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Phenol Content and Antioxidant and Antiaging Activity of Safflower Seed Oil (*Carthamus Tinctorius* L.)

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Abstract: The phenol content of vegetable oil and its antioxidant activity are of primary interest for human health. Oilseed species are considered important sources of these compounds with medicinal effects on a large scale. Total phenol content (TPC) and antioxidant activity (AA) of safflower oil were previously studied. Nevertheless, there is no report on genotypic differences and antiaging activity of safflower oil. The aim of this study was to determine the TPC, diphenyl-picrylhydrazyl (DPPH), and antiaging activity on three respective accessions from Syria, France, and Algeria of seed oil of safflower grown under semi-arid conditions during 3 consecutive years (2015, 2016, and 2017). The results showed that phenol content as well as antioxidant and antiaging activity varied according to both genotype and years. In 2017, the mean value of TPC in oil seed was two times higher than in 2015 and 2016. Moreover, accessions presented different TPC values depending on the year. The highest antioxidant activity was observed among accessions in 2017 compared to 2015 and 2016. As expected, a positive correlation was found between TPC and antioxidant activity. The inhibition in the collagenase assay was between 47% and 72.1% compared to the positive control (83.1%), while inhibition in the elastase assay of TPC ranged from 32.2% to 70.3%, with the positive control being 75.8%. These results highlight the interest of safflower oil as a source of phenols with valuable antioxidant and antiaging activity, and uses for cosmetics.

Keywords: Safflower (*Carthamus tinctorius* L.); oil; phenol; antioxidant activity; anti-collagenase activity; anti-elastase activity; methanolic extract; genotypic variability; year effect

1. Introduction

Safflower, *Carthamus tinctorius* L., an Asteraceae, is cultivated in semi-arid regions mainly for its seed that contains a high level of oil. Safflower oil contains saturated and unsaturated fatty acids coupled by its tocopherol content [1]. Other compounds are also present in safflower oil. Among them, phenolic compounds are in the unsaponifiable phase of oil and are responsible for its stability and important nutritional value. The phenolic compounds have great biological activity mostly due to their antioxidant activity. Moreover, the primary role of antioxidants is to prevent or delay oxidative lipid damage produced in proteins and nucleic acids by reactive oxygen species, including reactive free radicals [2]. In recent years, the food industry has spent time and resources on finding natural antioxidants to replace synthetic compounds in applications and obtain a profit in the growing trend

in consumer preferences for natural antioxidants. Koyama et al. [3] confirmed the effects of safflower seed extract and its phenolic constituents on atherosclerosis. Several studies have been carried out to evaluate the phenolic compounds and antioxidant activity of Asteraceae seeds including safflower [4,5], sunflower [6], and artichoke [7]. Moreover, recent research has reported that the root of *Carthamus caeruleus* L. growing wild in Mediterranean regions and especially in Algeria has a potent antioxidant activity [8]. Furthermore, collagen, a major component of skin, plays an important role in its firmness, and elastin fibres lend elasticity and ensure tissue adhesion. Many enzymes are activated when the skin is exposed to UV radiation, indirectly leading to the production of reactive oxygen species (ROS), which generate oxidative stress [9]. The degradation of collagen is caused by enzyme collagenase [10]. Another type of skin degradation is caused by proteolytic enzymes present in the dermis such as elastase. It has been suggested that the degradation of elastin by elastase rises with age and/or repeated UV radiation [11]. Recently, phenolic compounds have been found to inhibit the activity of proteinases, which induce the degradation of skin proteins, such as collagen and elastin. Total phenol content (TPC) and antioxidant activity (AA) of safflower oil were already reported. However, there has been no study on the antiaging activity of oil from safflower.

The aims of the present study were thus to evaluate the total phenol content (TPC) and diphenyl-picrylhydrazyl (DPPH) radical scavenging activity (%) of safflower seed oil (*Carthamus tinctorius* L.) cultivated under semi-arid conditions and to investigate the anti-enzymatic activity of the methanolic extract of safflower oil against callogenase and elastase activity.

2. Materials and Methods

Three accessions of *Carthamus tinctorius* L., namely Alep (Syrie), Gila (France), and Toughourt (Algeria), were used in this study. The morphological characteristics of these accessions are presented in Table 1. Field experiments were conducted at the experimental station of Ibn Khaldoun University of Tiaret (Algeria) (35°20′01″ North, 1°18′48″ East) during 3 successive years: 2015, 2016, and 2017. A complete randomized block design was used with three replicates.

Table 1. Morphological characteristics of the three studied accessions.

Accession	Country	Flower Color	Absence/Presence of Thorns	Precocity
Toughourt	Algeria	y,r	-	Late
Gila	France	w,y,r	+	Early
Alep	Syria	y,r	+	Early

y: Yellow; w: White; r: Red; (+) present; (-) absent.

Table 2 presents rainfall and temperatures during the three plant cycles. The effects of rainfall and temperature on the TPC and antioxidant and antiaging activity of safflower oil were studied across contrasted growing seasons. In 2017, the climatic conditions were characterized by low rainfall amounts and high temperatures (Table 2). Whatever the year, the flowering stage for all genotypes was in July, while maturity took place in August. During maturation, plants were subjected to higher temperatures in 2017 than in the other years (Table 2). Therefore, 2017 could be considered as the hottest year; 2016, in contrast, was the more favorable year, and 2015 was considered intermediate.

Table 2. Rainfall (mm) and temperature (°C, mean, maximum, and minimum values) during the growing season of three safflower accessions during 3 successive years.

Month	Temperature (°C)									Rainfall (mm)		
	2015			2016			2017			2015	2016	2017
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min			
April	15.3	23.5	7.1	12.8	19.9	5.6	13.0	20.1	6.0	0.0	24.6	6.8
May	19.2	28.3	10.1	16.4	24.3	8.6	19.8	29.0	10.6	12.7	26.7	26
June	21.1	29.3	12.9	21.7	30.4	12.9	25.2	34.0	16.5	7.4	6.5	0.4

Month	Temperature (°C)									Rainfall (mm)		
	2015			2016			2017			2015	2016	2017
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min			
July	27.0	36.2	17.8	26.7	35.7	17.7	26.8	35.1	18.5	0.0	0.2	1.0
August	27.0	34.6	19.3	25.6	34.7	16.4	27.7	36	19.5	12.0	0	4.8
Mean	**21.9**	**30.4**	**13.4**	**20.6**	**29.0**	**12.2**	**22.5**	**30.4**	**16.3**			
Total										**32.1**	**58.0**	**39.0**

2.1. Oil Extraction

Safflower oil was extracted with the Soxhlet apparatus (NF EN ISO 659) from seeds harvested from the matured heads (browning of leaves and bracts heads), healthy and without impurities. This method consists of oil extraction with an organic solvent (cyclohexane) on 20 g of solid matrix (seed crushed) for 6 h with a ratio of 1:10 w:v. The solvent containing oil was removed using a rotary evaporator at a temperature of 45 °C. The extracted oil was recovered in suitable vials and stored in the dark in a cold room at 4 °C until analysis.

2.2. Polyphenol Extraction

The total phenolic compounds were extracted according to the method described by Ollivier et al. [12] with few modifications. A 0.5-mL aliquot of a methanol/water solution (80/20 *v*/*v*) was added to 0.5 g of safflower oil in a centrifuge tube. After 10 min of vigorous mixing, the tubes were centrifuged for 15 min at 500 g, and the methanolic phase was recovered. Generally, this operation was repeated two times (three times in total) to ensure a good extraction of TPC, and the volume was brought to 1.5 mL using the methanol/water solution (80/20 *v*/*v*).

2.3. Total Phenol Content

The total phenol content was determined according to the method described by Merouane et al. [13], using Folin-Ciocalteu reagent and gallic acid as the standard. In brief, 500 µL of Folin-Ciocalteu reagent and 450 µL of distilled water were added to a tube containing 50 µL of extract with vigorous stirring. After 3 min, 400 µL of Na_2CO_3 (75 g·L^{-1}) were added. The tubes were incubated at 25 °C in the dark for 40 min.

The absorbance was determined at 725 nm against a blank that contained methanol instead of the extract. The phenol content of the extract was determined from the gallic acid calibration curve, and the results were expressed in mg of gallic acid equivalent per kg of safflower oil (mg GAE/kg of oil).

2.4. Antioxidant Activity Determination

The antioxidant activity was determined according to the method recommended by Nogala-Kalucka et al. [14]. The method involves the spectrophotometric measurement of the intensity of the color change in solution depending on the amount of DPPH. The reaction was initiated by mixing 1 mL of the methanolic extract with 3 mL methanol, and then adding 1 mL of DPPH (0.012 g/100 mL). Absorbance at a λmax of 517 nm was checked after 15 min. The activity of the extract in scavenging DPPH was calculated as follows:

% DPPH scavenging = [(Absorbance of control − Absorbance of sample)/Absorbance of control] * 100

2.5. Determination of Collagenase and Elastase Inhibition

Collagenase from *Clostridium histolyticum* (Sigma Aldrich, Lyon, France) was used. The activity of collagenase was assessed using N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (Sigma Aldrich, Lyon, France) as a substrate following the protocol of Wittenauer et al. [15]. Absorbance decrease was surveyed at 335 nm during 20 min using a microplate reader (BioTek ELX800; BioTek Instruments, Colmar, France). The activity of collagenase in the presence of each genotype for each year was determined in

triplicate, and the anti-collagenase activity was expressed as the inhibition percentage relative to the corresponding control (phenol extraction by adding the same volume).

The elastase assay was carried out using porcine pancreatic elastase (Sigma Aldrich, Lyon, France). The elastase activity was evaluated using N-Succ-Ala-Ala-Ala-*p*-nitroanilide (AAAVPN; Sigma Aldrich) as a substrate [15]. The release of *p*-nitroaniline was done at 410 nm using a microplate reader (BioTek ELX800; BioTek Instruments). Measurements were performed in triplicate, and the anti-elastase activity was expressed, for each genotype and year, as the inhibition percentage relative to the corresponding control (phenol extraction by adding the same volume of the same solvent).

2.6. Statistical Analyses

Results are presented as the mean ± standard deviation of three replicates for each parameter. A *p*-value of 0.05 was used to denote significant differences between mean values determined by the analysis of variance (ANOVA) using Statistica 8.0. Two-way ANOVAs were used in order to determine the effect of accession, year, and their interaction. A correlation analysis between antioxidant activity and TPC was also performed.

3. Results

The total phenol content of safflower oil and its antioxidant activity were strongly influenced by the used genotypes and growing conditions (Table 3). Same results were highlighted for both anti-collagenase activity and anti-elastase activity parameters. Significant interaction of year and genotype effects was observed on all measured parameters (Table 3).

Table 3. Effects of accession, year and their interaction on total phenol content, antioxidant and antiaging activity measured in three accessions of safflower grown during three years in Tiaret (semi-arid conditions), Algeria.

Source of Variation	df	Phenol Content	Antioxidant Activity	Anti-Collagenase Activity	Anti-Elastase Activity
Accession	2	7.63 **	53.48 ***	33.84 ***	86.21 ***
Year	2	407.69 ***	744.44 ***	79.6 ***	102.3 ***
Accession Year	4	43.25 ***	281.72 ***	124.2 ***	187.9 ***

** significant at $p < 0.01$; *** significant at $p < 0.001$.

3.1. Total Phenol Content

The highest phenol content was found for 2017 compared with 2015 and 2016. In 2017, the highest content was recorded for the Syrian genotype, while the French accession had the lowest content (Table 4). However, different results were recorded in 2015 and 2016, with the French genotype showing the highest phenol content. Furthermore, the Algerian accession showed intermediate values among the studied genotypes in all years (Table 4).

Table 4. Total polyphenol content and antioxidant activity of the three safflower accessions in 2015, 2016, and 2017.

Year	Accession	Total Phenol Content (mgEAG/kg of oil)	Antioxidant Activity (%)
2015	Syria	140.9 ± 7.0a	20.6 ± 0.6a
	France	199.5 ± 2.9c	33.1 ± 1.0c
	Algeria	168.1 ± 7.1b	24.7 ± 0.7b
	Mean	**169.5 ± 16.9**	**26.15 ± 3.7**
2016	Syria	186 ± 4.0a	27.6 ± 0.6a
	France	210.9 ± 0.4b	38.8 ± 0.0c
	Algeria	192.6 ± 4.3a	33 ± 0.8b
	Mean	**196.5 ± 7.4**	**33.13 ± 3.2**

Table 4. *Cont.*

Year	Accession	Total Phenol Content (mgEAG/kg of oil)	Antioxidant Activity (%)
2017	Syria	412.8 ± 1.3b	68.9 ± 0.4b
	France	289.2 ± 8.1a	38.9 ± 1.3a
	Algeria	305.8 ± 17.9a	40.5 ± 0.7a
	Mean	**335.9 ± 38.7**	**49.4 ± 9.7**

In the same column, for each year, means with the same letter were not significantly different at $p < 0.05$.

3.2. Antioxidant Activity

The methanolic extract from safflower oil revealed an antioxidant activity of 20% for all the used genotypes (Table 4). In addition, a similar profile to that of TPC was observed when evaluating antioxidant activity. This activity (%) showed higher values in 2017 compared to 2015 and 2016. In 2017, the highest antioxidant activity was reported for the Syrian accession. In contrary, in 2015 and 2016, this genotype exhibited the lowest antioxidant activity compared to the other genotypes (Table 4). Expectedly, a positive correlation was observed between the total phenol content of safflower oil and its antioxidant activity (Figure 1).

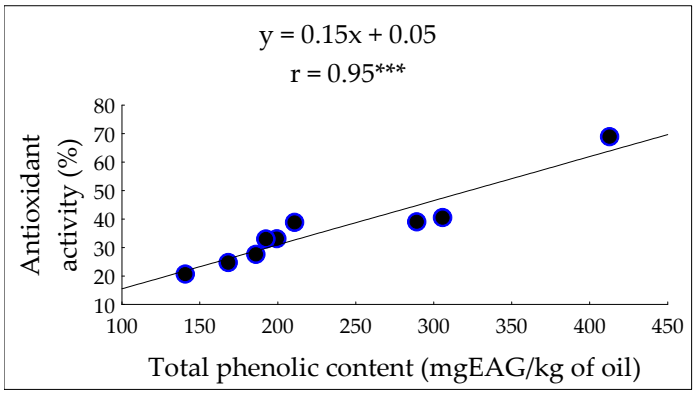

Figure 1. Correlation between total phenol content and antioxidant activity of the three safflower accessions in 2015, 2016, and 2017.

3.3. Antiaging Activity

Antiaging activity was assessed in safflower oil. The results indicated that this oil inhibited collagenase and elastase activity. Moreover, a wide diversity of antiaging activity was observed for all the used genotypes (Table 5).

Separately, a high anti-collagenase activity was found in 2016 and 2015, including a higher value recorded for the Syrian accession. Conversely, in 2017, the French accession shower higher activity compared to the other accessions (Table 5). For the second anti-enzymatic activity, as shown in Table 5, the highest anti-elastase activity was revealed in 2015 and 2017, which was approximately the same as the control. However, in 2016, the inhibition of anti-elastase activity decreased until it reached a minimum value; the lowest value was shown by the Algerian accession, which was half of the control value at 1000 µg/mL (Table 5).

Table 5. Anti-collagenase and anti-elastase activity of the three safflower accessions in 2015, 2016, and 2017.

Year	Accession	Anti-Collagenase Activity		Anti-Elastase Activity	
		IC_{50} (µg/mL)	Inhibition % at 500 µg/mL	IC_{50} (µg/mL)	Inhibition % at 1000 µg/mL
Control	*Control*	38.7 ± 0.2b	83.1 ± 0.2a	32.3 ± 0.2c	75.8 ± 0.1a
2015	Syria	135.9 ± 0.3a	65.2 ± 0.1b	202.8 ± 0.4a	66.7 ± 0.4b
	France	132.7 ± 0.2a	59.1 ± 0.2c	180.7 ± 0.1b	59.8 ± 0.3c
	Algeria	133.8 ± 0.4a	63.2 ± 0.3b	198.4 ± 0.2a	64.2 ± 0.5b
	Mean	**134.1 ± 0.9**	**62.5 ± 1.8**	**194 ± 6.7**	**63.6 ± 2.0**
2016	Syria	130.1 ± 0.2a	72.1 ± 0.6b	178.6 ± 0.9a	42.4 ± 0.4b
	France	124.6 ± 0.2b	61.6 ± 0.3c	163.7 ± 0.8c	49.1 ± 0.01b
	Algeria	123.4 ± 0.2b	64.9 ± 0.4c	171.4 ± 0.9b	32.2 ± 0.2b
	Mean	**126.03 ± 2.1**	**66.2 ± 3.1**	**171.2 ± 4.3**	**41.2 ± 4.9**
2017	Syria	144.5 ± 0.2a	47.0 ± 0.4b	298.1 ± 1.2a	70.3 ± 0.6ab
	France	134.7 ± 0.2b	52.8 ± 0.3b	254.3 ± 0.9a	63.2 ± 0.4b
	Algeria	136.1 ± 0.2b	49.9 ± 0.8b	274.6 ± 1.1a	67.2 ± 0.8b
	Mean	**138.4 ± 3.1**	**49.9 ± 1.7**	**275.7 ± 12.6**	**66.9 ± 2.0**

In same column, for each year, means with the same letter are not significantly different at $p < 0.05$.

4. Discussion

Polyphenolic compounds are the most important groups of secondary metabolites in medicinal herbs and dietary plants. The results of phenol content observed in safflower oil ranged from 140.9–412.8 mg GAE/kg of oil (Table 4). The high amount of TPC confirmed that safflower oil presents an important source of these components. Similar results were observed in previous studies [4,16–19]. The results showed a high antioxidant activity for all accessions, which ranged from 20.6% to 68.9% (Table 4). This activity has been described by several works [5,19,20]. Shirvani et al. [21] reported that this activity had a rate of 50% at the beginning of safflower seed germination. Kim et al. [21] mentioned that the antioxidant activity of safflower seeds is lower than that of other botanical sections of the safflower plant. In detail, they reported 114.2%, 113.6%, 94.4%, and 86.1% of DPPH radical scavenging activity in petals, leaves, buds, and shoots, respectively.

Interestingly, great importance has been given to anti-inflammatory activity in pharmaceutical and cosmetic uses. Nevertheless, no study has been carried out to date to evaluate the effects of climatic conditions on the antiaging activity of safflower oil. Likewise, and for the first time, the anti-elastase and anti-collagenase properties of the phenol content of safflower oil were assessed in this study to identify a new source of antiaging agent. During the three years, the results suggest that safflower oil has important anti-collagenase activity with 72.1% inhibition at 500 µg/mL, a value corresponding to IC_{50} = 130.1 µg/mL for the Syrian accession. This genotype showed an anti-elastase activity of 70.3% inhibition at 1000 µg/mL, a value corresponding to IC_{50} = 298.1 µg/mL (Table 5). Using essential oils extracted from some medicinal herbs and food plants, Aumeeruddy-Elalfi et al. [22] demonstrated a minimal anti-collagenase activity and anti-elastase activity of 52.2% inhibition at 400 µg/mL and 32.23% inhibition at 800 mg/mL, respectively. A methanolic extract of water-pepper sprout inhibited collagenase activity in a concentration-dependent manner with an IC_{50} value of 156.7 µg/mL [23].

Genotypic variability showed different responses to changes in weather conditions during the three years. Thus, these climatic variations, which were mainly due to variations in temperature and precipitation (Table 2), had significant effects on the studied parameters. Roche et al. [24] reported the effect of climatic conditions on the chemical composition of safflower seeds. In 2017, high temperatures and low rainfall were recorded during the growing season of safflower (Table 2). In the same year, the highest values of TPC (412.8 mg EAG/kg of oil) were reported for the Syrian accession (Table 4). In contrast, a decrease in temperature in 2015 and 2016 (Table 2) induced a decrease in the phenol

content and antioxidant activity for all the accessions. Therefore, it appears that these contents are influenced primarily by the increase in mean temperatures. Indeed, heat stress affected the accumulation of phenolic compounds in durum wheat seeds [25]. Another study confirmed that TPC increased significantly with the rise of temperature in sesame [26]. Unexpectedly, the total phenol content in 2016 was higher than 2015 (Table 4). This could be explained by the increase in rainfall, which led to an increase in the phenol content of safflower oil. These results are in accordance with those reported by Palese et al. [27] and in contradiction with those reported by Gucci et al. [28] regarding olive oil.

The influence of the growing conditions during the safflower plant cycle on its antioxidant activity was also investigated. A high antioxidant activity (Table 4) was found for all accessions with the rise of temperatures, confirming results already reported [26]. Taha and Matthäus [16] showed a significant increase in the antioxidant activity of safflower seeds caused by the roasting process. Britz and Kremer [29] reported that heat and drought influenced the tocopherol content in soybean during seed maturation. The content and quality of oil from plant material depends on the nature of the used solvents during its extraction. In our study, cyclohexane, an apolar solvent like hexane, may influence the oil content of safflower [30] and the extracted phenolic of the solid material [31,32]. Terpinc et al. [33] showed the effect of solvent type on the variation of total phenol content and antioxidant activity of different oil cake extracts. A higher percentage of unsaponifiable matter was demonstrated in safflower oil extracted by cyclohexane [34]. The use of a mixture of water and other organic solvents such as methanol in our study remains essential to ensure a perfect extraction of phenolic compounds from safflower seed oil.

The phenylpropanoid biosynthetic pathway is responsible for the synthesis of phenolic compounds [35]. However, biosynthesis stimulation of these compounds is mainly due to the regulation of many genes encoding the main enzymes of the phenylpropanoid pathway according to environmental conditions [36], such as drought stress [37] and high temperature [38,39]. The effect of genotype on phenolic compounds has been previously shown [40]. Moreover, as already seen in safflower and soybean [20,41], we found a positive correlation between TPC and antioxidant activity.

Furthermore, anti-elastase activity increased significantly with the increasing mean temperature recorded in 2017 (Table 5). In contrast, the anti-collagenase activity decreased with increasing temperature. However, the anti-collagenase activity remained higher than 47% inhibition at 500 µg/mL regardless of the temperature.

A great interest in the phenol content and antioxidant activity of the diet was reported among consumers and the scientific community. Phenols, antioxidant activity, and their impact on human health, even if not assessed in our study, have been reported broadly [42–49].

Today, many plants with high antioxidant activity are of interest for pharmaceutical and cosmetic applications. Indeed, a large range of oilseed has been used as skin products and hair cosmetics for a long time in several cultures, including sunflower and olive oil. The application of sunflower seed oil has been shown to preserve the stratum corneum integrity and improve hydration of the adult skin without inducing erythema [50]. Budiyanto et al. [51] reported that olive oil topically applied after UVB exposure can effectively reduce UVB-induced murine skin tumors, possibly via its antioxidant effects in reducing DNA damage by reactive oxygen species, and that the effective component may be labile to UVB. This antioxidant activity presents high potential as a UVB sunscreen agent [10,52]. Argan oil can improve skin elasticity [53] and skin hydration by restoring the barrier function and maintaining the water-holding capacity [54]. Moreover, a natural skin toning cream has been developed from safflower oil [55]. The oil body bound oleosin-rhFGF9 expressed in safflower seed stimulates hair growth and wound healing in mice [56]. Dakhil et al. [57] have reported that safflower oil characteristics can make it a main ingredient in the preparation of topical agents for the treatment of various skin problems. Abdul Karim et al. [52] recommended the possible use of cocoa pod extract as an ingredient in functional cosmetic products, specifically for anti-wrinkles as well as skin whitening or sunscreen products in combination with natural plant extracts to widen the spectrum of protection from sun rays.

Furthermore, many studies showed that skin aging and skin wrinkling may be reduced by the action of the antioxidant activity of various botanical extracts [58,59]. Aumeeruddy-Elalfi et al. [22] showed that the inhibitory potential of essential oils extracted from some medicinal plants make them potential candidates for the cosmetic (skin aging) and pharmaceutical industries.

In this study, the excellent anti-collagenase and anti-elastase activity of the phenolic compounds of safflower oil highlight its potential as a natural source of antiaging agents for cosmetic formulations. Besides the phenol content of safflower oil reported in our study, other research has confirmed that safflower oil contains also high proportions of polyunsaturated fatty acids and sterols [24]; the characteristics of all of these compounds could grant safflower oil high importance for pharmaceutical and industrial use.

5. Conclusions

Synthetic antioxidants are often associated with problems of carcinogenticity and toxicity; there is an increasing interest in oilseed as sources of natural antioxidants for cosmetic and pharmaceutical uses. This study focused on the quantitative profiling of methanol extractible (TPC) obtained from safflower seed oil grown in a semi-arid climate, and its antioxidant and antiaging activity. We revealed that safflower seed oil could be an important source of polyphenols with resulting antioxidant and antiaging activity. Genotype, year (climatic conditions), and their interaction significantly affected these properties. This was confirmed by the improvement in total phenol content and DPPH assay, and maintenance of appreciable antiaging activity of safflower oil with increasing temperature and drought. Recently, several studies reported that these compounds have an important role in human health. High levels of phenols as well as antioxidant and antiaging activity were reported for safflower oil seed. Their content and activity depend on both genotype and climatic conditions. Therefore, this highlights the potential interest of this source of valuable compounds for pharmaceutical and cosmetic applications. Moreover, these traits could be managed by modulations according to genotype and climatic conditions.

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