Unripe Fruits of *Pistacia Terebinthus* from Algeria: Fatty Acid Composition, Tocopherols, Sterols, Carotenoids, Phenolic Compounds, and Free Radical Scavenging Activity

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Abstract:

This study aimed to determine the lipid and phenolic compound content of immature *Pistaciaterebinthus*fruits and their anti-radical activity. The results show that red fruits have a low lipid yield (0.98 and 2%), rich in tocopherols, sterols, and carotenoids. The proportion of unsaturated fatty acids is higher (65.19 to 77.30%) than saturated fatty acids in all lipid classes except glycolipids and phospholipids. Immature fruit cakes are high in total phenols (4.05 and 271.58 mg EAG/g cake), low in tannins (0.50 to 3.40 mg ECAT /g cake), and lacking in flavonoid compounds. The antioxidant activity of total lipids, neutral lipids, and phenolic extracts from immature fruit is high (1.50, 1.076 mg EVE/g lipid and 43.17µg EVC/g oilcake), respectively.

Keywords: Pistaciaterebinthus, unripe fruit, lipids, phenolic compounds and antioxidant activity.

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Introduction

*Pistaciaterebinthus*is a shrub belonging to the Anacardiaceae family. It has a single or divided trunk. It generally has a well-balanced, rounded crown. Its leaves are deciduous, consisting of 7 to 11 oval leaflets, an uneven number that induces the presence of a terminal leaflet (differentiating it from pistachio lentiscus). Mature terebinth fruits are small, globular, dark green, and ripe in September-October, widespread in Mediterranean countries, Western Asia, the Middle East, and the USA. Pistachio usually grows on dry, rocky slopes, hillsides, and forests [1]. In Algeria, pistachio terebinth is found in western Algeria and the steppe [2].

Lipids are biomolecules that are practically insoluble in water and soluble in apolar organic solvents and are divided mainly into two classes: saponifiable fractions, such as fatty acids, and unsaponifiable particles, such as tocopherols, sterols, and carotenoids [3].

Antioxidants play an essential role in human metabolism. The biochemical reactions in our bodies produce free radicals, initiating oxidation chain reactions that harm our body's cells, damaging them and accelerating the aging process. Typically, the human body maintains the balance between antioxidants and free radicals by simultaneously producing both types of substances in the metabolic process. The imbalance between these two types of compounds leads to a phenomenon known as oxidative stress. The initiation of oxidative chain reactions in the human body can lead to pathologies such as atherosclerosis, cancer, heart attacks, allergies, rheumatism, and others [4].

Extracts of pistachio species have antioxidant activity in addition to antimicrobial, anti-inflammatory, and cytotoxic activities due to their high phenolic compound content. The extracted oil from *Pistaciaterebinthus*fruits is an alternative to vegetable oils, as it contains high amounts of monounsaturated (oleic acid) and omega-3 (linolenic acid) fatty acids and has a pleasant smell and taste [5].

In traditional medicine, the fruit treats gastralgia, rheumatism, coughs, eczema, diarrhea, throat infections, asthma, and heartburn. In addition, some effects are described as stimulating, diuretic, antitussive, astringent, antipyretic, and antibacterial [1].

*Pistaciaterebinthus*is a tree that could be more abundant in our country and supplies fruits rich in used fats.

Experimental

Methods and materials

The chemicals used in this work are of a high analytical standard: acetone, chloroform, methanol, absolute ethanol, hexane, ethyl acetate, dichloromethane, anhydrous sodium sulfate, sodium methanoate ($NaOCH_3$ 0.5% prepared in our laboratory), cyclohexane, silica gel, α-tocopherol (vitamin E), β-sitosterol, β-carotene, 1,10-orthophenanthroline, 1,1-diphenyl-2-picrylhydrazyl (DPPH.), Folin-Ciocalteu agent, sodium bicarbonate, catechin, ascorbic acid (vitamin C) and vanillin were brought from Sigma-Aldrich. BIOCHEM-Chemopharma supplied chloroform.

The unripe fruits of *Pistaciaterebinthus*used in this study were collected from a pistachio tree in the GueltetSidiSaad region (about 130 km northwest of Laghouat) in November 2020. The fruits were ground by hand, then stored in a dark, dry place until used.

Lipid extraction and quantitative analysis

Lipids were extracted by two methods, initially using Folch's solvent system (chloroform: methanol, 2:1 V/V) to obtain total lipids (neutral lipids (NL), glycolipids (GL) and phospholipids (PL)), then using hexane to extract apolar lipids. Lipids are extracted using the Soxhlet device. A precisely defined quantity of ground plant material was extracted separately using the above solvents. The extract obtained with Folch's solvent was washed thrice with a 100 ml chloroform to recover the total lipids (TL). The aqueous solution of NaCl (9% w/v) helps reduce the loss of lipids, thereby increasing extraction yield and eliminating phenolic and protein compounds. The two extracts obtained were dried over anhydrous sodium sulfate and filtered. The solvents were vaporized under reduced pressure using a rotary evaporator at 40°C. The lipid extracts obtained were kept refrigerated in hermetically sealed bottles until analysis.

Lipid class fractionation

Fractionation is performed on an open chromatographic column. A quantity of 15 g of silica gel is put into the column with a volume of 100 ml of chloroform while allowing the solvent to flow freely, followed by adding 450 mg of total lipid extract, which has been solubilized in the same solvent. Various pure solvents were used for lipid elution: chloroform to elute neutral lipids (NL), acetone to collect glycolipids (GL), and methanol to recover phospholipids (PL). After drying the solvents on anhydrous sodium sulfate and filtration, the solvents are evaporated under reduced pressure at 40°C. The dry extracts obtained are weighed to determine the percentage of each lipid class.

Fatty acid methyl ester (FAME) synthesis

In a flask, 200 mg of the lipid extracts obtained by different solvents were mixed with 25 mL of sodium methanoate CH_3ONa (0.5%, m/v). The mixture was boiled under the reflux for 20 minutes, then 20ml of distilled water was added. The aqueous phase comprising the fatty acids was extracted twice with cyclohexane. The organic phase obtained was washed several times with distilled water until neutralized and dried with anhydrous sodium sulfate before evaporating under reduced pressure.

The resulting fatty acid methyl esters (FAMEs) were stored in a refrigerator until further analysis.

The FAMEs were analyzed by aqueous phase chromatography (GPC):

- Instrument model: SHIMADZU GCMSQP2020 ;
- Injector temperature: 250°C in split mode (30:1);
- Injection volume: 0.5µl (10% dilution with hexane);
- Oven temperature setting:

- One step at 70°C for 1 min;
- Heating 15°C/min to 160°C;
- Level at 160°C for 2 min;
- Heating 7°C/min to 260°C;
- 260°C for 25 min;
- Heating 7°C/min to 330°C;
- One step at 330°C for 10 min.

– The used column: Rxi®-5ms (fused capillary column: 5% diphenyl / 95% dimethyl polysiloxane);

– Column dimensions: 30 m × 0.25 mm and 0.25 µm film thickness;

– Carrier gas: Helium;

– Flow rate 1mL/min.

Determination of total tocopherols

We followed the colorimetric method of Emmerie-Engel [6, 7]. The reductive properties of tocopherols reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in an ethanolic solution. The latter are complexed by orthophenantroline, producing a stable red-orange complex with a high molar extinction coefficient at 510 nm. A calibration line has been derived from commercial α-tocopherol, linking optical density and tocopherol concentration in grams per liter (g/L). Using a commercial vitamin E solution, we prepared solutions with fixed concentrations ranging from 0.01 to 0.05 g/L in ethanol. Each solution was mixed with 1 ml orthophenantroline reagent (0.4%) and 0.5 mL ferric chloride (0.12% ethanolic solution). The mixture was incubated in the dark for 5 min. Absorbance readings were taken at 510 nm by UV/Vis spectrophotometer (Shimadzu 1800) against a blank. Lipid extracts from each sample were processed similarly to the calibration curve for α-tocopherol. Results are expressed as mg tocopherol equivalent per gram of lipid (mg TE/g lipid). All measurements were performed in triplicate.

Determination of total sterols

According to the Liebermann-Burchard test [8, 9], this involves spectrophotometric absorption based on a color reaction specific to 3 β-hydroxysteroïdes possessing a double bond in position 5-6. Sterols form a stable complex with acetic anhydride in acidic media that absorbs in the visible at a wavelength of 550 nm.

We produced a calibration curve for this phytosterol using chloroform solutions of β-sitosterol at different concentrations ranging from 0.447 to 2.235 g/L. Each 1 mL of diluted solution was mixed with 2 mL of Liebermann's reagent. The mixture was incubated in the dark for 25 min at room temperature. The absorbance of each solution was measured at 550 nm against a blank on a UV/Vis spectrophotometer (Shimadzu 1800). Lipid extracts were treated in the same way, and

the total sterol content of each extract was determined from the β-sitosterol calibration curve. All measurements were performed thrice, and the mean readings were recorded. Results are expressed as mg β-sitosterol equivalent per gram of lipid (mg EβS /g lipid).

Determination of total carotenoids

β-carotene is generally the most abundant and joint compound in fats of plant origin. The total carotenoid content was obtained by modifying the Talcott and Howard method according to the following procedure:

Various concentrations of β-carotene in chloroform ranging from 0.012 to 0.07 g/l were prepared for the calibration curve. Each 2 mL diluted solution was sampled and measured at 464 nm against a solvent-blank medium. The same steps were followed for lipid samples, with carotenoid content based on the calibration curve performed with β-carotene. All measurements were performed thrice, and average readings were recorded. Results are expressed as mg β-carotene equivalent per gram of lipid (mg EβC/g lipid) [9].

Extraction and quantification of phenolic compounds

To extract phenolic compounds, we used six solvent systems: Methanol, Acetone, Methanol/Water with a ratio of (8/2 v/v), Acetone/Water with a ratio of (7/3 v/v), Ethanol/Water with a ratio of (8/2 v/v), and Methanol/Acetone/Water with a ratio of (4/4/2 v/v/v).

About 5g of delipidated oilcake was macerated in 100 mL of each solvent system for twenty-four hours at room temperature in the dark.

Extracts were filtered through filter paper. The phenolic compounds were obtained by removing the organic solvent under reduced pressure in a rotary evaporator at 45°C, then performing a liquid-liquid extraction with ethyl acetate. The extracts were dried using anhydrous sodium sulfate (Na_2SO_4) to eliminate any remaining water. After filtration, the ethyl acetate was evaporated under a vacuum using a steam extractor at 40°C. The dry residue obtained was weighed and solubilized in 5 mL of methanol, except for the methanolic extract, which was solubilized in 20 ml of the same solvent. The resulting extracts were transferred to hermetically sealed vials and stored in the refrigerator until analysis.

Total phenolic compounds analysis

The Folin-ciocalteu reagent, which consists of phosphotungstic acid and phosphomolybdic acid, reacts with phenols in an alkaline medium to give a blue mixture of tungsten and molybdenum oxides. This coloration is proportional to the number of phenols and absorbs at a wavelength of 760 nm [8].

We prepared a range of gallic acid concentrations from 0.06 to 0.3 mg/mL for the calibration curve. Subsequently, 500 µL of Folin-Ciocalteu reagent (diluted 10-fold by distilled water) is mixed with 100 µL of each prepared solution. After two minutes, two milliliters of a 2% sodium bicarbonate solution (m/v) were introduced into the mixture. The whole was left to react for 30 min in the dark. Absorbance readings were taken at 760 nm by UV/Vis spectrophotometer (Shimadzu 1800) against a blank, and the results thus obtained were used to plot the calibration curve for gallic acid.

Sample extracts were processed according to the protocol used to prepare the gallic acid calibration curve. Total phenol contents are expressed as (mg EAG/g oilcake). All measurements were performed thrice, and the average reading was recorded.

Determination of condensed tannins

This test relies on the acid-induced condensation of polyphenolic compounds with vanillin and is specific for flavone3-ols [10]. Extracts of 200 µL are added to 1 mL of a vanillin/hydrochloric acid mixture (1%/8%: V/V) in test tubes. The latter are placed in a water bath for 20 min at 30°C. Absorbance readings are performed at 500 nm using a UV/Vis spectrophotometer (Shimadzu 1800) against a blank. Different concentrations ranging from 0.10 to 1.12 mg/mL were prepared from a catechin stock solution, enabling the calibration curve to be plotted. Tannin contents are expressed as mg catechin equivalent per g oilcake (mg EC/g oilcake). All measurements were repeated thrice, and the average readings were recorded.

Anti-free radical activity

Due to its analytical simplicity, we selected the DPPH assay to investigate the anti-free radical activity of the various extracts. 1,1-diphenyl1-2-picrylhydrazyl (DPPH.) is a stable radical with a specific absorption at 517 nm, giving it a violet color [11].

Antioxidant activity is measured by the decrease in absorbance of an alcoholic solution of DPPH- due to its reduction to the non-radical form DPPH-H by the hydrogen donor antioxidants (AH) present in the plant extract or by another radical species [12].

We assessed the antioxidant activity of the various extracts using calibration curves for vitamin C and E. Anti-radical capacity is expressed by VEEAC (mg EVE/g lipid) for lipid extracts and by VCEAC (µg EVC/g oilcake) for phenolic compounds, The concentration of vitamin E or vitamin C in a solution is defined as (g/L) with an antioxidant capacity equivalent to a 1(g/L) solution of the studied extract.

Lipid extracts were solubilized in dichloromethane. To 1 mL of each solution was added 1 mL of DPPH solution (250µM). Immediately afterward, the reaction mixture was vortexed and stored in the dark for 30 min at room temperature to allow the reaction to proceed. The absorbance of the reaction medium was measured at 517 nm against a blank by UV/Vis spectrophotometer

(Shimadzu 1800). Vitamin E was used as the lipid standard. All measurements were performed in triplicate.

It should be noted that for phenolic extracts, we proceeded with the same protocol adopted in the case of lipid extracts, where vitamin C was used as the standard antioxidant.

The free radical scavenging powers (FRSP %) of the DPPH radical were determined according to the following formula (1);

$$\mathrm{FRSP}(\%) = \left(\frac{\mathrm{Ac}-\mathrm{Ae}}{\mathrm{Ac}}\right) * 100$$

(1)

Results and discussion

Extraction yields

Lipid yields from *Pistaciaterebinthus*fruits are reported in Table 1. The yield of simple lipids is 0.98% (w/w) relative to plant material. However, this yield is slightly higher for total lipids extracted by the solvent system (chloroform: methanol 2:1 V/V), where it reaches 2% (w/w).

Table 1 shows that neutral lipids occupy much more of the crude extract than glycolipids and phospholipids. The fractionation of total lipids reveals the mass proportions of the different lipid classes (neutral lipids NL, glycolipids GL, and phospholipids PL), where the values of these proportions are: 65.87%, 27.91%, and 6.22%, respectively, compared to total lipids TL.

When looking at lipid yields, hexane extract lipids (HE) from Pistachios was compared to yields from *Pistaciaatlantica*fruits (which range from 2.78 to 28.50% m/m) during their ripening period and unripe *Pistacialentiscus*fruits (which contain 11.7% m/m) [13]. We conclude that the studied *Pistachio terebinth* fruits are very poor in fat, possibly due to the non-completion of their ripening stage (incomplete biosynthesis).

Table 1: Lipid yield (%)

Lipid type	**Yield (m/m) %**	
HexanolicextractlipidEH	0.98	relative to plant matter
Total lipidLT	2.00	
NeutrallipidLN	65.87	
GlycolipidGL	27.91	relative to total lipid LT
PhospholipidPL	6.22	

Fatty acid composition

Findings regarding the fatty acid composition of lipids in *Pistaciaterebinthus*fruits can be used to assess stability and nutritional quality. Accordingly, a higher degree of oil formation makes it more susceptible to oxidative degradation.

Gas chromatographic analysis reveals the presence of fatty acids commonly found in vegetable oils. The results, illustrated in Table 2, indicate that the fatty acids contained in the various extracts studied are: myristic acid C14:0 (0.79 to 5.89%), palmitic acid C16:0 (16.81 to 49.91%), linoleic acid C18:2 (25.45 to 43.01%) and linolenic acid C18:3 (46.40 to 51.85%). Although the high proportion of unsaturated fatty acids in *Pistaciaterebinthus*fruit lipids, C18:1 oleic acid was deficient (0.71 to 0.77%). Moreover, it is noteworthy that low proportions of behenic acid C22:0 (0.85 to 3.55%), lignoceric acid C24:0 (1.38 to 5.03%), and montanic acid C28:0 (1.85 to 4.17%), very long carbon chain fatty acids, were found in the lipid extracts obtained by the extraction system (Chloroform/methanol) analyzed in this study.

The saturated fatty acids in our lipids obtained by the two extractive systems are palmitic, myristic, behenic, lignoceric, and montanic acids. Palmitic acid is the most dominant of the saturated fatty acids, where its proportion reaches a maximum value of 49.91% in phospholipids (PL) and a minimum value of 16.81% in hexanolic extract lipids (HE). Myristic acid is typically found in all lipid extracts but in lower amounts, usually less than 0.79% in phospholipids (PL).

Unsaturated fatty acids include oleic, linoleic, and linolenic acids. Linolenic acid (C18:3) was the primary fatty acid detected in hexanolic extract lipids (HE) and total lipids (TL), with proportions of 51.85 and 46.87%, respectively. Linoleic acid was found in all lipids with proportions between 25.45 and 43.01%. Second place went to linoleic acid, with 25.45 and 43.01% proportions in hexanolic extract lipids (HE) and phospholipids (PL). Oleic acid came last, with proportions of 0.77% in glycolipids and 0.71% in total lipids (TL). On the other hand, the high percentage of omega fatty acids has been attributed to the early stage of development for linoleic (C18:2) and linolenic (C18: 3) acids [7].

The high proportion of linolenic acid in the lipids of unripe fruit may be explained by the high activity of the desaturase enzyme, where *Pistaciaterebinthus*fruits are still in the developmental (unripe) stage.

In addition, UFA/SFA quotient values ranged from 0.70 to 3.40 (Table 2). These quotients in hexane extract lipids (HE), total lipids (TL), and neutral lipids (NL) are higher than those in glycolipids (GL) and phospholipids (PL). However, the quotients are higher than those found in Argan oil seeds (2.1) [14] and Algerian Atlas Pistachio fruit oil (2.7) [15] and lower than those of Sorghum seed oils in Algeria (over 5.7) [16].

Table 2: Fatty acid composition of Pistaciaterebinthusfruit lipids %

	Hexanolic extract lipids	Lipids from (Chloroform/methanol) extracts			
	HE	TL	NL	GL	PL
C14 : 0	5.89	4.93	4.79	3.87	0.79
C16 : 0	16.81	24.09	20.06	37.91	49.91
C18 : 1	-	0.71	-	0.77	-
C18 : 2	25.45	17.61	26.15	37.39	43.01
C18 : 3	51.85	46.87	46.40	-	-
C22 : 0	-	0.85	-	3.55	1.16
C24 : 0	-	1.38	-	5.03	1.74
C28 : 0	-	1.85	2.60	4.17	-
Others	-	1.71	-	7.31	3.39
Total SFA	22.70	33.1	27.45	54.53	53.6
Total UFA	77.30	65.19	72.55	38.16	43.01
UFA / SFA	3.40	1.97	2.64	0.70	0.80

SFA: Saturated Fatty Acids; **UFA:** Unsaturated Fatty Acids.

Total tocopherol analysis

Identifying homologous tocopherols in *Pistaciaterebinthus*fruit lipids is significant due to their antioxidant effects and positive nutritional influences on human metabolism as biological antioxidants [17].

We calculated the total tocopherol content of various extracts from the α-tocopherol calibration curve. Values are expressed as milligram equivalents of α-tocopherol (vitamin E) per g lipid (mg EVE/g lipid).

The following formula (2) gives the "m" contents of tocopherols, sterols, and carotenoids

$$\mathrm{m} = \frac{A(moy)}{k} * d * v * \frac{1}{p} * 1000 \qquad (2)$$

Where;

A (avg): Average absorbance,

k: the inclination of the calibration curve,

d: dilution factor,

V: dilution volume in liters,

P: Test sample (lipid) in grams.

The results of total tocopherol quantification are reported in Table 3. Total tocopherol content varied between extracts, ranging from 17.62 to 36.45 mg EVE/g lipid. The maximum value was recorded in hexanolic extract lipids (HE), while the minimum value was recorded in neutral lipids (NL). This result demonstrates that tocopherols are more soluble in hexane than in the solvent system (chloroform: methanol), as the latter is more polar than hexane, and tocopherols are considered fat-soluble vitamins (extractable by apolar organic solvents, such as hydrocarbons).

Given the unavailability of data on oils from unripe *Pistaciaterebinthus*fruit, we compared our results with those of the ripened *Pistaciaterebinthus*fruit studied by Özcan& al (396.8 - 517.7 mg/kg oil) [1], the oils from the ripening fruits of *Pistaciaatlantica*in Chelghoum& al(9.09 - 18.34 mg/g oil) [5], and the immature fruits of *Pistaciaatlantica*in Bentireche& al (219.55 mg /100 g oil) [18]. Based on this comparison, we can conclude that our lipids from immature *Pistaciaterebinthus*fruits are highly rich in total tocopherols. Therefore these lipid extracts could be put to therapeutic use. This result is unsurprising, as previous studies have stated that immature fruit oils are always richer in tocopherols than mature fruit oils.

Table 3: Total tocopherol, sterol and carotenoid contents

	Tocopherols content	Sterols content		Carotenoids content	
Hexane extractlipid (HE)	36.45± 0.88	759.08	105.29	7.99	0.04
Total lipid (TL)	21.51 ± 1.10	150.35	32.63	34.63	1.67
Neutrallipid(NL)	17.62± 0.14	711.93	22.3	0.76	0.06

Tocopherols (mg EVE/g lipid) ; Sterols (mg EβS/g lipid) and Carotenoids (mg EβC/g lipid).

Determination of total sterols

The results of total sterol quantification are shown in Table 3, expressed as mg β-sitosterol equivalent per gram of lipid (mg EβS / g lipid). Total sterol values ranged from 150.35 to 759.08 mg /g lipid. The lowest amount was recorded in neutral lipids (NL), while hexanolic extract lipids (HE) recorded the highest total sterols. In the absence of data on sterol levels in immature

*Pistaciaterebinthus*fruit oils, and in comparison with other studies such as mature *Pistaciaterebinthus*Chia fruit oils, where total sterol levels ranged from (1341, 3 to 1802.5 mg/kg oil) [19] and *Pistaciaatlantica*gall lipids with (42.14 to 112.75 µg/g oil) [20], it can be seen that the sterol contents in the lipids studied are very high, particularly in the apolar lipids of (HE and NL).

Total carotenoid quantification

The total carotenoid quantification values are summarized in Table 3, reported as milligrams of β-carotene equivalent per gram of lipid (mg EβC/g lipid).

Carotenoid values range from 0.76 to 34.63 and mg /g lipid. Neutral lipids (NL) were the most abundant, while total lipids (TL) were the least abundant. No studies have been conducted to determine the carotenoid content of immature Pistachio fruit oils, except for Chelghoum& al [5]. Compared to our results and those of Chelghoum& al (206.96 to 1248.20 µg/g oil), we can conclude that lipids in unripe *Pistaciaterebinthus*fruit are 1000 times higher than in *Pistaciaatlantica*fruit oils.

The location of this tree at altitudes of 1200 m and its exposure to solar radiation explain the carotenoid content, given that the UV-Visible spectroscopic analysis of this work revealed the absence of flavonoids in the unripe fruits of *Pistaciaterebinthus*, hence the antioxidant defense load has shifted to these compounds.

Determination of total phenols

From the gallic acid calibration curve, we calculated the total phenols in milligrams of gallic acid equivalent per gram of oilcake (mg GAE /g oilcake).

The results are reported in Table 4, showing that total phenol content ranged from 4.05 to 271.58 mg GAE /g of oilcake. The highest value was recorded in the methanolic extract, while the lowest was in the acetone extract.

Table 4: Phenolic compound (mg EAG /g oilcake) and tannin (mg ECAT /g oilcake) contents

Extraction system	Total phenols	Tannins
Methanol	271.58 ± 12.26	1.76 ± 0.07
Methanol/Water	80.80 ± 4.33	2.78 ± 0.06
Acetone	4.05 ± 0.32	0.84 ± 0.02
Acetone/ Water	22.31 ± 5.53	3.40 ± 0.04

Ethanol/ Water	10.07 ± 0.36	0.50 ± 0.01
Méthanol/Acetone/ Water	24.72 ± 0.69	2.23 ± 0.07

These results suggest that methanol is suitable for extracting total phenols from our plant. The findings enable us to classify our extracts in decreasing order of total phenol content: Methanol > methanol/water > methanol/acetone/water > acetone/water > ethanol/water > acetone.

We compared our results with the total phenolic compounds in the galls of *Pistaciaatlantica*, which have a maximum total phenol content of 243.85 mg GAE /100g dry matter [20], and with the phenols contained in the oil of immature *Pistaciaatlantica*fruits, where the content is equal to 2.51 mg /100g oil cake [18], as well as with mature fruits of the same species, where the total phenol content reaches a value of 251.25 mg GAE /100g oil cake [1]. This comparison shows that the immature *Pistaciaterebinthus*fruits studied are rich in total phenolic compounds.

However, it should be noted that the phenolic extracts of immature fruit cakes do not contain flavonoids. This result was confirmed by a qualitative test in which we did not record a UV spectrum characteristic of flavonoids. Indeed, the only band recorded in these spectra is equal to 320 nm, whereas flavonoids are characterized by two absorption bands at 250 and 350nm).

Condensed tannin quantification

The catechin calibration curve was used to calculate the tannin content of *Pistaciaterebinthus*samples. Tannin quantities are expressed in milligrams of catechin equivalent per gram of oilcake (mg ECAT /g oilcake).

The results reported in Table 4 show that the quantities of tannins in our extracts range from 0.50 to 3.40 mg CATE/g of oilcake. The high tannin content (3.39 mg CATE /g oilcake) was recorded in the acetone: water (7:3 V/V) solvent system conversely, the low content was recorded in the ethanol: water (8:2 V/V) mixture extract. These results demonstrate that the 70% hydro-acetone extract is a better solvent for condensing tannins. Comparing our results with those of tannin contents in *Pistaciaatlantica*galls (55.71 to 708.70 mg CATE /g dry matter) [20], we deduce that our extracts are low in tannins.

Anti-free radical activity

Based on the vitamin E inhibition curve, we calculated the VEEAC contents, ranging from 0.32 to 1.50 mg EVE/g lipid for hexanolic extract lipids (HE) and (NL), respectively (Table 5). These results enable us to rank the extracts in increasing order of free radical scavenging capacity: HE <NL <TL. This is explained by the VEEAC value, which induces the free radical scavenging capacity of the extract to increase. The activity of total lipids (TL) could be due to the presence

of phenolic compounds other than tocopherols, which are masked by lipids and extractable by the relatively polar solvent system (Chloroform/methanol 2/1 v/v) [18].

Similarly, bioactive compounds, such as tocopherols, carotenoids, and omega-3 (C18:3) fatty acids, play a crucial role in the antioxidant defense model and may contribute to protection against reactive oxygen species (ROS) [5].

It is also remarkable that the VEEAC values corresponding to total lipids (TL) and neutral lipids (NL) vary in the same direction with their tocopherol and carotenoid contents.

Comparing the extracts studied with the standard antioxidant and based on the definition of VEEAC, the extracts tested (TL and NL) appear more active than vitamin E.

We used several solvent systems with different polarities for phenolic extracts to discover the influence of solvent polarity on total phenol content and antioxidant capacity.

Based on the vitamin C free radical scavenging capacity curve, we calculated VCEAC values expressed in µg EVC/g oilcake. We found that they range from 5.66 to 43.17 µg EVC/g of oilcake. The higher the EVC value, the better the antioxidant performance of the extract. Accordingly, we have ranked our extracts in descending order of free radical scavenging capacity:

Methanol > acetone/water > methanol/water> ethanol/water = acetone > methanol/acetone/water.

This ranking does not vary in the same order as that for total phenol content. This result may reflect that antioxidant capacity depends on total phenol content and individual phenols soluble chemical structures in appropriate solvents.

Given these results (Table 5), it can be seen that the methanolic extract has the highest free radical scavenging activity of the other extracts, indicating that the molecular species responsible for antioxidant activity are mainly extractable by this solvent.

Table 5: VEEAC and VCEAC contents of lipid and phenolic extracts.

Lipidextracts	
Extract	**VEEAC extract (mg EVE/g oil)**
HE	0.328 ± 0.02
T L	1.502 ± 0.03
NL	1.076 ± 0.01

Phenolicextracts

Extract	VCEAC extract (µg EVC/g oilcake)	Polarity index
Methanol	43.17 ± 0.27	5.2
Methanol/Water	14.64 ± 0.12	6.2
Acetone	8.39 ± 0.05	5.1
Acetone/ Water	16.83 ± 0.34	6.33
Ethanol/ Water	8.39 ± 0.08	5.48
Methanol/ Acetone/ Water	5.66 ± 0.12	6.16

Conclusion

This work contributes to a better understanding of the chemical composition of fatty acids and the content of tocopherols, sterols, and carotenoids, as well as the free radical scavenging properties of lipid and phenolic extracts from immature *Pistaciaterebinthus*fruits.

The study findings revealed that the unripe fruits of *Pistaciaterebinthus*are very low in lipids, irrespective of the solvent used in the study, making them suitable for pharmaceutical and cosmetic applications.

Fatty acid composition in all lipid classes is dominated by high proportions of unsaturated fatty acids (UFA): 77.30% for hexanolic extract lipids(HE) and 72.55% for neutral lipids (NL). Linolenic acid is the predominant acid in hexanolic extract lipids(HE)(51.85%), total lipids TL (46.87%), and neutral lipids NL (46.40%). Linoleic acid was present in all lipid classes, ranging from 17.61% in TL total lipids to 43.01% in PL phospholipids. Oleic acid recorded a deficient proportion (0.71- 0.77%). Among saturated fatty acids, palmitic acid was present in relatively high proportions (49.91% in PL phospholipids and 37.91% in GL glycolipids) and myristic acid in low proportions (no more than 5.89%).

This fatty acid composition enables us to classify *Pistaciaterebinthus*unripe fruit lipids as linolenic-linoleic vegetable oils.

The greatest levels of tocopherols and total sterols were recorded in hexanolic extract lipids (HE), with contents of 36.45 and 759.08 mg/g lipid, respectively. The carotenoid content is significant, equal to 34.63 mg/g lipid, attributed to neutral lipids (NL).

Neutral lipid (NL) and total lipid (TL) extract from the unripe fruit showed significant free radical scavenging capacity compared to the reference antioxidant (1.076 and 1.502 mg EVE/g lipid, respectively).

Total phenol quantification on the different extracts obtained with different solvent systems from unripe *Pistaciaterebinthus*fruit cakes indicated that the methanolic extract was the richest in total phenols (271.58 mg EAG /g cakes) and the most potent antioxidant (43.17µg EVC/g cakes).

This research aims to enhance our comprehension of the chemical makeup of fatty acids and the levels of tocopherols, sterols, carotenoids, total phenols, and antioxidant capacity in lipid and phenolic extracts obtained from immature *Pistaciaterebinthus*fruit.

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