted with 70 mL petroleum ether in a Twisselmann apparatus for 6 h. The solvent was removed with a rotary evaporator at 40°C. The obtained oil was dried by a stream of nitrogen and stored at -18°C until use.

Fatty acid composition: The fatty acid composition of the oil samples obtained above was analyzed by gas chromatography (GLC) of the fatty acid methyl esters (FAME) following the standard method DGF-C-VI0[12], in combination with DGF-C-VI 11d. Briefly, 12 mg of safflower seed oil was dissolved in 1 mL of n-heptane. Then 50 µg of sodium methylate was added and the closed tube was agitated vigorously for 1 min at room temperature. After addition of 100 µL of water, the tube was centrifuged at 4500 rpm for 10 min and the lower aqueous phase was removed. For neutralization of the aqueous phase 50 µL of HCl (1 mol with methyl orange) was added, the solution was shortly mixed and the lower aqueous phase was rejected. The organic phase was dried with about 20 mg of sodium hydrogen sulphate (monohydrate, extra pure, Merck, Darmstadt, Germany) and after centrifugation at 4500 rpm for 10 min, the top n-heptane phase was transferred to a vial and injected into a gas chromatograph (Agilent 5890, Waldbronn, Germany) with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 µm, Varian Deutschland, Darmstadt, Germany). The temperature program was as follows: From 155-220°C with 1.5°C min^{-1}, after reaching 220°C/10 min isotherm. Other settings were: Injector: 250°C, detector: 250°C, carrier gas: 36 cm/sec hydrogen, split ratio: 1:50, detector gas: 30 mL min^{-1} hydrogen, 300 mL min^{-1} air and 30 mL min^{-1} nitrogen, automated injection volume 1 µL. The retention time in comparison to fatty acids from edible oils with known composition used to identify fatty acids in the samples. The peak areas were computed by Chemstation as integration software and percentages of FAME were expressed as area percent by direct internal normalization.

Sterol composition of the safflower seed oil: Sterol composition of the oil samples was obtained by the ISO 12228 method[11]. In brief, about 250 mg of oil was saponified with a solution of ethanolic potassium hydroxide by boiling under reflux. The unsaponifiable matter was isolated by solid-phase extraction on an aluminium oxide column (Merck, Darmstadt, Germany) on which fatty acid anions were retained and sterols passed through. The sterol fraction was separated from unsaponifiable matter by thin-layer chromatograph (Merck, Darmstadt, Germany), re-extracted from the TLC material and afterwards, the composition of the sterol fraction was determined by GLC using betulin as internal standard. The compounds were separated on a SE 54 CB (Macherey-Nagel, Düren, Germany, 50 m long, 0.32 mm ID, 0.25 µm film thickness). Further parameters were as follows: Hydrogen as carrier gas, split ratio 1:20, injection and detection temperature adjusted to 320°C, temperature program, 245-260°C at 5°C min^{-1}. Peaks were identified either by standard compounds (β-sitosterol, campesterol, stigmasterol) by a mixture of sterols isolated from rape seed oil (brassicasterol) or by a mixture of sterols isolated from sunflower oil (Δ7-avenasterol, Δ7-stigmasterol and Δ7-campesterol). All other sterols were identified by GC-MS for the first time and afterward by comparison of the retention time[7].

Lipid composition of safflower seed oil: The lipid composition of safflower seed oil has been determined according to method DGF-C-VI5b(02)[12]. Briefly, about 100 mg of sample material were weighed within ± 0.1 mg into a 10 mL volumetric flask and filled up to the mark with tetrahydrofuran (THF). Then the flask was placed into a ultrasonic bath for about 5-10 min to ensure the complete dissolving of the sample. Finally 20 µL of the solution was injected directly onto 2×Plgel columns, 5J1m, 100 A, 300×7.5 mm tempered to 40±0.1°C by an oven. As mobile phase tetrahydrofuran stabilized with 250 mg kg^{-1} BHT was used with a flow rate of 1.0 mL min^{-1}. The analytes were detected by an RI detector.

Preparation of safflower seeds extracts

Ultrasonic extraction method (USE): For the extraction of phenolic compounds from plant materials by ultrasonic extraction 2 g of dried ground material were treated by ultrasonic two times with 50 mL methanol/water (60:40, v/v) at 50°C for 15 min according to Taga *et al.*[13]. After centrifugation the solvent phases were combined and then the solvent was evaporated to dryness under vacuum at 40°C using a rotary evaporator. The dry residue was weighed.

Accelerated solvent extraction method (ASE): Accelerated solvent extraction of safflower seeds was done by using an

automated extraction system (Model ASE 200, Dionex, Germany). The system was set for the control of extraction parameters, such as temperature, pressure, extraction time and volume of solvent. Two grams of the ground seeds were heated-up for 6 min, the samples were extracted for 10 min at a pressure of 6.67 Mpa and 40°C with methanol/water (60:40, v/v) at four successive cycles. After finishing the extraction, the extract was combined with 15 mL petroleum ether in a separation funnel to remove lipid compounds. The aqueous phase was given into round flask and the solvent was evaporated under vacuum at 40°C using a rotary evaporator. The dry residue was weighed.

Shaking extraction procedure: Two grams of safflower seed were used to extract the phenolic compounds by a simple shaking process. Ground seeds were extracted using methanol/water (60:40, v/v) by shaking for 24 h at room temperature. The solution was given into a round flask after centrifugation and the residue was extracted using methanol/water (60:40, v/v) by shaking for additional 1 h twice. Then the extracts were combined and the volume was reduced until about 10 mL under vacuum at 40°C. The solution was transferred into a separation funnel and the round flask was washed firstly with 15 mL methanol/water (60:40, v/v) and secondly with 15 mL petroleum ether to remove residual lipids. Then the solvent was evaporated to dryness under vacuum at 40°C using a rotary evaporator. The dry residue was weighed.

Preparation of safflower flower extracts: Sample extracts were obtained by stirring 0.2 g of dry safflower flower powder with 6 mL of methanol/water (60:40, v/v) for 30 min at 120 rpm using a magnetic stirrer plate. Extract obtained was kept for 24 h at 4°C, filtered through a Whatman No. 4 filter paper and freed of solvent under reduced pressure at 40°C, using a rotary evaporator. The dried crude concentrated extracts were stored at -20°C until used for analyses. The additional two extraction procedures were done for safflower flower extraction (ASE and ultrasonic extraction) with 0.2 g of material each as mentioned above for safflower seeds.

Determination of total phenolic compounds (TPC) and antioxidant activity with the 1,1-diphenyl-2-picrylhydrazyl radical scavenging method and with β-carotene bleaching method: Total phenolic compounds of the crude extracts were determined by the Folin-Ciocalteu method described by Taga *et al.*[13]. Absorbance was measured at 750 nm on a spectrophotometer. The amount of the phenolic compounds was determined by calculation with a standard calibration curve prepared of gallic acid in concentrations of 0.05-0.4 mg mL^{-1} and specified as mg TPC/g extract given as gallic acid equivalents (GAE).

The antioxidant capacities of the crude extracts were evaluated in the same extracts Hatano *et al.*[14] and expressed by IC_{50}. The IC_{50} is defined as concentration of an antioxidant which was required to quench 50% of the initial DPPH radicals under the experimental conditions given.

The determination of the antioxidant activity using the β-carotene/linoleic acid system was carried out according to the method described by Lu and Foo[15]. The decrease of absorbance of β-carotene resulting from the co-oxidation by linoleic acid at 55°C was measured spectrophotometrically. The antioxidant activity coefficient (AAC) was calculated by a modified version of the formula:

$$AAC = 1000 \times \frac{AA_{60}\text{-}AB_{60}}{AB_0\text{-}AB_{60}}$$

in which, AA_{60}, AB_0 and AB_{60} are the absorbencies of the test and blank samples at 0 and 60 min, respectively[16].

Statistical analysis: Data analysis was performed using the SAS software (Version 9.1, SAS Institute). Mean separation of data was carried out using least significant difference LSD test at 5% probability levels[17].

RESULTS

Proximate composition of safflower seeds: The proximate compositions of the analyzed safflower seeds are presented in Table 1. Moisture content was low for all safflower varieties with 3.9, 3.3 and 3.6% for Kharega 1, Kharega 2 and Giza1, respectively. Crude protein was high in Kharega 2 (15.24%) followed by Kharega 1 (13.67%) and Giza1 (13.24%). Crude fat ranged from 29.4% in Kharega 2 to 31.0% in Giza1. No significant changes ($p<0.005$) of the gross chemical composition among the studied samples for moisture, oil ,ash and carbohydrates whereas Kharega 2 variety varied significantly ($p<0.005$) for protein content comparing with Kharega 1 and Giza1.

The result of proximate analysis of two safflower varieties studied by Ingale and Shrivastava[18] showed total lipid and crude protein content close to the present results. Ash content of the studied varieties ranged between 1.87 and 2.72%. Total carbohydrates, calculated by the difference of the other

Table 1: Proximate composition of some safflower seed varieties

Samples	Moisture (%)	Oil (%)	Ash (%)	Protein (%)	Carbohydrate (%)
Kharga1	3.9±0.08[a]	30.1±0.54[a]	2.20±0.05[a]	13.67±0.09[b]	50.14±0.34[a]
Kharga2	3.3±0.06[a]	29.4±0.47[a]	2.72±0.10[a]	15.24±0.19[a]	49.33±0.48[a]
Giza1	3.6±0.03[a]	31.0±1.43[a]	1.87±0.05[a]	13.24±0.10[b]	50.29±0.42[a]

*Mean values of chemical composition within column that have the same letter are not significantly different p<0.05

Table 2: Fatty acid profile, total lipids composition and sterols fractions of safflower seeds oil

		Samples		
Fatty acid	Carbon chain	Kharega 1 (%)	Kharega 2 (%)	Giza1 (%)
Palmitic	16:0	7.47	6.21	6.73
Stearic	18:0	1.17	2.53	2.85
Oleic	18:1 D9	13.35	10.90	12.34
Vaccenic acid	18:1 D11	0.74	0.62	0.65
Linoleic	18:2	75.06	75.33	76.42
Nervonic acid	24:1	0.00	2.65	0.64
Unsaturated/saturated		10.31	10.24	9.39

	Samples		
Sterols compounds	Kharega 1 (mg kg^{-1})	Kharega 2 (mg kg^{-1})	Giza 1 (mg kg^{-1})
Cholesterol	6.2	8.1	5.8
Cholestanol	367.7	319.0	352.9
Campesterol	321.1	274.0	250.0
Campestanol	57.0	53.1	49.1
Stigmasterol	226.0	193.6	212.0
7-Campesterol	139.3	129.7	120.8
Sitosterol	1152.3	1144.1	929.7
Sitostanol	115.9	101.5	104.6
5-Avenasterol	42.8	46.3	55.56
5,24-Stigmastadienol	41.2	41.8	35.4
7-Stigmastenol	501.0	559.3	428.5
7-Avenasterol	81.1	87.6	108.1
Total sterols (mg kg^{-1})	3051.60	2958.10	2652.46

	Samples		
Lipid compositions	Kharega 1 (%)	Kharega 2 (%)	Giza 1 (%)
Oligomer TG	0.08	0.26	0.17
Dimer TG	0.15	0.33	0.14
Monomer TG	98.15	97.46	98.39
Diglyceride	1.02	1.15	0.80
Monoglyceride	0.00	0.01	0.04
FFA	0.59	0.80	0.48

ingredients to 100% ranged between 49.33 and 50.30%. These results are in good agreement with those reported by Mariod *et al.*[19]

Fatty acid profile, total lipids composition and sterol fractions of safflower seeds oil

Fatty acid composition: The fatty acids composition in percentage of total fatty acid methyl ester (FAMEs) of safflower oils obtained by using gas chromatography (GC) is shown in Table 2. As predominant saturated, monounsaturated and polyunsaturated fatty acids, respectively, were found palmitic acid (C16:0, 6.2-7.5%), oleic acid (C18:1, 10.9-13.4%) and linoleic acid (C18:2, 75.6-76.4%), respectively. In the analyzed safflower oils PUFAs presented the predominant part of the fatty acid compositions which is in agreement with Orsavova *et al.*[20]. They analyzed seven vegetable oils with the highest amount of PUFAs found in safflower oil reaching a content up to 79.1%.

Lipid fraction: The lipid composition of safflower seed oil is shown in Table 2. The results revealed that, triacylglycerols (TAG) represent more than 97% of total lipid fraction of all varieties. The results showed higher amounts of TAG than those reported by Al Surmi *et al.*[21], who revealed that triacylglycerols constituted from 81.70-85.34% of the total lipids fraction of safflower oil while[22], found that safflower seed oil contain 90.5% of TAG.

Table 3: Content of total extractable and total content of phenolic compounds obtained from different extraction techniques

	Shaking method		Ultrasonic method		ASE method	
Samples	Total extract (mg g^{-1} sample)	Total phenolic compounds (mg GAE/g sample)	Total extract (mg g^{-1} sample)	Total phenolic compounds (mg GAE/g sample)	Total extract (mg g^{-1} sample)	Total phenolic compounds (mg GAE/g sample)
Kharega 1	73.1	3.9	75.9	3.6	608.0	4.4
Kharega 2	56.4	3.2	71.1	4.8	53.9	4.0
Giza1	54.9	3.4	66.5	4.9	56.3	4.2

Table 4: Antioxidant activity for safflower varieties under different extraction techniques

	Shaking method		Ultrasonic method		ASE method	
Samples	DPPH IC_{50}	AAC (β-carotene)	DPPH IC_{50}	AAC (β-carotene)	DPPH IC_{50}	AAC (β-carotene)
Kharega 1	0.88	120.82	0.91	212.79	1.31	64.60
Kharega 2	0.84	145.92	0.86	208.02	1.29	39.98
Giza1	0.84	177.56	0.80	273.11	1.21	72.66

Sterol composition: The sterol composition of safflower seed oils is shown in Table 2. The concentrations of campesterol, campesterol, stigmasterol, Δ7-campesterol, β-sitosterol, sitostanol, Δ5-avenasterol, Δ7-stigmastenol and Δ7-avenasterol the total amount of sterols was found in the range from 2326.0-2715.5 mg kg^{-1} which is in agreement with the Codex Standard for Named Vegetable Oils giving values from 2100-4600 mg kg^{-1}. The phytosterol content of solvent extracted safflower oil was reported by Nogala-Kalucka *et al.*[23] as 290, 190, 1,450, 640, 710 and 190 mg kg^{-1} fat for campesterol, stigmasterol, sitosterol, avenasterol, Δ7-stigmasterol and campestanol, respectively.

Phenolic compounds content of safflower seeds as affected by extraction method: In this study safflower seeds were extracted by three different extraction methods (shaking extraction, ultrasonic extraction and accelerated solvent extraction) using a mixture of methanol/water (60:40, v/v). The results for the content of total extractable and total of phenolic compounds of safflower seed are shown in Table 3. The total content of phenolic compounds ranged between 3.17 mg g^{-1} (Kharega 2, shaking extraction) and 4.89 mg g^{-1} (Giza1, ultrasonic extraction). These results are in agreement with Sreeramulu and Raghunath[24], who found the content of phenolic compounds in Indian safflower seeds with 5.99 mg g^{-1} determined as gallic acid equivalent. Indeed among the examined varieties Kharega 1 had the highest amount of total extract while Giza1 had the highest amount of total phenolic compounds by the same extraction method (ultrasonic extraction method). For all seed materials high amounts of total extractable compounds and total phenolic compounds were found by ultrasonic extraction compared to the other extraction methods although extraction by ASE was a little more efficient for the extraction of the phenolic compounds in the case of Kharega 1. These results are in agreement with Taha *et al.*[25], who found no correlation between the amount of total extractable and total phenolic compounds for some Sudanese samples extracted by methanol/water (60:40 v/v) by shaking and accelerated solvent extraction method.

Antioxidant activity of extracts from safflower seeds as affected by different extraction methods: Antioxidant activity for safflower seed extracts obtained by shaking, ultrasonic and accelerated solvent extraction methods using methanol/water (60:40, v/v) was determined by DPPH radical scavenging activity and β-carotene bleaching methods.

DPPH assay: The results in Table 4 show that the extracts of all tested safflower materials possessed free radical scavenging properties measured by the DPPH method but to varying degrees. The calculated IC_{50} values ranged from 0.80 (ultrasonic) to 1.31 (ASE). Using the shaking extraction technique, all samples showed better DPPH scavenging activity than other extraction procedures. No difference among varieties was observed for their DPPH scavenging activity for the same extraction procedure, at the same time the lowest antioxidant activity was recorded by ASE extraction procedure for all examined plants. The results are in agreement with that of earlier findings of Sultana *et al.*[26], who showed that medicinal plant material extracts, prepared using shaking exhibited better DPPH scavenging activity than the corresponding extracts, obtained by reflux technique.

β-Carotene-linolenic acid method: The measurement of the inhibition of linoleic acid peroxidation with β-carotene

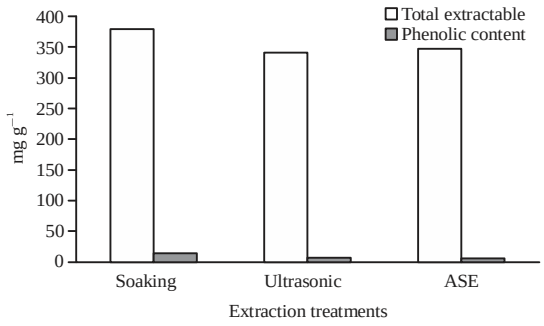

Fig. 1: Effect of extraction procedures on total extractable and total phenolic compounds of safflower flower

co-oxidation was also used to measure antioxidant activity of safflower seed extracts. The AAC of safflower extracts are shown in Table 4. Among the three different varieties, maximum inhibition was noted for Giza1 followed by Kharega 1 and Kharega 2 extracted by ultrasonic. The present data revealed that, the extracts of all plant materials, prepared using the ultrasonic extraction, exhibited higher levels of inhibition of linoleic acid oxidation than those obtained by shaking or ASE extraction. All plant material extracts were affected by the extraction technique however each material tested retained the same efficacy order as displayed in the case of ultrasonic extraction. This order was very similar to the order found as result for the DPPH assay. Therefore, the effective processing step to liberate antioxidant compounds from different plant species may not be similar[27]. The results are in agreement with Taha *et al.*[25], who found a good correlation between the amount of total phenolic extract and the AAC values for some Sudanese samples.

Phenolic compounds of safflower flowers: Three kinds of extraction procedures (soaking, ultrasonic and ASE) with a mixture of methanol and water (60%)were used to extract the antioxidant active compounds from safflower flowers (Fig. 1). The results showed that the content of phenolic compounds of safflower flowers ranged between 6.4 and13 mg GAE/g. This result was in accordance with literature data which reported amounts of polyphenolic compounds of saffron of526 mg/100 g on dry weight[28].

Antioxidant activity of safflower flower: All extracts of safflower flower showed higher scavenging ability on DPPH radicals for soaking (IC_{50} = 1.68) than for ASE (IC_{50} = 2.88) and for ultrasonic (IC_{50} = 3.21) extracts (Table 5). Concerning β-carotene bleaching assay (Table 5), flowers of safflower presented a good ability to prevent the bleaching of β-carotene with AAC-values ranging from 106.94-201.24 for ultrasonic and soaking extracts, respectively. It is noticeable in Table 5 that the ASE extract of safflower flower shows pro-oxidant activity for the β-carotene bleaching test.

Table 5: Antioxidant activity of safflower flower

Extraction procedure	Antioxidant activity	
	DPPH IC_{50}	AAC
Soaking	1.68	201.24
Ultrasonic	3.21	106.94
ASE	2.88	ND

NDL: Not detected

DISCUSSION

The chemical composition of safflower seeds showed low moisture content which indicates that the raw material can be stored for a long time also the composition indicates that all varieties are good sources for edible oil compared with other oilseeds, such as sunflower seed. The contents found in the present study for ash and carbohydrate showed the suitability of seed as human or animal feed.

Fatty acid composition of vegetable oils is formed by a mixture of saturated (SFAs) and unsaturated (UNFAs) fatty acids classified into monounsaturated (MUFAs) or polyunsaturated fatty acids (PUFAs) depending on the number of unsaturated double bonds. In the present study the amount of free fatty acid indicates not much hydrolysis of triacylglycerols by agricultural treatment or climate conditions during growing of seeds. Moreover, oligomeric and dimeric TAG recorded a small amount of the total lipid composition, showing only a very small oxidative degradation of the oils.

The type and amount of phytosterols vary with the source of oil, the total sterols (sum of esterified and non-esterified sterols) generally account for 0.2-1.0% of total lipids for most vegetable oils[29]. The structure of plant sterols such as (sitosterol and stigmasterol) is assumed as reinforcing the membrane adhesion and that structure is adapted to large temperature variations in plants[30].

Although the investigated varieties were grown under hot and dry climate of upper Egypt the resulted safflower seed oils had only moderate amounts of sterols.

Phenolic compounds are important antioxidants of commonly consumed foods, seed oils contain considerable amounts of phenolic compounds that have a great effect on the stability of oil[30]. Safflower seeds are known to contain several phenolic compounds, such as serotonin derivatives, lignans and flavones[6]. Extraction of plant materials by a mixture of water and other organic solvents such as methanol, ethanol, acetonitrile, acetone, hexane and diethyl

ether is a commonly used method because of a higher extraction yield for phenolic compounds[31]. Extraction by shaking method seems to be a little more selective for the extraction of phenolic compounds from safflower seeds. The extraction of phytochemical components from plant material depend on the type of plant part (leaf, fruit, seed, bark), the presence of a waxy layer as well as on the selected compounds such as carotenoids, non polar molecules, simple phenolic compounds, polar compounds or complex phenolic compounds. The results indicated that a high amount of extractable compounds did not necessarily corresponds to a high amount of phenolic compounds.

Lower IC_{50} values and higher AAC values indicated higher antioxidant activity. The Folin-Ciocalteu method is the one adopted method for screening of natural antioxidants, being considered the best method for the determination of total phenolic content[32]. Free radical scavenging activity of plant extracts is mainly due to phenolic compounds[33]. Recovery of antioxidant compounds from plant materials is typically accomplished through different extraction techniques taking into account their chemistry and uneven distribution in the plant matrix[34]. The reduction in the radical scavenging activity of the extracts, obtained by the reflux technique might be ascribed to the thermal decomposition of phenolic compounds during the extraction process. On the other hand the highest amount of total phenolic compounds was recorded by ASE extraction technique. However, it is important to point out that optimal extraction yield may not corresponds to higher antioxidant activity. The aqueous based solvents may just solubilized a larger range of compounds, some of which may have little or no antioxidant activity. Amongst other contributing factors, efficiency of the extracting solvent to dissolve endogenous compounds might also be very important[35].

The measurement of the inhibition of linoleic acid peroxidation with β-carotene co-oxidation was also used to measure antioxidant activity of safflower seed extracts. Linoleic acid (C18:2) is a polyunsaturated fatty acid with two double bonds, which easily can form hydroperoxides by oxidation. By changing the absorption maximum of β-carotene during oxidation the prevention can be determined spectrophotometrically at 470 nm. A high antioxidant activity coefficient (AAC) indicates a greater inhibition of linoleic acid oxidation for extracts of safflower plant materials as affected by extracting procedure. The results allow to claim that soaking is a particularly advantageous technique for the extraction of compounds from safflower flowers due to the higher amount of total extractable compounds in comparison to the extraction by ultrasonic and ASE. That was also the same for total phenolic compounds showing that soaking procedure was the best technique for reaching a higher phenolic content.

Flower of safflower was good source for phenolic compounds with good antioxidant activity. The cause of the high antioxidant activity of safflower flower can possibly be due to including serotonins, flavonoids and lignans, compounds which are characteristic to possess antioxidant effects[36]. Also Hiramatsu *et al.*[37] found that water extract of safflower flower has free radical scavenging activity against DPPH radicals and the component responsible for this activity was especially carthamin. *Crocus sativus* was rich in phenolic constituents and demonstrated good antioxidant activity using DPPH assay[38]. Antioxidant activity of extracts was found to depend on the plant species, the plant part and the extraction technique[39]. In fact, the synergistic interactions between the antioxidants in the mixture of phenolic compounds make the antioxidant activity dependent not only on the concentration but also on the nature and the interactions between the antioxidants[40]. Therefore, content of phenolic compounds could not be the major criterion for assessing the antioxidant activity.

Comparison of antioxidant activity for both seed and flower: It is clear from the results that both the phenolic content and the antioxidant activity varied according to the different part of the studied plant. The complex relation between chemical components and phenolic compounds in plants has been previously studied by Maisuthisakul *et al.*[41]. They indicated that antioxidant activity correlated well with phenolic and flavonoid contents. In addition, fat and energy are useful parameters to differentiate seeds from other plant parts. The negative correlation between the ash and the antioxidant properties can be explained. The ash contains minerals and heavy metals (including iron) which can act as pro-oxidants. It is also noted that the DPPH radical scavenging activity is inversely associated with the dietary fiber. The major antioxidant mechanism of dietary fiber is as a metal chelating agent. Another mechanism is free radical scavenging due to some polyphenols which are associated with dietary fiber[42]. In addition, during photosynthesis there are many processes to translocate carbohydrates from leaves to various sink organs.

CONCLUSION

- Safflower seeds are characterized by a relatively high content of oil (about 30%)
- Safflower is a valuable source for the production of edible oil with a remarkable high content of polyunsaturated linoleic acid
- The seeds also contain noteworthy amounts of protein (14%) and carbohydrates (50%) making the residue of the oil extraction a valuable source for animal feeding
- Additionally to the fatty acids safflower oil also contains higher amounts of phytosterols which have some health promoting effects
- Comparable to the very low variation of the content of phenolic compounds obtained by different extraction methods also the antioxidant activity determined by DPPH assay were very close together with the highest activity for the shaking method
- The highest AAC measured by the β-carotene-linoleic acid assay was found for the ultrasonic extraction and again the lowest for the ASE
- Not only safflower seeds contain remarkable amounts of antioxidative compounds but also the flowers
- Although the antioxidant activity is not as strong as for the seed extracts from the flowers show a radical scavenging activity within the DPPH assay and also some effect on protecting linolenic acid against oxidation

SIGNIFICANCE STATEMENT

This study discovers the antioxidant capacity for both safflower seeds and flowers that related to their content of the phenolic compounds that can be developed as functional additives in food applications . This study will help the researchers to uncover the critical area of natural food additives that possess health benefits that many researchers were not able to explore. Thus a new theory on these natural extracts for food and medicine applications may be arrived at.

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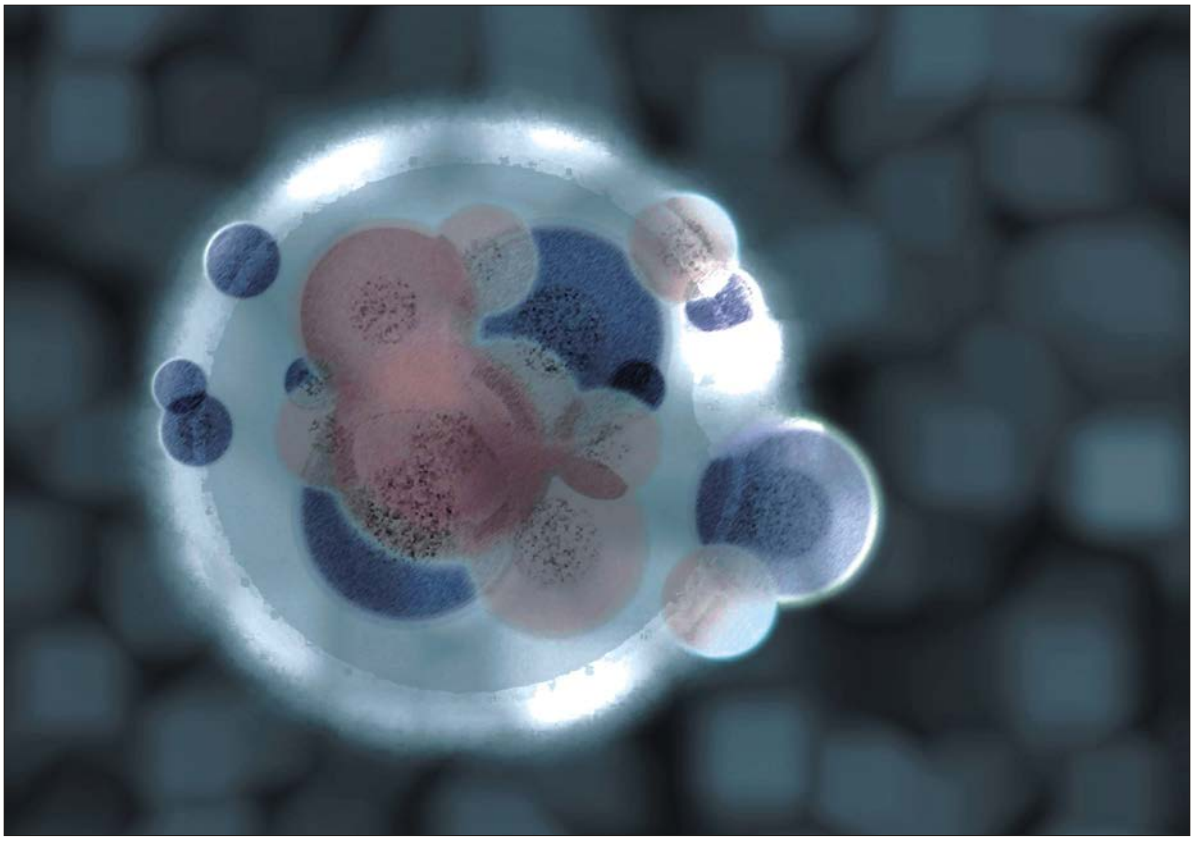

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