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Antibacterial activity of extracted bioactive molecules of *Schinus terebinthifolius* ripened fruits against some pathogenic bacteria

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ABSTRACT

The aim of this work is to identify the chemical constituents and the bioactivity of essential oil (EO), acetone extract (ACE) and *n*-hexane extract (HexE) of *S. terebinthifolius* ripened fruits using GC-MS. Total phenolic content and antioxidant activity of extracts were determined using the Folin-Ciocalteu and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assays, respectively. The toxicity against the growth of *Acinetobacter baumannii*, *Bacillus subtilis*, *Escherichia coli*, *Micrococcus flavus*, *Pseudomonas aeruginosa*, *Sarcina lutea*, and *Staphylococcus aureus* was determined with measuring the inhibition zones (IZs) using the disc diffusion method at the concentrations from 125 to 2000 μg/mL, also, the minimum inhibitory concentrations (MICs) using 96-well micro-plates and ranged from 4 to 2000 μg/mL. The major components in EO were *α*-pinene (36.9%), and *α*-phellandrene (32.8%). The major components in ACE were oleic acid (38.7%), *α*-phellandrene (13.33%), and *δ*-cadinene (11.1%), while the major methyl esters of fatty acids detected in HexE were oleic (12.8%), and palmitic (10.9%). The EO showed good activity against the growth of *Staph. aureus* and *P. aeruginosa* with MIC values of 16 μg/mL and 32 μg/mL, the ACE showed broad activity against the studied bacterial pathogens with MIC values ranged from of 4–128 μg/mL against the studied bacterial isolates, while HexE, however, showed weak antibacterial activity. The IC_{50} values of EO, ACE and HexE were 15.11 ± 0.99, 118.16 ± 1.7 and 324.26 ± 2.45 μg/mL, respectively, compared to IC_{50} of Tannic acid (23.83 ± 1.9 μg/mL) and butylated hydroxytoluene (BHT, 2.9 ± 0.1 μg/mL). Data suggested that the ripened fruits of *S. terebinthifolius* have potent antioxidant and antibacterial activities.

1. Introduction

Extracts and essential oils (EOs) from aromatic and medicinal plants have been possessed different bioactivity against certain human and plant bacterial pathogens [1–5]. Fruits of *Schinus terebinthifolius* Raddi (family Anacardiaceae) are commonly used throughout the world as cuisine spice [6], however, all parts of *S. terebinthifolius*, including oleoresin (or balsam), have been used medicinally throughout the tropical regions to treat inflammatory and hemostatic diseases [7], since they exhibit antioxidant and antimicrobial activities [8–11].

Other investigations have reported that phenolic compounds of *S. terebinthifolius* might be useful in the control of pathogenic fungi such as *Paracoccidioides brasiliensis* [10]. Among 24 plants, *S. terebinthifolius* methanol extracts showed the most activity against *Candida albicans* (MIC 1.25 mg/mL) [8]. Clear inhibition zones were also observed for *S. terebinthifolius* aqueous extract against *C. albicans* [12]. The plant has been reported to produce resin-like materials that contain monoterpenes that defend the plant against penetration by attacking pathogenic agents [13].

EO compounds such as *α*-pinene, *β*-pinene, *α*-phellandrene, *β*-phellandrene, limonene, *p*-cymene, *α*-terpineol, *α*-fenchene, *β*-isosylvestrene, *α*-cadinol, epi-*α*-cadinol, *δ*-cadinene, γ-cadinene, epi-*α*-muurolol, elemole, *δ*-elemene, *δ*-3-carene, germacrene D-4-ol, germacrene D, *β*-longipinene, (*E*)-*β*-cariophyllene, and *α*-funebrene have been

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found in *S. terebinthifolius* plants [14–24].

Other compounds, including phenols [25], tannins, flavonoids, anthraquinones [26], biphenyl esters, bioflavonoids [27], free steroids [28], and terpenes [29], have also been identified in *S. terebinthifolius*. Schinol and biphenyl 4′-ethyl-4-methyl-2,2′,6,6′-tetrahydroxy[1,1′-biphenyl]-4,4′-dicarboxylate were obtained from the stems of the plant and showed antifungal activity against *Paracoccidioides brasiliensis* [30]. Quercetin and kaempferol were identified in *S. terebinthifolius* and showed antifungal activity against *P. brasiliensis*, *Phytophthora megasperma*, and *Cylindrocarpon destructan* [31].

The aim of the present study was to identify the compositions of the methyl ester of fatty acids (fixed oil), essential oil, and acetone extract of the ripened fruits of *S. terebinthifolius*. Antioxidant activity and total phenolic compounds were analyzed using a 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay and the Folin-Ciocalteu method, respectively. Antibacterial activity was determined using the agar-disc diffusion method and by measuring the minimum inhibitory concentrations (MICs) against the growth of some bacterial pathogens. The chemical compositions of the extracts were analyzed using GC/MS.

2. Material and methods

2.1. Plant material

Ripened fruits (RFs) of *S. terebinthifolius* were collected from pruned branches of trees growing in Antoniades Gardens, Alexandria, Egypt, 2014 and all the chemical procedures and experiments were completed at the beginning of 2018. The plant was identified at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University and a sample was deposited (voucher number Zidan0031). The fruits were subjected to different extraction methods as shown in Fig. 1.

2.2. Extraction and Gas chromatography (GC)/mass spectrometry (MS) analysis of essential oil

The *S. terebinthifolius* RFs (100 g) were hydro-distillated for 3 h in a Clevenger apparatus [32]. The EO was dried over anhydrous Na_2SO_4 and measured with respect to the mass of fresh weight of the RFs (1.5 mL/100 g fresh weight). The EO was kept dry in sealed Eppendorf tubes and stored at 4 °C until chemical analysis. The GC-MS analysis procedure can be found in our previously work [24]. Chemical identification was performed on the basis of an MS library search (NIST and Wiley) [33]. Retention indices (RIs) for the most abundant compounds were calculated using a generalized equation for all the components that had a mixture of aliphatic hydrocarbons (C_8-C_{32}, Sigma-Aldrich) that were co-injected at the temperature program mentioned above equal to samples ones and computer matching with the Wiley 275.L and Wiley 7 n.L libraries.

2.3. Extraction with acetone

Approximately 50 g of ground RF was soaked in 150 mL acetone for 1 week, and then, the mixture filtered and the solvent was evaporated under reduced pressure using a Rotary Evaporator apparatus [34,35]. The filtrate was concentrated and weighed. The acetone extract (ACE) yielded 12.34% *w/w*. The extract was stored in refrigerator at 4 °C prior to use.

2.3.1. GC-MS analysis of acetone extract

The ACE was analyzed for their chemical composition using a Trace GC Ultra-ISQ Mass Spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS apparatus (30 m × 0.25 mm × 0.25 µm film thickness). The column temperature and program for the separation of the chemical compounds in the acetone extract was followed Salem et al. [36]. The components were identified by comparing their retention times and mass spectra with those in the WILEY 09 and NIST 11 mass spectral databases [33].

2.4. n-hexane extraction and methylation of fatty acid

Samples of RF (50 g) were extracts with 150 mL of *n*-hexane for one week and the extraction was repeated three times. The mixture was filtered and the solvent was evaporated using a Rotary Evaporator apparatus. GC apparatus with the method recommended by Salem et al. [37] was used to identify the methylated fatty acids (MFAs) in the *n*-hexane extract (HexE). The conditions used for characterization of MFAs by GC with standard fatty acids (C_2-C_{25}) that were previously injected into the GC at the same conditions for calibration purposes have been done [38,39].

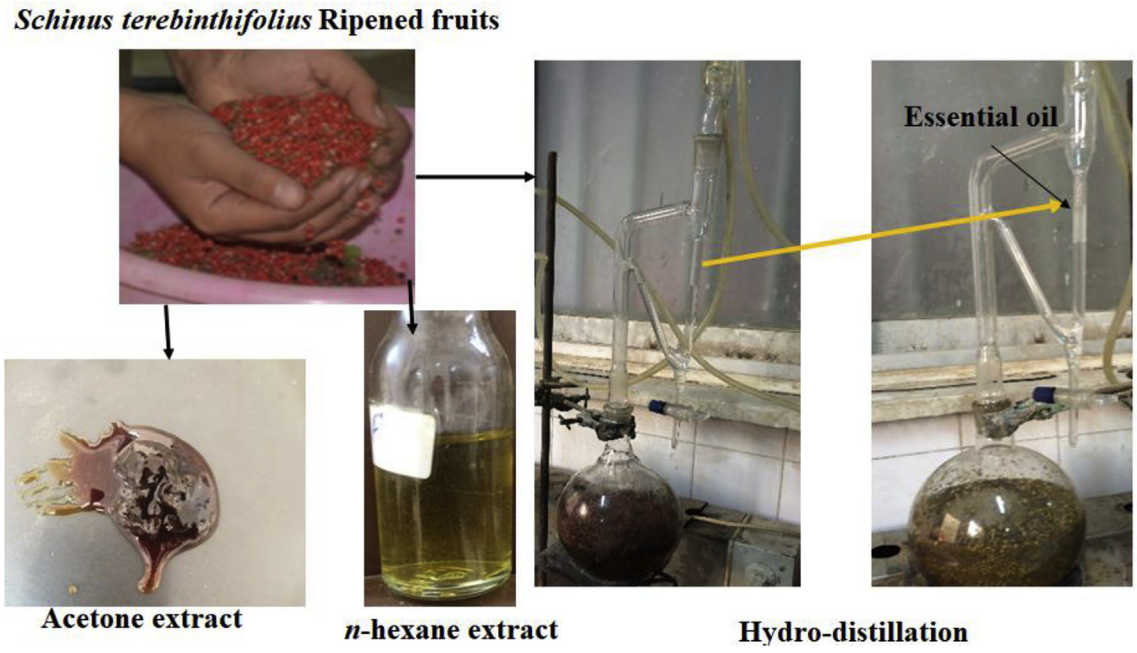
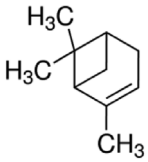
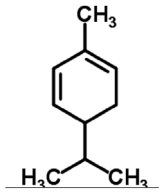
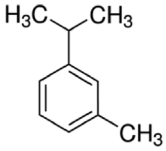
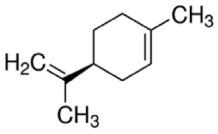
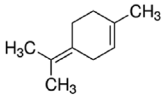
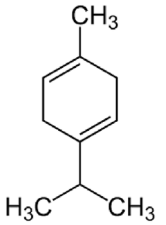
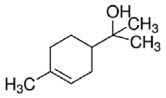
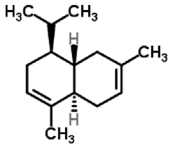
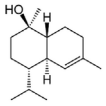
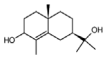
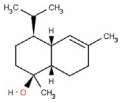
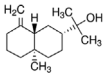


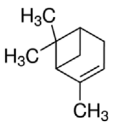
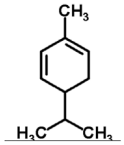
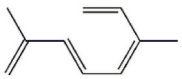
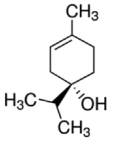
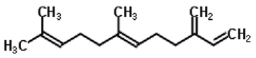
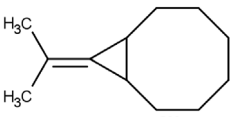
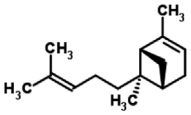
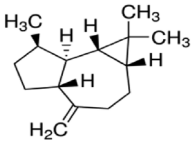
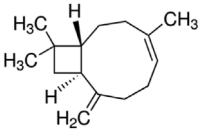
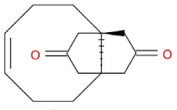
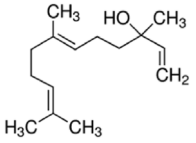
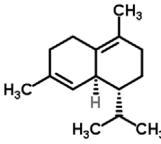
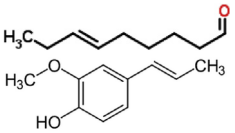
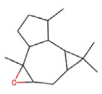
Fig. 1. Essential oil, acetone and *n*-hexane extracts from *S. terebinthifolius* ripened fruits.

Table 1
Essential oil composition of *S. terebinthifolius* fruits.

Constituent [24]	RI[a]	MW[b]	Molecular formula	Percentage in oil[c]	SI[d]	RSI[e]	Chemical structure
α-pinene	925	136	$C_{10}H_{16}$	36.9	896	896	
α-phellandrene	1005	136	$C_{10}H_{16}$	32.8	908	915	
m-cymene	1023	134	$C_{10}H_{14}$	0.4	876	922	
Limonene	1041	136	$C_{10}H_{16}$	11.9	859	859	
Terpinolene	1035	136	$C_{10}H_{16}$	3.1	915	921	
γ-terpinene	1067	136	$C_{10}H_{16}$	0.3	921	938	
α-Terpineol	1193	154	$C_{10}H_{18}O$	6.0	933	934	
β-cadinene	1500	204	$C_{15}H_{24}$	0.3	917	929	
α-Cadinol	1624	222	$C_{15}H_{26}O$	0.8	907	926	
γ-eudesmol	1629	222	$C_{15}H_{26}O$	0.2	898	919	
T-muurolol	1654	204	$C_{15}H_{24}$	0.3	917	929	
β-eudesmol	1667	222	$C_{15}H_{26}O$	0.2	846	870	

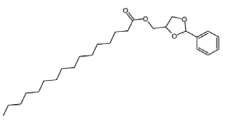
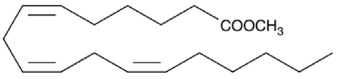
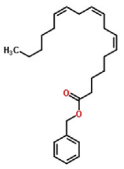
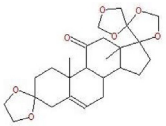
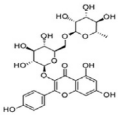
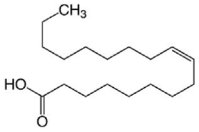
[a] RI; Retention index.
[b] MW: Molecular wight (g/mol).
[c] Percentage of total FID area obtained on HP-5 capillary column.
[d] SI: Standard index.
[e] RSI: Reverse standard index.

Table 2
Chemical composition of acetone extract from ripened fruits of *Schinus terebinthifolius*.

RT[a]	Compound Name	Molecular Formula	MW[b]	Peak Area %	SI[c]	RSI[d]	Chemical structure
7.08	α-Pinene	$C_{10}H_{16}$	136	4.71	749	847	
10.69	α-Phellandrene	$C_{10}H_{16}$	136	13.33	762	846	
10.82	Cosmene	$C_{10}H_{14}$	134	0.81	615	785	
15.25	Terpinen-4-ol	$C_{10}H_{18}O$	154	1.40	518	782	
18.95	β-Farnesene	$C_{15}H_{24}$	204	1.05	542	680	
19.26	9-(1-Methylethylidene)-bicyclo[6.1.0]nonane	$C_{12}H_{20}$	164	0.51	515	757	
20.32	α-Bergamotene	$C_{15}H_{24}$	204	1.44	584	790	
22.08	Aromadendrene	$C_{15}H_{24}$	204	1.14	712	774	
22.57	Isocaryophyllene	$C_{15}H_{24}$	204	0.57	400	745	
22.67	Tricyclo[6.3.3.0]tetradec-4-ene,10,13-dioxo-	$C_{14}H_{18}O_2$	218	0.80	467	659	
22.77	Nerolidol	$C_{15}H_{26}O$	222	0.13	426	659	
23.20	δ-Cadinene	$C_{15}H_{24}$	204	11.10	642	735	
23.37	(E)-6-Nonenal	$C_9H_{16}O$	140	0.20	373	725	
23.78	1-(3-hydroxy-1-propenyl)-, (Z)- Cyclooctanol	$C_{11}H_{18}O_2$	182	0.51	472	723	
25.72	Longipinene epoxide	$C_{15}H_{24}O$	220	0.66	603	660	

(*continued on next page*)

Table 2 (*continued*)

RT[a]	Compound Name	Molecular Formula	MW[b]	Peak Area %	SI[c]	RSI[d]	Chemical structure
26.35	Palmitic acid (2-phenyl-1,3-dioxolan-4-yl)methyl ester	$C_{26}H_{42}O_4$	418	0.22	434	526	
26.50	Linolenic acid methyl ester	$C_{19}H_{32}O_2$	292	6.67	581	662	
26.67	Benzyl (6Z,9Z,12Z)-6,9,12-octadecatrienoate	$C_{25}H_{36}O_2$	368	0.58	573	712	
27.64	Pregn-5-ene-3,11-dione, 17,20:20,21-bis[methylenebis(oxy)]-, cyclic 3-	$C_{25}H_{34}O_7$	446	0.43	412	481	
28.47	Kaempferol-3-O-rutinoside	$C_{27}H_{30}O_{15}$	594	2.62	405	430	
31.91	Oleic acid	$C_{18}H_{34}O_2$	282	38.74	735	743	

[a] RT: Retention time (min.).
[b] MW: Molecular wight (g/mol).
[c] SI: Standard index.
[d] RSI: Reverse Standard index.

2.5. Determination of total phenolic content and antioxidant activity

The total phenolic content (TPC) was determined using the Folin-Ciocalteu assay [36]. The data were expressed as milligrams of Gallic acid equivalents (GAE) per gram extract (mg GAE/gram dry extract).

The total antioxidant activity (TAA %) was measured by the DPPH assay (Sigma-Aldrich) [40]. In addition, the antioxidant activity of each extract was expressed in terms of IC_{50} (the concentration absorbance required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve [36]. The measurements of DPPH radical scavenging activity were carried in triplicate and compared with the radical scavenging activity of tannic acid (TA) and butylated hydroxytoluene (BHT).

2.6. Antibacterial activity

The following bacterial isolates *Acinetobacter baumannii* ATCC 17978, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 35210, *Micrococcus flavus* ATCC 10240, *Pseudomonas aeruginosa* D s0432–1 *Sarcina lutea* ATCC 9341 and *Staphylococcus aureus* ATCC 6538 were used for the bioassay. The inhibition zones (IZs) observed against the studied bacterial pathogens created by the EO, ACE, and HexE were measured using the disc diffusion method [41]. All the extracts were dissolved in 10% dimethyl sulfoxide (DMSO) and prepared at the concentrations of 125, 250, 500, 1000 and 2000 µg/mL. Negative (DMSO) and positive (tetracycline 20 µg/disc) controls were used and all tests were performed in triplicate.

2.7. Determination of minimum inhibitory concentrations

Minimum inhibitory concentrations (MICs) were determined by serial dilution of extracts (4, 8, 32, 64, 128, 250, 500, 1000 and 2000 µg/mL) and performed in 96-well micro-plates [42].

2.8. Statistical analysis

Data of the inhibition zones were analyzed using a one-way analysis of variance (ANOVA). $LSD_{0.05}$ was used for comparison between the means. In addition, the results of TPCs, TAA% and IC_{50} were presented as mean ± standard deviation of three measurements as analyzed with one way ANOVA.

3. Results and discussion

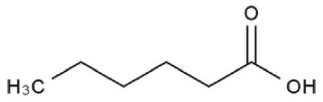
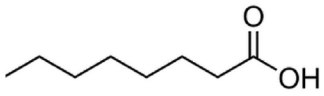
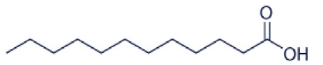
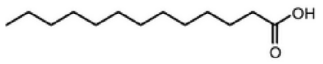
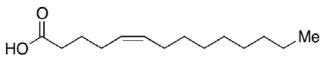
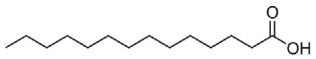
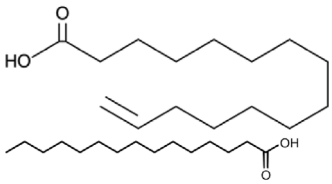
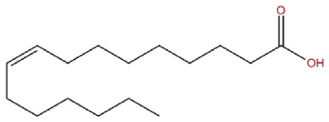
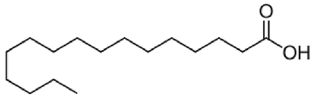
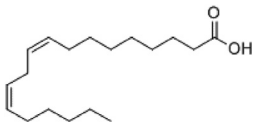
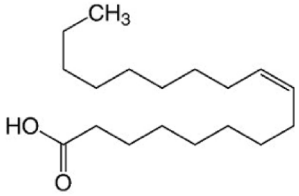
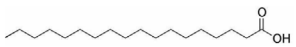
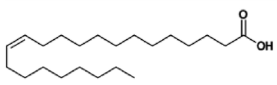
3.1. Chemical composition of extracts

3.1.1. Essential oil constituents of S. terebinthifolius fruits

The identified 12 molecules representing 93.2% of the total essential oil by GC–MS are presented in Table 1. The major chemical compositions of EO from *S. terebinthifolius* fruits were included α-pinene (36.9%), α-phellandrene (32.8%), limonene (11.9%), α-terpineol (6.0%), and terpinolene (3.1%). Other compounds can be found in previous work [24].

Previous studies have reported that α-fenchene, limonene, β-pinene, α-phellandrene, and β-isosylvestrene are the major components of *S. terebinthifolius* fruit EO and represent approximately 80% of the EO composition [19]. The EO composition from leaves, flowers, and fruits

Table 3
Concentration of methylated fatty acids identified in *Schinus terebinthifolius* fruits.

Fatty Acid	% FA	Conc. of FA (g/100 g of lipid)	Chemical structure	Conc. of FA (g/100 g of sample)
n-caproic acid (C6:0)	5.44	0.004		0.002
Caprylic acid (C8:0)	0.20	0.000		0.0008
Lauric acid (C12:0)	1.76	0.001		0.0007
Tridecanoic acid (C13:0)	5.59	0.004		0.0022
Tetradecenoic acid (C14:1)	0.86	0.001		0.0004
Myristic acid (C14:0)	8.31	0.007		0.004
14-pentadecenoic acid (C15:1)	2.87	0.002		0.002
Pentadecanoic acid (C15:0)	3.36	0.003		0.002
Hexadecenoic acid (C16:1)	1.14	0.001		0.0005
Palmitic acid (C16:0)	10.91	0.009		0.005
Linoleic acid (C18:2c)	6.69	0.005		0.003
Oleic acid (C18:1c)	12.85	0.005		0.003
Stearic acid (C18:0)	2.76	0.002		0.002
Erucic acid (C22:1, *cis*-13)	8.81	0.007		0.004

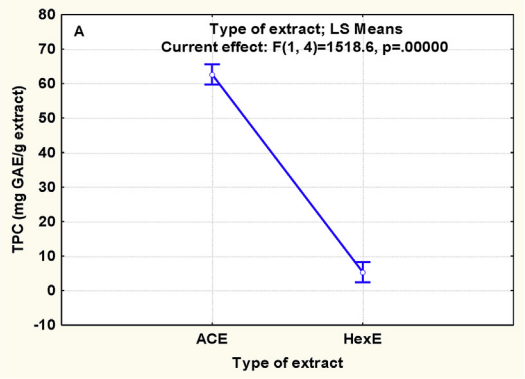
of *S. terebinthifolius* collected at different locations in India revealed the presence of *α*-pinene (15.01–51.82%) as the major component [15]. Other major components such as *α*-cadinol, *δ*-cadinene, *β*-pinene, and epi-*α*-muurolol were also detected [14]. Additionally, *α*-pinene (16.9%), *α*-phellandrene (21.1%), *β*-phellandrene (10.8%), and limonene (23.7%) were found to be the major constituents of *S. terebinthifolius* fruits EO [18].

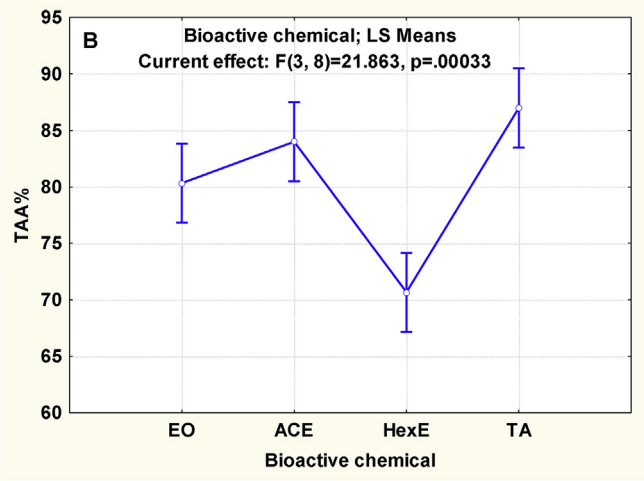
The major EO components extracted from the fruits of *S. terebinthifolius* grown at different regions in Brazil were *β*-phellandrene, elemole, *α*-cadinol, *δ*-cadinene, epi-*α*-cadinol, *δ*-3-carene, germacrene D-4-ol, and germacrene D [16]; *β*-pinene and *β*-longipinene, and germacrene D [20]; *α*-pinene, *β*-pinene, sabinene, *α*-funebrene, and limonene [21]; and *α*-pinene *δ*-3-carene, *α*-phellandrene, and limonene [23]. The EO of *S. terebinthifolius* berries grown in Tunisia contained the following major compounds: α-pinene, *β*-pinene, α-phellandrene, *β*-phellandrene, *p*-cymene, *α*-terpineol, and γ-cadinene [17].

3.1.2. GC-MS chemical composition of acetone extract

The chemical composition of the compounds identified in ACE of *S. terebinthifolius* ripened fruits is presented in Table 2. The main components in ACE were oleic acid (38.7%), *α*-phellandrene (13.3%), *δ*-cadinene (11.1%), linolenic acid methyl ester (6.6%), *α*-pinene (4.7%), kaempferol-3-*O*-rutinoside (2.6%), *α*-bergamotene (1.4%), terpene-4-ol (1.4%), 5*α*-androstan-16-one, cyclic ethylene mercaptole (1.3%), aromadendrene (1.1%), and *β*-farnesene (1.1%).

The most frequent substances present in the Anacardiaceae family are triterpenes, phenols, lipids, biflavonoids, and other classes of substances [43]. Additionally, terpenoids and fatty acids have been found





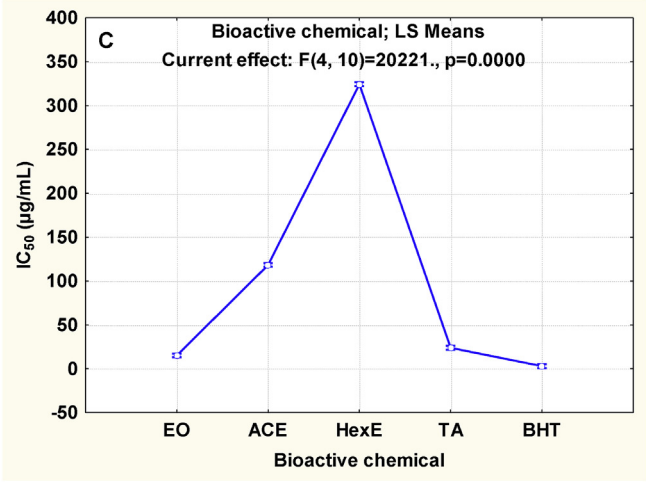


Fig. 2. Statistical analysis of total phenolic compounds (TPC), total antioxidant activity (TAA%) and IC_{50} values.

in *S. terebinthifolius* [29]. Other studies have revealed that the ethanol extract from the bark comprises phenols, triterpenes, and anthraquinones, while the leaf extracts has phenols, flavones, flavonoids, xanthones, leucoanthocyanidins, flavanones, and free steroids [28]. The leaves and bark are rich in tannins and EOs [44]. Ethyl gallate, myricetrin, quercitrin, methyl gallate and myricetin were isolated from leaves ethyl acetate fraction of the ethanolic extract and these substances are responsible for the anti-free radical [10].

3.2. Methylated fatty acids detected in S. terebinthifolius fruits

The lipophilic components from different parts of the plant are mainly composed of FAs and FA esters. GC analysis of methylated FAs from *S. terebinthifolius* ripened fruits with GC are presented in Table 3. The major methylated FAs we detected in the fruit were oleic acid (C18:1c) 12.85%, palmitic acid (C16:0) 10.91%, erucic acid (C22:1, *cis*-13) 8.81%, myristic acid (C14:0) 8.31%, linoleic acid (C18:2c) 6.69%, tridecanoic acid (C13:0) 5.59%, *n*-carpoic acid (C6:0) 5.44%, pentadecanoic acid (C15:0) 3.36%, 14-pentadecanoic acid (C15:1) 2.87%, and stearic acid (C18:0) 2.76%.

The major MFAs found in the bark were tridecanoic acid (C13:0) 7.05%, myristic acid (C14:0) 10.42%, palmitic acid (C16:0) 4.57%, and erucic acid (C22:1, *cis*-13) 6.57% [45], in wood were myristic acid (C14:0) 9.95%, 14-pentadecanoic acid (C15:1) 3.62%, pentadecanoic acid (C15:0) 3.66%, tridecanoic acid (C13:0) 6.19%, palmitic acid (C16:0) 5.17%, and erucic acid (C22:1, *cis*-13) 7.99% [46] and in leaves were heptadecenoic acid (C17:1) 22.56%, stearic acid (C18:0) 11.47%, and oleic acid (C18:1) 11.18% [47].

3.3. Total phenolic compounds and antioxidant activity

Fig. 2 presents the total phenolic compounds (TPCs) and the total antioxidant activity (TAA%) as well as the IC_{50} values. The TPC (Fig. 2A) of ACE was 62.66 ± 2.52 mg GAE/g extract. Also, HexE showed weak presence of phenolic compounds with minor amount (5.34 ± 0.39 mg GAE/g extract). The TAA% (Fig. 2B) of EO, ACE, and HexE were 80.33 ± 3.51, 84 ± 1, and 70.66 ± 2.31%, respectively. Subsequently, it was observed that these values were lower than or close to the value presented by TA (87 ± 3%). The IC_{50} values of EO, ACE and HexE were 15.11 ± 0.99, 118.16 ± 1.7 and 324.26 ± 2.45 µg/mL, respectively, compared to IC_{50} of Tannic acid (23.83 ± 1.9 µg/mL) and BHT (2.9 ± 0.1 µg/mL).

TPC: total phenolic compounds; TAA%: total antioxidant activity; IC_{50}: the concentration absorbance required to inhibit DPPH radical formation by 50%; EO: essential oil; ACE: acetone extract; HexE: *n*-hexane extract; TA: tannic acid; BHT: butylated hydroxytoluene.

Previously, it had been reported that the EO in the fruits of the *Schinus* species are characterized by a pungent-smell, which has been used in the therapy for respiratory disorders, and displays antioxidant and anticancer activities. However, these properties are attributed to the presence of high levels of monoterpenes [11,17].

3.4. Antibacterial activity

The antibacterial activities, reported as inhibition zones (IZs) and minimum inhibitory concentrations (MICs) of extracts from *S. terebinthifolius* ripened fruits are presented in Table 4 and Table 5, respectively.

From Table 4, with IZ values of 18.3 mm and 15.6 mm, the ACE observed the good activity against the growth of *A. baumannii* at 2000 and 1000 µg/mL level of concentrations, respectively, while EO didn't show any activity. At the concentration of 2000 µg/mL, EO and ACE showed the highest activity against *B. subtilis* with IZ value of 14.6 mm, as well as against *Sar. lutea* with IZ value of 13.3 mm. The ACE observed the highest IZs against *E. coli* with values of 15.3 mm and 13 mm at the concentrations of 2000 and 1000 µg/mL, respectively. ACE followed by EO showed the highest activity against the growth of *Staph. aureus* with IZ values of 18.3 mm and 16.3 mm, respectively. The ACE showed activity against *M. flavus* with IZs value of 20.3 mm and 17.3 mm at the concentrations of 2000 and 1000 µg/mL, respectively, however, EO observed an IZ value of 15.3 mm at 2000 µg/mL. For *P. aeruginosa*, the ACE and EO showed IZ value of 18.3 mm at 2000 µg/mL.

HexE, however, had only weak antibacterial activity against the studied bacterial isolates *B. subtilis*, *Sar. lutea*, *E. coli*, *M. flavus* and *A. baumannii*, while *Staph. aureus* and *P. aeruginosa* were resistant. The results observed that the ACE and EO extracted from fruits of *S. terebinthifolius* had a good activity against the growth of the studied bacterial isolates.

Table 4
Antibacterial activity (inhibition zones) of extracts from *S. terebinthifolius* ripened fruits against the studied bacterial strains.

Extract	Conc. (μg/mL)	Inhibition zone (mm)						
		A. baumannii	*B. subtilis*	*E. coli*	*M. flavus*	*P. aeruginos*	*Sar. lutea*	*Staph. aureus*
EO	125	0	0.0	0.0	0	7	0.0	6.6 ± 0.3
	250	0	0.0	0.0	5.3 ± 2.6	9.6 ± 0.3	0.0	8
	500	0	9.3 ± 0.3	0.0	10 ± 1.1	11.6 ± 0.3	0.0	10.6 ± 0.6
	1000	0	10.6 ± 0.6	5.3 ± 0.3	11.6 ± 0.8	14.3 ± 0.3	7.6 ± 0.3	12.3 ± 0.3
	2000	0	14.6 ± 0.6	11 ± 1	15.3 ± 0.3	18.3 ± 0.3	13.3 ± 0.8	16.3 ± 0.6
ACE	125	0	7	7.3 ± 0.6	9	7	0	0
	250	9.6 ± 0.3	8.6 ± 0.3	8.6 ± 0.3	11.3 ± 0.6	11 ± 0.6	6	7
	500	11.6 ± 0.3	10	10.6 ± 0.3	15.3 ± 0.3	13 ± 0.5	9 ± 0.6	12.3 ± 0.3
	1000	15.6 ± 0.3	12.3 ± 0.3	13.00 ± 0.6	17.3 ± 0.3	15.6 ± 0.3	11.3 ± 0.3	15.3 ± 0.3
	2000	18.3 ± 0.3	14.6 ± 0.6	15.3 ± 0.8	20.3 ± 0.3	18.3 ± 0.3	13.3 ± 0.3	18.3 ± 0.3
HexE	125	0	0	0	0	0	0	0
	250	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0
	1000	0	6.6 ± 0.3	0	6	0	8.3 ± 0.3	0
	2000	6.6 ± 0.3	8.6 ± 0.6	10.0 ± 0.6	7.3 ± 0.3	0	10.6 ± 0.6	0
	Negative control[a]	0	0	0	0	0	0	0
	Positive control[b]	23.3 ± 0.6	16.3 ± 0.6	19.3 ± 0.6	20.3 ± 0.6	26.6 ± 1.1	19.3 ± 1.5	21.6 ± 1.1

EO: Essential oil; ACE: Acetone extract; HexE: *n*-hexane extract.
Values are presented as mean ± SD.
[a] Dimethyl sulfoxide (DMSO 10%).
[b] Tetracycline (20 μg/disc).

Table 5
Antibacterial activity (MIC) of extracts from *Schinus terebinthifolius* ripened fruits against the studied bacterial strains.

Extract	MIC (μg/mL)						
	A. baumannii	*B. subtilis*	*E. coli*	*M. flavus*	*P. aeruginosa*	*Sar. lutea*	*Staph. aureus*
EO	> 2000	250	500	128	32	500	16
ACE	8	4	16	4	128	128	8
HexE	1000	1000	1000	1000	> 2000	2000	> 2000
Positive control[a]	4	4	32	8	16	16	8

MIC: Minimum inhibitory concentration (μg/mL).
EO: Essential oil; ACE: Acetone extract; HexE: *n*-hexane extract.
[a] Tetracycline.

According to MIC measurements (Table 5), EO showed good activity against the growth of *Staph. aureus* and *P. aeruginosa* with MIC values of 16 μg/mL and 32 μg/mL, while ACE showed broad activity against the studied bacterial pathogens with MIC values of 4, 128, 16, 8, 4, 8 and 128 μg/mL, against *B. subtilis*, *Sar. lutea*, *E. coli*, *Staph. Aureus*, *M. flavus*, *A. baumannii*, and *P. aeruginosa*, respectively.

Fruit extracts have been reported to possess antimicrobial, analgesic, anti-inflammatory, antioxidant, anti-allergic, anti-free radical, and insecticidal activity [25,26,48,49]. Additionally, fruits of *S. terebinthifolius* are rich in tannins, flavonoids, and EOs [18]. Limonene, a monoterpene hydrocarbon, is the most abundant component of the EOs extracted from *S. terebinthifolius* [6]. The isomer of 4′-ethyl-4-methyl-2,6,3′,5′-tetrahydroxy[1,1′-biphenyl]-4,4′-dicarboxylate, has been isolated from *S. terebinthifolius* fruit [27]. More details about the activity of extracts, especially the lyophilized powder materials in the concentration of 8–100%, have also been reported [50]. Extracts dissolved in ethanol and dichloromethane were induced inhibition zones against *E. coli*, *Staph. aureus* and *P. aeruginosa* [9].

The antimicrobial action of aqueous extracts was also evaluated [51]. Only the alcoholic extracts had an inhibitory effect on the growth of *S. aureus* and *B. cereus*. However, no inhibitory effect on the growth of *C. albicans*, *Aspergillus niger*, *E. coli*, *P. aeruginosa,* and *Salmonella cholera* were observed for either extracts. Moreover, the gel of *S. terebinthifolius* showed an 84% cure rate against bacterial vaginosis in non-pregnant women [52].

4. Conclusions

In the present study, the major chemicals composition of the EO from *S. terebinthifolius* ripened fruits were *α*-pinene, *α*-phellandrene, limonene, and *α*-terpineol. The major components in the ACE were oleic acid, *α*-phellandrene, *δ*-cadinene, linolenic acid methyl ester, *α*-pinene, and kaempferol-3-*O*-rutinoside. The antibacterial results showed that the ACE, and EO had good activity against the growth of *A. baumannii*, *P. aeruginosa*, *M. luteus*, and *S. aureus*. Good antioxidant activity with IC_{50} values of was reported with EO and ACE compared with the referenced compounds used. The results suggest that the ripened fruits of *S. terebinthifolius* are a good source of antioxidant and antibacterial activities. Overall, the extracts and EO from *S. terebinthifolius* ripened fruits could be a sustainable source for natural products and biomedicine against certain bacterial pathogens.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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