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Lipid classes and fatty acid composition of *Thapsia garganica* L. seeds oil

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Abstract. This study focused on characterizing the seed oil of *Thapsia garganica* (Apiaceae), a medicinal plant native to Laghouat, Algeria, and evaluating its antioxidant properties. Various solvent systems were employed to extract and fractionate the lipid content of *T. garganica* seeds oil. Chemical indices were determined, and fatty acids methyl esters were analyzed using GC/MS. Tocopherol composition was assessed via HPLC, and antioxidant activity was evaluated using the 2,2-diphényl 1-picrylhydrazyle (DPPH) method. The GC/MS analysis revealed distinct fatty acid profiles across various fractions, highlighting a notable presence of petroselinic acid and higher-than-usual levels of pentadecanoic acid in all fractions. *T. garganica* oil exhibited richness in tocopherols, particularly with α-tocopherol being the predominant homolog. The antioxidant activity assessment of different lipid fractions indicated potent activity within polar lipids (glycolipids and phospholipids). Furthermore, *T. garganica* oil was abundant in unsaturated fatty acids, notably petroselinic acid, displaying significant radical scavenging activity in its polar fractions.

Keywords: *T. garganica*, petroselinic acid, pentadecanoic acid, tocopherols, antioxidant activity.

Introduction

The Apiaceae family distinguishes itself among plant families with its umbel inflorescence and singular-seeded fruits. Comprising species native to temperate regions, they have garnered extensive use as spices or medicines due to their abundant secondary metabolites (Heywood, 1971). Numerous plants within the Apiaceae family, particularly those belonging to the genus *Thapsia*, are recognized for their significant concentration of petroselinic acid (*cis*-6 18:1) found in their seed oil (Avato *et al.*, 2001; Ngo-Duy*et al.*, 2009). Because of the unsaturation at carbon 6, this fatty acid has potential industrial significance (Cahoon, 1992).

T. garganica L. stands as a significant medicinal plant, the subject of numerous scientific studies (Makunga *et al*., 2003; Nebeg *et al*., 2019). Historically, this ancient medicinal herb featured in various European remedies until 1937 (Jäger *et al*., 1993). Widely distributed across several regions of Algeria, *T. garganica* has been traditionally employed to alleviate rheumatism (Aït Youssef, 2006). Notably, original data on the lipid fraction isolated from its seeds are lacking. Our study aims to characterize the lipid fraction of *T. garganica* seed oil, contributing to a deeper understanding and valorization of this medicinal species.

Materials and methods

Sample preparation

The fruits of *T. garganica* were collected from the Sidi Makhlouf region in Laghouat, Algeria. Professor Yousfi Mohamed identified the *T. garganica* plant, and a voucher has been deposited in the herbarium of the Laboratoire des Sciences Fondamentales at Laghouat University (voucher number: Tg-s 2022). These fruits exhibit an elliptical shape, ranging from 1 to 2 cm in length and nearly 1 cm in width, characterized by strongly winged margins. The wings, finely striated and bright yellow in color, adorn the green-hued fruit. Following collection, the fruits were air-dried at room temperature. Subsequently, the seeds were separated from the umbels and wings, then pulverized using a mortar in preparation for the extraction procedure.

Chemical reagents

All chemicals were purchased from Sigma (USA), Aldrich (Milwaukee, USA), FlukaChem (Buchs, Switzerland) and Merck (Germany).

Lipid quantification

The powdered seeds of *T. garganica* underwent maceration with petroleum ether as the extraction solvent for 24 hours, shielded from light. Following maceration, the extract underwent filtration and drying with an excess of anhydrous sodium sulfate. Subsequently, the petroleum ether extract was evaporated under reduced pressure at 40°C and then stored at 6°C.

Lipids were extracted using Folch's method (Folch *et al*., 1957) from *T. garganica* seeds. The lipid fraction was obtained by macerating and mixing the seeds at room temperature in chloroform:methanol (2:1) for 24 hours. After extraction, the mixture underwent filtration, and the recovered solution was washed with a 9% NaCl solution. The chloroform fraction, containing the total lipids, was then filtered and dried with an excess of anhydrous sodium sulfate before being evaporated under vacuum at 40°C. The resulting oil was stored at 6°C. Subsequently, the crude *Thapsia* oil was fractionated using silica gel column chromatography (Rouser *et al*., 1967). For this, 9 g of the lipid extract were added to the column along with 10 g of activated silica gel. Elution was carried out successively using chloroform, acetone, and methanol as eluents to separate neutral lipids, glycolipids, and phospholipids, respectively.

Determination of chemical indices

The acid value (AV), saponification value (SV), and iodine value (IV) were determined following the protocols outlined in the French norm AFNOR (1984), specifically AFNOR NFT 60-204, AFNOR NF T 60-206, and AFNOR NF T 60-203, respectively. AFNOR standards encompass a wide range of topics including analysis methods, quality standards, good manufacturing practices, and regulatory requirements. These standards play a crucial role in ensuring methodological consistency and quality control. Additionally, they establish a framework for assessing the safety, purity, and nutritional content of fats and oilseeds, thereby facilitating evidence-based research and informed decision-making across various disciplines such as nutrition, food science, agriculture, and biochemistry.

GC-MS analysis of fatty acids methyl esters

The fatty acid methyl esters (FAMEs) of total lipids, neutral lipids, glycolipids, and phospholipids were prepared using the standard boron trifluoride procedure BF3. Initially, the oil underwent hydrolysis in the presence of methanolic potassium, followed by esterification in the presence of a 10% w/v boron trifluoride-methanol complex. Subsequently, the methyl esters were recovered through liquid-liquid extraction using hexane after the addition of water. Fatty acid analysis was

conducted by Gas Chromatography/Mass Spectrometry (GC/MS) using a 1 HP 5890 Series II chromatograph. Separation was achieved using a silica gel capillary column (60 m x 0.25 mm) grafted with a DB-Wax stationary phase of 0.2 µm thickness. The analysis involved temperature programming and the use of hydrogen as the carrier gas. Identification of FAMEs was facilitated by comparing retention indices and mass spectra with reference standards.

Tocopherol content

The tocopherol composition was analyzed using a Waters HPLC system equipped with a nonpolar reversed-phase (RP18) column and a fluorescent detector. A diluted sample of twenty microliters in pentane (representing neutral lipids of *Thapsia* oil) was isocratically eluted with HPLC-grade methanol and water (92:8 v/v). Tocopherols were detected using fluorimetry, with excitation at 290 nm and emission at 330 nm. By comparing peak areas, the relative contents of tocopherols in the extract were determined using external standards for α, β, γ, and δ-tocopherol.

Antioxidant activity analysis using DPPH radical scavenging test

The antiradical activity of various lipid classes was assessed using the DPPH method (Molyneux, 2004). Different concentrations of the analyzed fraction were prepared, and each dilution (100 µL) was added to 1 mL of a 250 µM ethanolic solution of DPPH. After 30 minutes, the absorbance at 517 nm was measured. The following equation was utilized to determine the DPPH radical scavenging capacity:

$$\text{Inhibition (\%)} = [1 - (\text{Test sample absorbance}/\text{Control absorbance})] \times 100$$

The EC50 value represents the concentration of substrate needed to reduce DPPH activity by 50% (Molyneux, 2004). Graphical analysis of inhibition percentage variation against concentration curves for each oil fraction was used to derive the EC50 value. This process was repeated three times for each lipid fraction, and the EC50 value was determined as the average of three repetitions.

Statistical analysis

Hierarchical Cluster analysis (HCA) of chemical data was performed with XlStat 2014.5.03. Ward's linkage method was used to determine the distance between clusters and Euclidean distance for their agglomeration. Differences between means were assessed by one-way ANOVA using Fisher's test at a level of 0.001 with Origin b9.3.226.

Results

Lipid quantification

The crude fat content extracted using petroleum ether 9.45%. Conversely, a mixture of two solvents (chloroform:methanol) extracted 22.4% of the weight of *T. garganica* seeds. Significantly divergent extraction performances were observed between petroleum ether and the solvent mixture (chloroform:methanol). This variation stems from the differing polarities of the solvents used; petroleum ether, being nonpolar, may not efficiently extract all lipids, primarily targeting neutral lipids such as triglycerides. Conversely, miscible solvents like methanol and chloroform have the capacity to extract a broader spectrum of lipids, particularly polar lipids like glycolipids and phospholipids. Additionally, differences in extraction rates may also be influenced by sample type variability. Similar to many other plant species, *T. garganica* crude oil exhibited a high proportion of neutral lipids, constituting 83.37% of the total, in contrast to minor lipids represented by modest levels of glycolipids (5.82%) and phospholipids (1.93%).

Determination of chemical indices

Assessing the quality and structural stability of the oil can be achieved through the examination of its acid value (AV), saponification value (SV), and iodine value (IV), as detailed in Table 1.

Table 1. Chemical characterization of *T. garganica* oil

AV (mg KOH/g)	SV (mg KOH/g)	IV (Wijs)
8.16±0.3	206.04 ± 0.8	87.65±0.5

AV: acid value, SV: saponification value, IV: iodine value; SD: standard deviation.

Analysis of fatty acids methyl esters

Table 2 illustrates the relative proportions of various FAMEs found in crude oil and across different lipid classes. It highlights a prevalent presence of unsaturated fatty acids with diverse profiles within these classes. Specifically, total lipids, neutral lipids, and phospholipids exhibit notable concentrations of linoleic acid (41.37%, 40.32%, and 35.353%, respectively). Conversely, the glycolipid fraction demonstrates a higher abundance of linolenic acid (73.35%) and encompasses a broad spectrum of fatty acids, including short-chain (C8:0, C14:0), as well as an unusual fatty acid, pentadecanoic acid (C15:0).

In our investigation, we observed the presence of two isomers of C18:1 in the phospholipids chromatogram, manifested as two distinct peaks with respective retention times of 46.8 and 46.9 minutes. Upon analyzing chromatograms of total lipids, neutral lipids, and glycolipids, we identified peaks at 46.8, 46.7, and 46.7 minutes, respectively, corresponding to petroselinic acid. However, in the case of phospholipids, the ratio of C18:1 acid was calculated by summing the ratios of the two peaks representing the two isomers: petroselinic (C18:1ω6) and oleic (C18:1ω9) acids.

Multivariate analysis was employed to compare the fatty acid profiles of *T. garganica* oil with those of other species within the *Thapsia* genus and certain seed oils from the Apiaceae family (Avato *et al*., 2001; Ngo-Duy *et al*., 2009). This analytical approach helps to simplify the graphical representation of complex multivariate data and illustrate their underlying patterns.

As depicted in Fig. 1, notable differences in fatty acid levels are evident between the studied *T. garganica* (1) and the five species within the *Thapsia* genus, including *T. garganica* (2), as reported by Avato *et al*. (2001), who noted a very similar composition among these five species. To enable a meaningful comparison of this oil with other vegetable oils, a similar statistical analysis was conducted, as depicted in Fig. 2.

Table 2. The relative proportions on various fatty acids of crude oil and the various classes of lipids

Fatty acid	Sample			
	TL	NL	GL	PL
C8:0	-	-	14.458	-
C14:0	-	-	0.695	-
C15:0	-	-	0.336	-
C16:0	19.499	18.908	18.023	27.064
C16:1	-	-	0.808	-
C18:0	5.971	5.069	1.585	2.425
C18:1	28.364	33.911	31.813	32.801
C18:2	41.368	40.315	23.893	35.353
C18:3	2.612	1.069	7.335	2.07
C20:0	1.318	0.727	0.480	0.287
C22:0	0.573	-	0.573	-
C24:0	0.294	-	-	-

TL: total lipids, NL: neutral lipids, GL: glycolipids, PL: phospholipids.

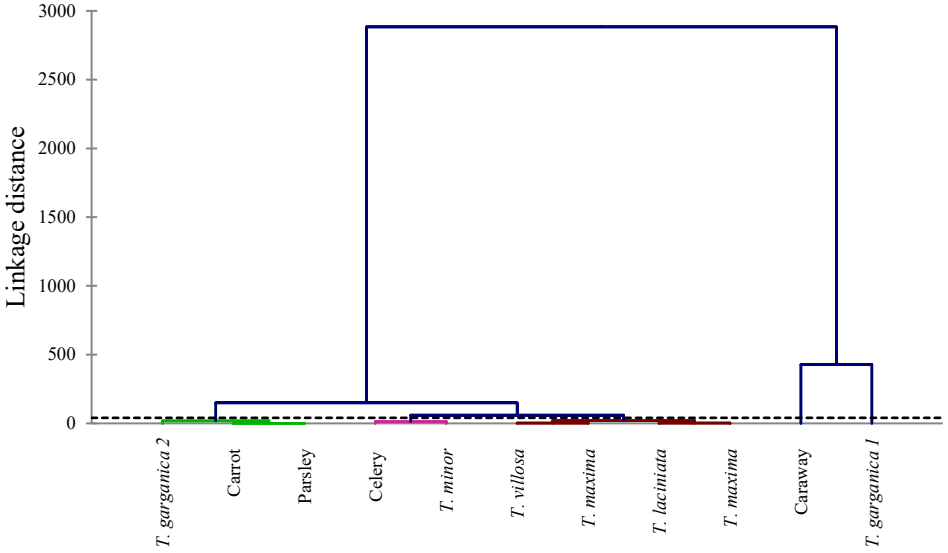

Figure 1. Cluster analysis (Ward's method) of *T. garganica* and some plants of same genus and family.

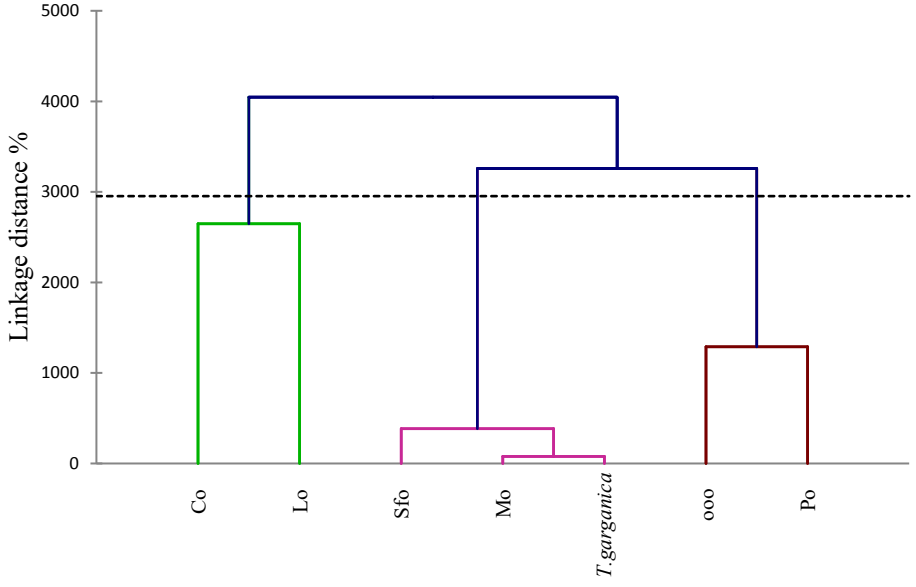

Figure 2. Cluster analysis (Ward's method) of *T. garganica* and some vegetable oils (MO, maize oil; SFO, sunflower oil; OOO, Orujo olive oil; CO, coconut oil; LO, linseed oil; PO, palm oil).

Tocopherol content

The total tocopherol content in *T. garganica* oil measured 940.62 mg/kg (Table 3). Comparatively, vitamin E-active compound levels in other plants range from 430 to 2680 mg/kg in rapeseed oil and 600 to 3370 mg/kg in soybean oil (Matthäus and Özcan, 2015). Consequently, *T. garganica* seed oil emerges as a potential source of antioxidants. The primary tocopherol compounds in *T. garganica* seed oil were α-tocopherol homologs, followed by (β+γ)-tocopherol. Notably, all obtained contents exhibited significant differences at $P \leq 0.001$. The α-tocopherol content (516.84 mg/kg), boasting the highest vitamin E concentration, aligns with ranges observed in corn (23–573 mg/kg) (Rossell and Pritchard, 1991), golden apple (514 mg/kg), starking apple (544 mg/kg), quince (496 mg/kg), and chufa (685 mg/kg) (Matthäus and Özcan, 2015).

Table 3. Tocopherol content of *T. garganica* oil (μg/g of oil)

α-tocopherol	[β+γ]-tocopherols	δ-tocopherol	Total tocopherols
516.84	394.49	29.29	940.62

Antioxidant activity

Table 4 presents the results of the DPPH test. Each lipid fraction displayed distinct inhibition potentials, with lower EC50 values indicative of higher antioxidant activity. Significantly divergent activities were observed among the lipid fractions ($P \leq 0.001$), as well as in comparison with Vit E and Vit C. Notably, the difference between the latter two was insignificant at this same significance level.

Table 4. The results of DPPH test

Lipid fractions	IC50 (g/l)§
NL	5.38± 0.28
TL	2.41 ± 0.042
PL	1.44 ± 0.12
GL	0.976 ± 0.1
Vit E	0.026 ±0.001
Vit C	0.008 ± 0.002

TL: total lipids, NL: neutral lipids, GL: glycolipids, PL: phospholipids, Vit-E: vitamin E, Vit-C: vitamin C. § The values are significantly different at P≤0.001 according to Fisher's test except for Vit-E and Vit-C which are not significantly different at this level.

Discussion

The seeds of *T. garganica* cannot be classified as oleaginous akin to peanut, olive, and sunflower seeds, which typically contain 30-40% oil (Karleskind, 1992). The elevated acidity value suggests a significant presence of free fatty acids, likely stemming from either grain maturity or inadequate oil preservation. A high iodine value may indicate a substantial quantity of unsaturated bonds (unsaturated fatty acids). According to our analysis, the saponification number (206.04 mg KOH/g) closely resembles that of date seed oil (207.8 mg KOH/g) (Boukouada *et al*., 2014) and is relatively comparable to those of olive (184–196 mg KOH/g), corn (187–195 mg KOH/g), and cottonseed (189–198 mg KOH/g) oils (Rossell and Pritchard, 1991). Additionally, *T. garganica* oil is characterized by medium chain-length and unsaturated fatty acids, as inferred from the inverse relationship between saponification value and fatty acids weight.

In this study, a comparison was made with several other investigations focusing on oils within the Apiaceae family, revealing the abundant presence of petroselinic acid, an isomer of oleic acid, among its members (Avato *et al*., 2001; Ngo-Duy *et al*., 2009). Chromatographic separation of oleic and petroselinic acids indicates that these two isomers possess similar chain lengths (LCE) on a polar stationary phase, differing by approximately 0.04 to 0.06 carbon units. Theoretically, this slight difference enables some separation between the two fatty acids, although resolution largely depends on operational conditions (Wolff, 1995). Furthermore, a multivariate analysis was employed to compare the fatty acid profiles of *T. garganica* oil with those of other *Thapsia* genus species and selected seed oils within the Apiaceae family (Avato *et al.*, 2001; Ngo-Duy *et al.*, 2009).

The multivariate analysis reveals that this dissimilarity stems from variations in the percentages of C18:1 acid isomers, likely influenced by differences in sample variety and origin. Within the same cluster, *T. garganica* oil exhibits a comparable fatty acid profile to that of caraway seeds (carrot, celery, and parsley). The hierarchical cluster analysis (Fig. 2) delineates three structures, segmented into two levels. *T. garganica* is positioned at the second level, clustering with corn rather than sunflower, forming a unified class with similar fatty acid profiles (Pinzi *et al*., 2011).

Despite its lower concentration, δ-tocopherol exhibits greater antioxidant potency in the oil compared to other tocopherols. Notably, its concentration surpasses that found in other oils such as coconut (ND-2 mg/kg), cottonseed (ND-17 mg/kg), groundnut (ND-3–22 mg/kg), and sunflower (ND–7 mg/kg) (Rossell and Pritchard, 1991). Glycolipids and phospholipids emerged as more

effective DPPH radical scavengers, representing a promising finding given their significantly lower EC50 values compared to standards (vitamin E and vitamin C). While phospholipids are recognized natural antioxidants, further research is warranted to elucidate the mechanism behind the radical scavenging activity of these two fractions from *T. garganica* oil. The antioxidant activity of total lipids exceeded that of neutral lipids, likely attributed to the presence of various molecules such as tocopherols, glycolipids, phospholipids, and other polar active compounds present in the crude oil, which are retained in the polar mixture of two solvents (chloroform/methanol).

Conclusion

Describing the seeds of *T. garganica* as oil-bearing would be inaccurate, given the relatively low oil content obtained using non-polar solvents (9.45%). However, this oil is notable for its richness in unsaturated fatty acids, particularly with a significant presence of petroselinic acid. Moreover, its tocopherol content, primarily consisting of α-tocopherol, along with the pronounced radical scavenging activity of its polar fractions (PL and Gl), positions *T. garganica* oil within the realm of other plant materials sought after for their bioactive components or pharmaceutical applications.

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