

## Fatty acid composition, phytochemicals and antioxidant potential of *Capparis spinosa* seeds

✉A. Bodaghzadeh<sup>a</sup>, ✉K. Alirezalu<sup>b,✉</sup>, ✉S. Amini<sup>a</sup>, ✉A. Alirezalu<sup>a</sup>, ✉R. Domínguez<sup>c</sup> and ✉J.M. Lorenzo<sup>c,d,✉</sup>

<sup>a</sup>Department of Horticultural Sciences, Faculty of Agriculture, Urmia University, Urmia, Iran.

<sup>b</sup>Department of Food Science and Technology, Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Tabriz, Iran.

<sup>c</sup>Centro Tecnológico de la Carne de Galicia, Parque Tecnológico de Galicia, rúa Galicia n° 4, San Cibrao das Viñas, Ourense 32900, Spain

<sup>d</sup>Área de Tecnología de los Alimentos, Facultad de Ciencias de Ourense, Universidad de Vigo, 32004 Ourense, Spain

✉Corresponding authors: [jmlorenzo@ceteca.net](mailto:jmlorenzo@ceteca.net); [kazem.alirezalu@tabrizu.ac.ir](mailto:kazem.alirezalu@tabrizu.ac.ir)

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**SUMMARY:** The present study evaluates the contents in bioactive compounds, antioxidant activity, oil content and fatty acid composition of *Capparis spinosa* seeds. Samples were collected from 5 different habitats (AH: Ahar; KU: Kurdistan; U1, U2 and U3: Urmia) in Iran. The oil content in the seeds ranged from 16 to 27%. The predominant fatty acid was linoleic acid (45–50%) followed by oleic acid (30–39%), palmitic acid (2–8%) and stearic acid (2–3%). Total phenolic content (TPC) varied from 16.3 to 24.2 mg GAE/ g DW; total flavonoid content (TFC) ranged from 1.48 to 3.05 mg QE/g DW; and the antioxidant activity (DPPH assay) of the seeds was between 35 and 63%. The compounds obtained from different genotypes of *C. spinosa* seeds had different compositions, great antioxidant capacity and unsaturated fatty acids, and therefore could be a prospective source of natural bioactive molecules for the food and health industry.

**KEYWORDS:** Antioxidant activity; Bioactive compounds; Fatty acids; Flavonoids; Phenolic compounds

**RESUMEN:** *Composición de ácidos grasos, fitoquímicos y potencial antioxidante de las semillas de Capparis spinosa.* El presente estudio evaluó el contenido de compuestos bioactivos, actividad antioxidante, contenido de aceite y composición de ácidos grasos de las semillas de *C. spinosa*. Se recolectaron muestras de cinco hábitats diferentes (AH: Ahar; KU: Kurdistán; U1, U2 y U3: Urmia) en Irán. El contenido de aceite de las semillas osciló entre el 15,66 y el 27,50%. El ácido graso predominante fue el ácido linoleico (45–50%) seguido por el oleico (30–39%), el palmítico (2–8%) y el esteárico (2–3%). El contenido fenólico total varió de 16,3 a 24,2 mg GAE/g DW; el contenido total de flavonoides entre 1,48 y 3,05 mg QE/g DW; la actividad antioxidante de las semillas estuvo entre 34,68 y 62,74%. Los compuestos obtenidos de semillas de *C. spinosa* tienen gran capacidad antioxidante y ácidos grasos insaturados, por lo que podrían ser una fuente de moléculas bioactivas naturales en la industria alimentaria y de la salud.

**PALABRAS CLAVE:** Ácidos grasos; Actividad antioxidante; Compuestos bioactivos; Compuestos fenólicos; Flavonoides

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## 1. INTRODUCTION

The caper is a perennial shrub plant and is the common name of the genus *Capparis*, in the family *Capparidaceae*. It is distributed in the subtropical and tropical regions with 250 species (Wojdyło *et al.*, 2019). The taxonomy of the genus in Iran has been the subject of much debate. Boissier (1867), combined *Capparis parviflora* and *Capparis mucronifolia* species in the *Capparis spinosa* as varieties. However, Zohary (1960) introduced five species with several varieties in Iran. *Capparis spinosa*, with three varieties, var. *spinosa*, var. *parviflora* and var. *Mucronifolia*, has been cited in In Flora Iranica (Hedge and Lamond, 1970). *Capparis* species are mostly distributed in the south of Iran, however, *Capparis spinosa* is widely distributed in all regions of Iran.

Different organs of caper-like fruits and flower buds are commonly used by the local population for their anti-inflammatory, antidiabetic, antihyperlipidemic, antihypertensive, antihepatotoxic and anticarcinogenic activities since the caper and its derivatives, such as pickled caper buds are rich in vitamins, essential minerals, fatty acids and proteins (Özcan *et al.*, 2004; Arslan and Özcan, 2007; Özcan, 2008; Bakr and El Bishbishy, 2016). *C. spinosa* seeds are rich in lipids, containing unsaturated fatty acids (Oleic acid, vaccenic acid, linoleic acid), bioactive (phenolic compounds) and nutraceutical (flavonoids and sterols) compounds (Matthäus and Özcan, 2005). However, Duman and Özcan (2014) reported that capers had high levels of Ca, K, Mg, Na, and S minerals but caper seeds involved more mineral matter than oils. During the last decade, several natural compounds such as synthetic additive replacers (Domínguez *et al.*, 2020; Lorenzo *et al.*, 2018) have been investigated in the food industry. *C. spinosa* is a possible source of these compounds. *C. spinosa* seeds contain a relatively high amount of oil (23–33%) (El-Waseif and Badr, 2018), rich in phospholipids (phosphatidylinositol), with a high content in tocopherols ( $\gamma$ -tocopherols and  $\delta$ -tocopherols), sterols (sitosterol, campesterol and stigmaterol), carotenoids ( $\alpha$ -tocopherol) and glucosinolates (Glucocapperin) (Matthäus and Özcan, 2005; Tlili *et al.*, 2009; Zhang and Ma, 2018).

It is well known that the genotype and region could exert a great impact on the oil composition and functional and nutritional properties (Alirezalu *et al.*, 2016). However, there are limited data relating to the effects of genotype and location on *C. spinosa* oil composition. In addition to environmental conditions that have considerable effects on the biosynthesis of secondary metabolites, genetic factors can also contribute to the biosynthesis and accumulation of these compounds. The interaction of environmental factors and the genetic background of plants play a substantial role in the accumulation of metabolites (Alirezalu *et al.*, 2018; Shaghghi *et al.*, 2019; Alirezalu *et al.*, 2020). Some genes

contribute to the biosynthesis of fatty acids and phenolics in medicinal plants. The expression of these genes varies in various species. Moreover, cytochromes-P450 isoforms contribute to the phenylpropanoid metabolism and by effecting several reactions, they regulate phenolic synthesis (Ayabe and Akashi, 2006).

The purpose of the present study was to determine, for the first time, oil content, fatty acid composition, phytochemicals and antioxidant activity of the oil from caper seeds collected from 5 different regions.

## 2. MATERIALS AND METHODS

### 2.1. Sample preparation

The ripe fruits of *C. spinosa* genotypes (accession) from 5 region of West Iran were collected from 27 June until 10 July 2016 (Table 1). Details of collection sites are presented in Table 1. The seeds were obtained from ripened fruit (5 plants from every region). The freeze-dried fruits were squashed in a mortar and sifted using a 60-mesh screen to separate the seeds from the pulp. After air-drying at 30 °C for 72 h, seeds were removed, mixed and used for additional analysis (Fazio *et al.*, 2013).

### 2.2. Oil extraction

For this study, 5 g of a powdered sample from each plant seed were extracted by hexane solvent (300 mL) using a Soxhlet extractor for 5 h at 80 °C in order to prevent damage to minor compounds. The extract was filtered through filter papers containing sodium sulphate and vacuum evaporated (around 150 mbar) in a rotary evaporator until the n-Hexane portions were removed at 40 °C and then stored -20 °C for further analysis. The oil content in the seeds was expressed on a percent basis, based on whole samples. Oil content was then measured using Equation 1.

$$(\%) \text{ Oil content} = (W_1 - W_2) / W_3 \times 100 \quad \text{Eq. 1}$$

$W_1$  = Weight of dried sample before extraction + filter bag

$W_2$  = Weight of dried sample after extraction + filter bag

$W_3$  = Original weight of simple

### 2.3. Fatty acid analysis

Fatty acid methyl esters (FAMES) were prepared from the oil samples according to the method described by Savage *et al.* (1997). FAME) and were derived from the esterification of 20 mg extracted oil with 2 mL 0.01 M NaOH in dry methanol at 60 °C for 30 min under continuous shaking and transesterification of glycerolipids with boron trichloride/methanol. The FAMES were determined by GC (Agilent technologies, 6890N, USA) according to the method reported by Azadmard-Damirchi and Dutta

TABLE 1. Sampling locations of the different caper genotypes.

Code	Species	Collection sites	Geographical location		
			Longitude (N)	Latitude (E)	Altitude (m)
AH	<i>C. spinosa</i>	East Azerbaijan /Ahar (Qareh daq)	38°47'32.72"	47°11'54.84"	2172
KU	<i>C. spinosa</i>	Kurdistan / Kurdistan	34°16'58.41"	47°01'07.86"	1553
U1	<i>C. spinosa</i>	West Azerbaijan/Urmia(1)	37°45'17.82"	44°42'31.68"	1735
U2	<i>C. spinosa</i>	West Azerbaijan/Urmia(2)	37°20'31.37"	45°08'57.87"	1350
U3	<i>C. spinosa</i>	West Azerbaijan/Urmia(3)	37°27'39.13"	44°57'05.86"	1624

(2006). The GC instrument was equipped with a capillary injection valve and DB-Wax capillary column (30 m x 0.25 mm i.d and 0.25 µm of thickness), which was treated with polyethylene glycol and a flame ionization detector (FID). The FAMES were analyzed by comparison of their retention times with standard FAMES and the peak areas are reported as a percentage of the total fatty acids.

#### 2.4. Preparation of seed extract

One g of caper seed was powdered by liquid nitrogen and extracted with 20 mL of 80% methanol, then mixed gradually for 1 h using a magnetic stirrer. The extract was filtered using filter paper (Whatman No.1). The resulting extract was kept at -70 °C for corresponding analysis.

#### 2.5. Total phenolic content (TPC)

The TPC in the seed extracts was determined by Folin-Ciocalteu method Singleton *et al.* (1999) with some modification. Briefly, 100 µL of methanolic extracts were shaken for 1 min with 1 mL of diluted (1:10) Folin-Ciocalteu reagent and held at 25 °C for 5 min. Then 800 µL of sodium carbonate (10%) were added and the final volume was made up to 5.0 mL with distilled water. After that, the mixture was left at room temperature for 2 h. Finally, the absorbance at 760 nm was measured by a spectrophotometer (UNICO, China). Gallic acid was expressed as a standard solution and TPC was used as mg GAE/g DW.

#### 2.6. Total flavonoid content (TFC)

The TFC was measured by an adapted colorimetric method using aluminium chloride (AlCl<sub>3</sub>) (Ordoñez *et al.*, 2006). Briefly, 400 µL of seed extract were added to 0.3 mL distilled water followed by 0.03 mL NaNO<sub>2</sub> (5%). The mixture was held for 5 min at 25 °C, and then 0.03 mL of AlCl<sub>3</sub> (10%) were added. After 5 min, the mixture was treated with 0.2 mL of 1 mM NaOH. Finally, the reaction was diluted to 100 mL with distilled water. The absorbance versus prepared blank was measured at 420 nm. TFC

was reported as mg of quercetin equivalents per g of sample dry weight (mg of QE/g DW).

#### 2.7. Antioxidant activity

To measure the antioxidant activity by the DPPH (2, 2'-diphenyl-1-picrylhydrazyl) free radical scavenging method, 500 µL of methanolic extracts were mixed with 1 mL of the DPPH solution. The mixture was held in the dark at 20 °C for 30 min. Absorbance was determined at 517 nm using UV-Vis spectrophotometer (UNICO, China). The percentage of inhibition (I%) of free radical DPPH was calculated using the formula:  $RSA\% = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$  (Nakajima *et al.*, 2004).

#### 2.8. Statistical analysis

All of the analyses (5 regions × 5 sampling point × 3 experiments) were carried out in a completely randomized design. SAS 9.1.3 software package (SAS Institute, US) was used for statistical analysis of the data. Normal distribution and variance homogeneity had been previously tested (Shapiro-Wilk). The data were submitted to one-way analysis of variance (ANOVA). The different parameters studied in the present research were included in the model as dependent variables, while genotype was included as a fixed effect. Duncan's test was carried out when the ANOVA was significant ( $P < 0.05$ ), in order to determine differences between means. The principal component analysis (PCA) and hierarchical cluster analysis (HCA) based on Ward's method and correlation analysis based on Pearson's method were performed among the variables (fatty acid composition) by MINITAB 13.2.

### 3. RESULTS AND DISCUSSION

#### 3.1. Oil content

The oil contents in the seeds from wild fruits of the caper genotypes are reported in Figure 1. There were significant differences ( $P < 0.05$ ) in oil content among the

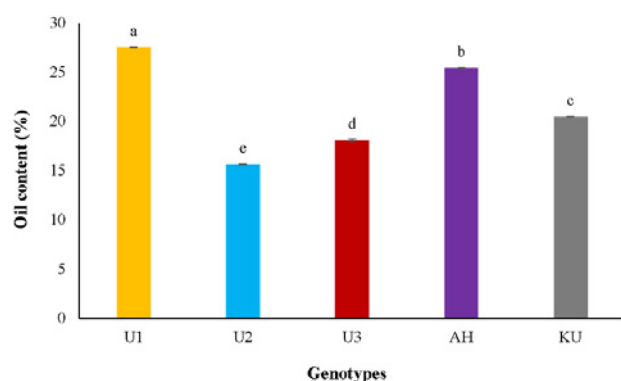


FIGURE 1. Oil content (g/100 g) of caper seeds in different genotypes. a-e Means (Three experiments) with different letters within treatments were significantly different at the level of  $P < 0.05$ . Mean comparisons of genotypes were carried out by Duncan's Multiple Range Test (DMRT) at  $\alpha=0.05$ .

genotypes. Oil contents in samples ranged from 16 to 27%. Two genotypes (U1 (Urmia) and AH (Ahar)) had significantly higher oil contents than the other genotypes. The highest and the lowest oil contents were found in the U1 (27.50%) and U2 (16.66%) (Urmia) genotypes, respectively. El Amri *et al.* (2019) revealed that the oil content in *C. spinosa* was not only affected by location, but also the highest oil content was observed in big seeds.

These results agree with Tlili *et al.* (2009), who revealed that oil content in caper seeds ranged from 23 to

34% in Tunisia. Zia-Ul-Haq *et al.* (2011) obtained a similar oil content (29.1%) in *Capparis decidua*. Matthäus and Özcan (2005) found that habitat had a high impact on the oil content of Turkish *C. spinosa* seeds. The range of oil content in the present study was higher than that reported in Italian *C. spinosa* subsp. *Rupestris* (Argentieri *et al.*, 2012), which can be related to agro-ecological growing conditions (Čolić *et al.*, 2017). These differences could be due to geographic distribution, soil quality, climatic conditions of an area, and size of seeds and/or using different analysis methods. Caper seeds with high oil content are considered to have potential for food and pharmaceutical applications.

### 3.2. Fatty acids composition

The fatty acid composition of the oil samples is reported in Table 2. Eleven fatty acids were detected in the extracted oils from *C. spinosa* seeds and ranged from C14:0 to C20:0. The results showed significant differences ( $P < 0.01$ ) in the fatty acid composition of the oil. Among the fatty acids, the predominant constituent was linoleic acid (C18:2n-6) followed by oleic acid (C18:1n-9), palmitic acid (C16:0) and stearic acid (C18:0). The presence of these fatty acids was also reported in other studies (Akgül and Özcan, 1999; Matthäus and Özcan, 2005; Saadaoui *et al.*, 2015; Tlili *et al.*, 2009; Yuldasheva *et al.*, 2008). The monounsaturated fatty acids in the caper oil were

TABLE 2. Fatty acid composition (g/100 g) in seed oil genotypes of capers.

Fatty acid composition	Genotypes				
	U1	U2	U3	AH	KU
Myristic acid; C14:0	0.34 ± 0.01 <sup>b</sup>	0.25 ± 0.00 <sup>d</sup>	0.40 ± 0.01 <sup>a</sup>	0.27 ± 0.00 <sup>c</sup>	0.27 ± 0.01 <sup>c</sup>
Palmitic acid; C16:0	1.87 ± 0.02 <sup>c</sup>	6.46 ± 0.05 <sup>c</sup>	7.05 ± 0.04 <sup>b</sup>	6.39 ± 0.04 <sup>d</sup>	7.88 ± 0.02 <sup>a</sup>
Palmitoleic acid; C16:1n-7	0.59 ± 0.09 <sup>c</sup>	1.68 ± 0.055 <sup>a</sup>	1.49 ± 0.07 <sup>ab</sup>	1.29 ± 0.03 <sup>b</sup>	1.35 ± 0.04 <sup>ab</sup>
Stearic acid; C18:0	2.75 ± 0.05 <sup>a</sup>	2.29 ± 0.01 <sup>c</sup>	2.37 ± 0.0 <sup>b</sup>	2.23 ± 0.02 <sup>c</sup>	2.71 ± 0.02 <sup>a</sup>
Oleic acid; C18:1n-9	38.88 ± 0.03 <sup>a</sup>	34.78 ± 0.03 <sup>b</sup>	34.18 ± 0.02 <sup>c</sup>	34.73 ± 0.03 <sup>b</sup>	30.52 ± 0.05 <sup>d</sup>
Linoleic acid; C18:2n-6	47.30 ± 0.02 <sup>c</sup>	45.59 ± 0.02 <sup>d</sup>	45.62 ± 0.02 <sup>d</sup>	47.71 ± 0.02 <sup>b</sup>	49.41 ± 0.05 <sup>a</sup>
α-Linolenic acid; C18:3n-3	1.33 ± 0.01 <sup>a</sup>	1.39 ± 0.1 <sup>a</sup>	1.19 ± 0.02 <sup>ab</sup>	0.69 ± 0.01 <sup>c</sup>	1.07 ± 0.0 <sup>b</sup>
γ-Linolenic acid; C18:3n-6	0.46 ± 0.00 <sup>c</sup>	0.49 ± 0.01 <sup>b</sup>	0.56 ± 0.00 <sup>a</sup>	0.43 ± 0.00 <sup>d</sup>	0.47 ± 0.00 <sup>c</sup>
Stearidonic acid; C18:4n-3	0.72 ± 0.03 <sup>bc</sup>	0.80 ± 0.03 <sup>a</sup>	0.78 ± 0.04 <sup>ab</sup>	0.71 ± 0.01 <sup>c</sup>	0.73 ± 0.02 <sup>bc</sup>
Arachidic acid; C20:0	0.59 ± 0.02 <sup>b</sup>	0.74 ± 0.02 <sup>a</sup>	0.69 ± 0.05 <sup>a</sup>	0.53 ± 0.04 <sup>b</sup>	0.51 ± 0.03 <sup>b</sup>
UFA	89.28 ± 1.32 <sup>a</sup>	84.73 ± 1.75 <sup>bc</sup>	83.82 ± 1.01 <sup>c</sup>	85.56 ± 0.71 <sup>b</sup>	83.55 ± 0.91 <sup>c</sup>
SFA	5.55 ± 0.45 <sup>d</sup>	9.74 ± 0.71 <sup>bc</sup>	10.51 ± 0.54 <sup>ab</sup>	9.42 ± 0.09 <sup>c</sup>	11.37 ± 0.42 <sup>a</sup>

UFA: Unsaturated fatty acid; SFA: Saturated fatty acid; Values are expressed as means ± standard deviation (n=3). <sup>a-c</sup> Means (Three experiments) with different letters within the same row were significantly different at the level of  $P < 0.05$ . Mean comparisons of genotypes were carried out by Duncan's Multiple Range Test (DMRT) at  $\alpha=0.05$ .

palmitoleic and oleic acid. Our results were in agreement than those reported by Matthäus and Özcan (2005), who determined that linoleic acid (24.6-50.5%) was the major fatty acid in *C. spinosa* genotypes. In contrast, other authors found oleic acid as the major fatty acid in caper seed oil (Akgül and Özcan, 1999; Saadaoui *et al.*, 2015; Tlili *et al.*, 2009). Zia-Ul-Haq *et al.* (2011) reported that the major fatty acids were linoleic acid (47.3%) followed by oleic acid (33.2%) in *Capparis decidua*, which agree with the values reported in the present study. The KU (Kurdistan) genotype showed the highest content in linoleic acid, while U2 and U3 (Urmia) the lowest ones. As mentioned above, the second most abundant fatty acid was oleic acid, ranging from 30.52 (KU) to 38.88% (U1). Myristic acid (C14:0),  $\gamma$ -linolenic acid (C18:3n-6), stearidonic acid (C18:4n-3) and arashidic acid (C20:0) were present below 1% in all genotypes. The presence of these minor fatty acids in *C. spinosa* seed oil was also reported in different studies (Givianrad *et al.*, 2011; Saadaoui *et al.*, 2015; Tlili *et al.*, 2009). The palmitoleic acid (C16:1n-7) in caper genotypes ranged from 0.59% in U1 to 1.68% in U2. The linolenic acid content varied from 0.69 to 1.33% in AH and U2. Stearidonic acid (C18:4n-3) oscillated from 0.71 (AH) to 0.8% in the (U2) genotype. The lowest amount of  $\gamma$ -linolenic acid (C18:3n-6) was present (> 0.56%) in most of the caper genotypes investigated.

The predominant saturated fatty acids in the caper seed oil were palmitic acid (1.87-7.88%), followed by stearic acid (2.23-2.71%), arachidic acid (0.51-0.69%) and myristic acid (0.25-0.40%). The highest levels of palmitic and stearic acid were observed in the KU genotype and U3 genotype, which contained the highest contents in myristic acid and arashidic acids. A similar fatty acid profile has also been reported for Tunisian *C. spinosa*. Tunisian genotypes showed higher myristic, palmitic, palmitoleic, and oleic acid contents and the lowest amount of linoleic acid compared to our genotypes. There was a large variation in palmitic, oleic, linoleic and palmitoleic acids in comparison with stearic and stearidonic acids. The U1 genotype showed the highest content in unsaturated fatty acid. According to some reports, ecological conditions, genetic variation, seed maturity, seed production environment and geographical origin can affect the fatty acid composition of seeds (Acar *et al.*, 2008; Saadaoui *et al.*, 2015; Saxena *et al.*, 2017), which could explain the differences found in the present research.

Several investigations were conducted to demonstrate the effect of different fatty acids on human health. To this regard, it is well known that saturated fatty acids have negative effects on health and promote the appearance of various diseases. A recent study published by The World Health Organization about the effects of SFA on serum lipids and lipoproteins concluded that each 1% of dietary energy as SFA replaced with an equivalent amount of cis-

PUFA or cis-MUFA resulted in a significant reduction in total, LDL and HDL cholesterol and in triglyceride contents (Mensink, 2016). In a similar way, a study reviewed the effect of fatty acids on health found that the intake of SFA increased LDL-cholesterol, favored inflammation processes and was associated with an increased incidence of type 2 diabetes (Calder, 2015). However, this evidence has been questioned by recent systematic reviews and meta-analyses of data on mortality in relation to exposure to SFA either through the diet or in the bloodstream.

On the other hand, the replacement of SFA with MUFA, particularly oleic acid, may improve glucose control and insulin sensitivity and would be expected to lower the risk of cardiovascular disease (Calder, 2015). In the same way, the consumption of PUFA, especially n-3 fatty acids, has a high impact on health and plays an important role in the prevention of cardiovascular disease. (Nagy and Tiuca, 2017).

Therefore, with the aforementioned, the caper could be an excellent source for obtaining healthy oil with potential use in the food industry. In fact, caper seeds presented higher amounts of oil content and unsaturated (linoleic and oleic acids) fatty acids. In addition, the U1 and AH genotypes showed the higher oil and unsaturated fatty contents and the lowest amounts of saturated fatty acids. Therefore, these two genotypes could be the best options for food application.

### 3.3. Correlation analysis

The results of the simple correlation coefficients of oil content and fatty acids are presented in Table 3. Studies have shown fatty acid levels do not affect oil content (Johansson *et al.*, 2000; Onemli, 2012). A significant positive correlation was observed between myristic acid and  $\alpha$ -linolenic acid ( $r=0.646^{**}$ ).

Palmitic acid was positively correlated ( $r= 0.833^{**}$ ) with palmitoleic acid and negatively correlated ( $r=-0.920^{**}$ ) with oleic acid. The negative correlation was revealed when palmitic acid increased and oleic acid decreased. The results agreed with previous reports by multiple authors (Lamaisri *et al.*, 2015; Raheja *et al.*, 1987). Stearidonic acid showed to correlate positively with palmitoleic acid,  $\gamma$ -linolenic acid and arachidic acid and negatively with linoleic acid.

A positive significant correlation was observed between stearic acid and linoleic acid ( $r=0.566^*$ ). However, observed differences between *C. spinosa* seeds from different habitats were similar to the data published by Onemli (Onemli, 2012). Negative correlations existing between linoleic and  $\gamma$ -linolenic acid can be shown by  $\alpha$ - and  $\gamma$ -linolenic acids synthesized by the desaturation of linoleic acid in plants. This is in agreement with results found by other reports by Johansson *et al.* (Johansson *et*

TABLE 3. Correlation coefficients among fatty acid composition of the caper genotypes

Fatty acids	C14:0	C16:0	C16:1n-7	C18:0	C18:1n-9	C18:2n-6	C18:3n-3	C18:3n-6	C18:4n-3	C20:0
C14:0	1									
C16:0	-0.253	1								
C16:1n-7	-0.236	0.833**	1							
C18:0	0.112	-0.414	-0.606*	1						
C18:1n-9	0.342	-0.920**	-0.655**	0.053	1					
C18:2n-6	-0.423	0.107	-0.256	0.566*	-0.439	1				
C18:3n-3	0.232	-0.327	-0.073	0.321	0.300	-0.460	1			
C18:3n-6	0.646**	0.345	0.422	-0.144	-0.186	-0.598*	0.457	1		
C18:4n-3	0.062	0.381	0.698**	-0.373	-0.177	-0.614*	0.368	0.625*	1	
C20:0	0.232	0.082	0.388	-0.390	0.160	-0.852**	0.654**	0.666**	0.658**	1

\* Correlation is significant at the 0.05 level, \*\* Correlation is significant at the 0.01 level. C14:0; Myristic acid, C16:0; Palmitic acid, C16:1n-7; Palmitoleic acid, C18:0; Stearic acid, C18:1n-9; Oleic acid, C18:2n-6; Linoleic acid, C18:3n-3;  $\alpha$ -Linolenic acid, C18:3n-6;  $\gamma$ -Linolenic acid, C18:4n-3; Stearidonic acid, C20:0; Arachidic acid

*al.*, 2000). Arachidic acid in caper oil was positively correlated with  $\alpha$ -linolenic acid and  $\gamma$ -linolenic acid.

### 3.4. Phytochemicals and antioxidant activity in seeds

The TPC in the caper seed extracts of the 5 studied genotypes are shown in Figure 2. The amount of TPC varied from 16.3 to 24.2 mg GAE/g DW in the different genotypes. In a recent study, some authors found values between 3 and 10 mg of polyphenols/g DW of *C. spinosa* flowers (Wojdyło *et al.*, 2019). In contrast, other authors reported that the TPC of the aerial part of *C. spinosa* ranged between 14.8 and 87.5 mg GAE/g in roots and 4.49-58.6 mg GAE/g in roots (Baghiani *et al.*, 2012). Baghiani *et al.* (2012) reported that the extraction solvent exerted a great influence on TPC contents. They found that the chloroform extract showed the highest levels of TPC followed by ethyl acetate extract and crud extract. This fact could explain the differences reported by different researchers (Alirezalu *et al.*, 2020).

The highest TPC was observed in the KU and U1 genotypes and the lowest content was observed in the U2 seeds. Indeed, climatic factors influenced oxidative stress and the generation of reactive oxygen species in perennial plants and led to excessive phenolic compound production in aggressive environments (Lamien-Meda *et al.*, 2010). Phenolic compounds in plant extracts are considered as minor components for eliciting nutraceuticals, antimicrobial activity and antioxidant properties (Alirezalu *et al.*, 2019). In the present research, the TPC variation was lower than those reported in a previous study (Wojdyło *et al.*, 2019). This authors also reported that cultivar had a significant influence on polyphenol content, which agreed with our results (Wojdyło *et al.*, 2019).

As shown in Figure 3, the concentration of flavonoids in seed extracts varied from 1.48 to 3.05 mg QE/g DW. All the extracts analyzed showed high TFC. The data showed that the highest TFC was measured in the KU genotype and the lowest content was observed in U2 genotype. According with Mamati *et al.* (2006), different biosynthetic pathway of phenolic compounds and related enzyme expression in the growth stages can be effected by the phenolic content in plants. Baghiani *et al.* (2012) reported values of TFC which range between 23.5 and 298 mg QE/g in aerial parts and between 0.25 and 2.12 mg QE/g in roots. These authors found that ethyl acetate was the best solvent to extract flavonoids from plant material. This aspect could explain, in part, the differences found between this research and our data. Health benefits, especially the antioxidant potential of food, depend on the type and amount of flavonoids (Wojdyło *et al.*, 2019). As mentioned for TPC, the cultivar, genotypes and different caper organs (flowers, berries, leaves, seeds) also had a significant influence on the TFC content (Baghiani *et al.*, 2012b; Wojdyło *et al.*, 2019), which agree with the variations observed in the present study.

The antioxidant activity of extracts from the caper seeds of different genotypes is presented in Figure 4. The values for DPPH radical scavenging activity were directly dependent on the level of total phenolic compounds present in the methanolic extracts. The results of DPPH radical scavenging activity of seed extracts ranged between 35 and 63%. The highest free radical scavenging properties were obtained in the seed extract from the KU genotype followed by the extracts from AH>U1>U3>U2. The phenolic compounds and related antioxidant activity of different seeds were analyzed, and it was observed that there was a significant ( $P < 0.05$ ) linear correlation among TPC, TFC and antioxidant capacities (Katalinic *et al.*, 2006).

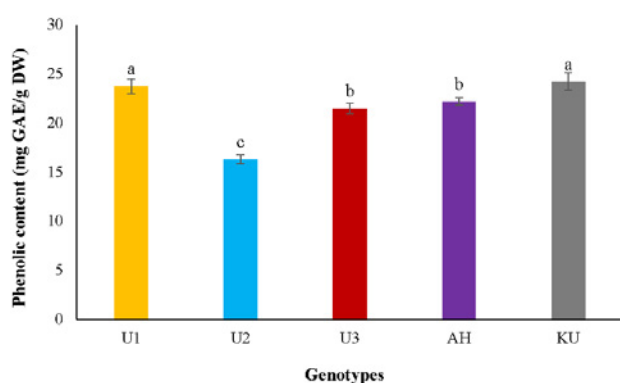


FIGURE 2. Phenolic contents (mg GAE/g DW) of methanolic extracts from caper seeds of different genotypes. <sup>a-c</sup> Means (Three experiments) with different letters within treatments were significantly different at the level of  $P < 0.05$ . Mean comparisons of genotypes were carried out by Duncan's Multiple Range Test (DMRT) at  $\alpha=0.05$ .

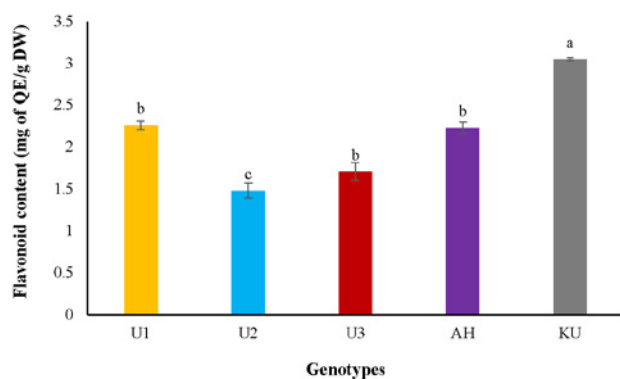


FIGURE 3. Flavonoid contents (mg QE/g DW) of methanolic extracts from caper seeds in different genotypes. <sup>a-c</sup> Means (Three experiments) with different letters within treatments were significantly different at the level of  $P < 0.05$ . Mean comparisons of genotypes were carried out by Duncan's Multiple Range Test (DMRT) at  $\alpha=0.05$ .

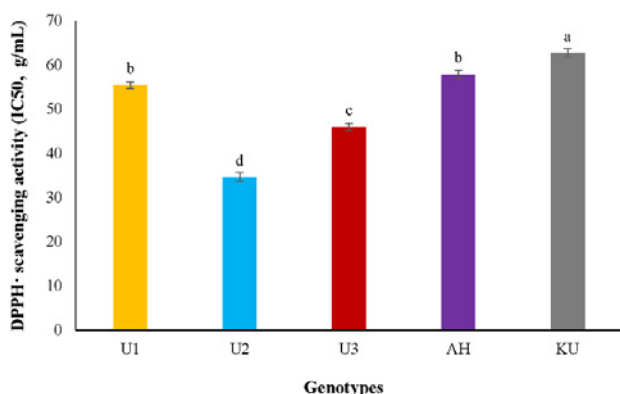


FIGURE 4. Antioxidant activity (%) of methanolic extracts from caper seeds in different genotypes. <sup>a-d</sup> Means (Three experiments) with different letters within treatments were significantly different at the level of  $P < 0.05$ . Mean comparisons of genotypes were carried out by Duncan's Multiple Range Test (DMRT) at  $\alpha=0.05$ .

Wojdyło *et al.* (2019) found 34 flavonols, 10 hydroxycinnamic acids, and 5 flavan-3-ols in *C. spinosa* flowers. From flavonols, these authors found derivatives of quercetin, kaempferol, isorhamnetin, myricetin and rutin. They also found (+)-catechin and (-)-epicatechin and dimers and trimers from procyanidins (flavan-3-ols) and ferulic, sinapic, quinic, coumaroylquinic and caffeoylquinic acids and their derivatives (hydroxycinnamic acid derivatives). It is well known that these compounds present high antioxidant activity. El-Ghorab *et al.* (2007) found that the dichloromethane and methanol extracts from caper buds and leaves exhibited higher antioxidant activities than those of their essential oils in testing systems.

The KU genotype contained high amounts of TPC and TFC and showed high antioxidant activity. Several studies have reported that the phenolic compound in plants through their scavenging or chelating activity are associated with their antioxidant activities, their free radical scavenging activity is attributed to free hydroxyl groups (Chang *et al.*, 2001; Ghafar *et al.*, 2017; Ghimire *et al.*, 2011; Ibrahim and El-Masry, 2016). In addition, flavonoids, in fact are a large group of plant phytochemicals as powerful antioxidants with potent free radical scavenging properties and whose utilization as regulators of redox-sensitive signaling pathways is of great interest (Izzi *et al.*, 2012). Thus, the variation in antioxidant activity between genotypes was directly related with the phenolic contents. Some studies on the phytochemical composition of various extracts from *C. spinosa* have proven the presence of several bioactive compounds such as vitamins, polyphenols and flavonoids, which are known for antioxidant properties which extend the shelf-life of foods (Matsuyama *et al.*, 2009). Tlili *et al.* (2010) reported that *C. spinosa* extract is rich in phenolic compounds and vitamin antioxidants and can be used as a promising constituent for increasing the nutritional and medicinal properties of foods.

### 3.5. Principal component (PCA) and hierarchical cluster analysis (HCA)

The scores for the principal component analysis of caper genotypes are presented in Figure 5A. The first two principal components accounted for 78% (PC1 = 25% and PC2 = 53%, respectively) of the total variation. The characteristics that contributed positively to PC1 were oil content, linoleic acid, stearic acid, antioxidant activity, TPC, and TFC; whereas negative contributions were observed for stearidonic acid, arachidic acid,  $\gamma$ -linolenic acid, palmitoleic acid,  $\alpha$ -linolenic acid and palmitic acid. PC2 was mainly correlated positively with palmitic acid and palmitoleic acid.

The biplot showed three distinct groups. The first group, comprised of two genotypes (U2 and U3) and was collated from the Urmia province. It was characterized by high amounts of stearidonic acid,  $\gamma$ -linolenic acid and arachidic acid along with the lowest amount of total phenols.

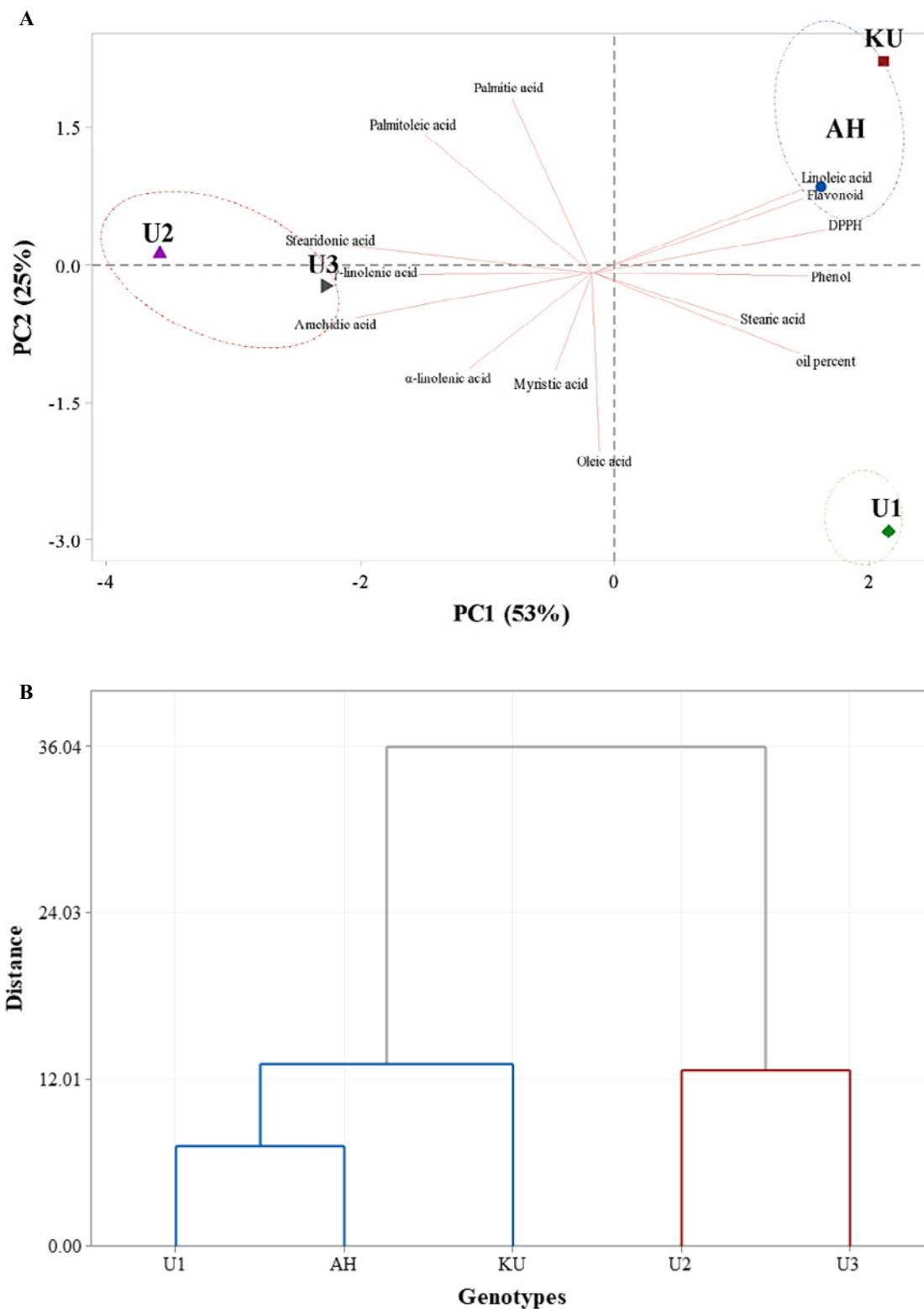


FIGURE 5. A. Principal component analysis (PCA) of caper genotypes; B. Hierarchical cluster analysis (HCA) caper genotypes



The second group included AH and KU genotypes and was characterized by the highest contents in linoleic acid, TFC and antioxidant activity by DPPH assay confirmed that antioxidant activity was mainly due to the presence of flavonoid compounds in caper seeds. The third group (U1 genotype) had the highest amount of oil content, stearic acid and phenols.

A cluster analysis (HCA) of 5 genotypes of capers is shown in Figure 5B. The cluster analysis was performed using antioxidant activity (by DPPH assay), total phenol, total flavonoid, oil content and fatty acid composition. In this case, 5 genotypes were divided into two main clusters. The U2 and U3 genotypes were grouped into the first cluster. They had high amounts of stearidonic acid,  $\gamma$ -linolenic acid and arachidic acid and the lowest amounts of total phenols, total flavonoids, antioxidant activity and oil content. The second cluster was comprised of three genotypes including U1, AH and KU with high amounts of linoleic acid, stearic acid, total phenols, total flavonoids, oil content and antioxidant activity.

Generally, variation in fatty acid composition, TPC, TFC and antioxidant activity was detected among the genotypes grown in West Iran. Genetic variability in capers may be utilized in trait-specific breeding programs. These results showed that the seeds of capers are promising sources of natural antioxidants, unsaturated fatty acids and bioactive compounds which are beneficial to the food and pharmaceutical industries. Based on the obtained results, U1 and KU genotypes are the most suitable to be used in breeding programs due to their unsaturated fatty acids, phenolic compounds and antioxidant activity.

#### 4. CONCLUSIONS

To the best of our knowledge, this report is the first study on oil content and fatty acid composition combined with phenolic compounds and antioxidant activity in *C. spinosa* seeds collected from different genotypes from Iran. The oils obtained from *C. spinosa* seeds of different genotype had different chemical compositions and antioxidant activity. The extracted oil from U1 constituted high levels of oil content and unsaturated fatty acids. The extract from KU was rich in TPC, TFC and showed the highest free radical scavenging activity. In conclusion, the *C. spinosa* seeds showed efficient antioxidant potential and can be an alternative for synthetic antioxidants in food and pharmaceutical products. In addition, the high oil content and special unsaturated composition make *C. spinosa* seeds a potential healthy oil source which can be used in the reformulation of several foods.

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