

Chemical composition, antioxidant and antibacterial activities of extracts from *Schinus molle* wood branch growing in Egypt

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Abstract In the present work, for the first time, the chemical components of essential oils (EOs) and extracts from wood branch (WB) resulted from the tree pruning wastes of *Schinus molle* L. grown in Egypt were evaluated for their antioxidant and antibacterial activities. EOs, methanol (ME), dichloromethane (DCME) and water (WE) extracts as antioxidant and antibacterial activities were measured. Total phenolic and flavonoid contents as well as analysis of extracts by gas chromatography–mass spectrometry (GC–MS) were reported. The major components in EOs were α -elemol, β -pinene, and α -phellandrene, in ME were 6-(4-chlorophenyl)-3-cyano-4-(*N*-benzylpiperazino)-2H-pyran-2-one, and 2-naphthalene methanol, decahydro- $\alpha,\alpha,4a$ -trimethyl-8-methylene, in DCME were 12-methyl-E,E-3,13-octadecadien-1-ol, and 1,2-benzenedicarboxylic acid, dioctyl ester, and in WE were β -eudesmol, and (Z,Z,Z)-9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester. The highest total antioxidant activity was found with EOs (90 ± 1.23 %) and WE (86.30 ± 1.40 %). The lowest IC_{50} values of

13.11 ± 3.00 , and 12.66 ± 2.15 $\mu\text{g/mL}$ were found with WE and EOs, respectively. EOs and WE were observed to have good antibacterial activity against *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Sarcina lutea*, *Pseudomonas aeruginosa*, and *Micrococcus luteus*. In conclusion, the *Schinus molle* L. WB EOs and extracts might, indeed, be used as a potential source for pharmaceutical or food industries.

Keywords *Schinus molle* L. · Wood branch · Extracts · Antioxidant activity · Antibacterial activity

Introduction

Pepper tree (*Schinus molle* L.), belonging to the family Anacardiaceae, is frequently grown as an ornamental tree in Mediterranean region, and originated from South America and its habitat ranged from southern Brazil to Chile and Mexico [1–3]. Fresh leaves and fruits (berries) essential oils (EOs) have been shown to have a significant activity against the growth of some bacterial and fungal strains [3–9]. Other biological effects like antitumor, anti-inflammatory, and antispasmodic have been reported [10–12].

Ethnopharmacologically, the tree is widely used in traditional medicine to treat a variety of diseased conditions including colds, asthma, coughs, diarrhea and dysentery, hemorrhage, laryngalgia, laryngitis, sore throat, spasm, trachagia and vermifuge [13]. Pepper tree is one of the medicinal plants traditionally used in many Brazilian medical literature for folk medicine [10, 14]. Various parts of this plant have been traditionally used to treat various diseases such as ulcers, respiratory problems, wounds, rheumatism, gout, diarrhea, skin disease, toothache,

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rheumatism, menstrual disorders, and respiratory and urinary tract infection [10, 14].

Schinus molle L. extracts have been used as topical antiseptic, antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant, antitumor, astringent, antispasmodic, digestive stimulant, tonic, wound healing, diuretic, an analgesic agent, a stimulant and an antidepressant [10, 14].

α -phellandrene and β -phellandrene were found as the main constituents in the leaf EOs of *Schinus molle* L. grown in Tunisia [15], and Turkey [16], bicyclogermacrene from leaf EOs of *Schinus molle* L. grown in Uruguay [17]. Limonene, (E)-caryophyllene, and bicyclogermacrene were reported as major components from the EOs of aerial parts (leaves and twigs) of *Schinus molle* L. grown in Southern Brazil [18], and in Tunisia, α -phellandrene, β -phellandrene, and β -pinene were reported as major components from berries EOs [3].

There are few studies on the chemical composition of *Schinus molle* L. extracts and most of them refer to EOs from leaves, aerial parts and fruits, while the information on chemical components of wood branch (WB) and their biological activity are still not determined. In our continuing research on the biological activities of Egyptian medicinal higher plants, as well as to the best of our knowledge, since no information is available on the chemical composition of WB resulted from the tree pruning wastes of *Schinus molle* L. grown in Egypt, the present study was carried out to evaluate the EOs, methanol, dichloromethane and water extracts for antioxidant and antibacterial activities. The total phenolic and flavonoid contents as well as analysis of extracts by gas chromatography–mass spectrometry (GC–MS) were reported.

Materials and methods

Branches of *Schinus molle* L. and essential oils preparation

Fresh branches resulted from the tree pruning wastes of *Schinus molle* L. were collected from Alexandria, Egypt, in the middle of September 2013, and the laboratory work was completed at the end of 2015. The plant was identified at the Department of Forestry and Wood Technology and with the voucher No. Zidan00310. Bark was removed and wood was cut into small pieces (100 g) and then hydro-distilled for 3 h [8]. The oil was dried over anhydrous Na_2SO_4 , and measured with respect to fresh weight of WB (0.75 mL/100 g fresh weight). The oil was kept dry in sealed Eppendorf tubes and stored at 4 °C until used for GC–MS analysis and biological activity tests.

Preparation of extracts

One hundred grams of air-dried powder of WB was used for the extraction for each of the following solvents: methanol, dichloromethane, and water. About 250 mL of each solvent was poured in a 500-mL conical flask containing the air-dried WB and covered with cotton wool plug and wrapped with aluminum foil. Extraction was allowed to proceed for 48 h under laboratory conditions. The extracts were filtered using filter paper Whatman No. 1, and then, the solvents were evaporated to dryness using a rotary evaporator. The yields for methanol (ME), dichloromethane (DCME) and water (WE) extracts were 15.12, 17.05; and 21.30 g/100 air-dried samples, respectively. All the extracts were kept dry in sealed brown vials and stored at 4 °C until used for GC/MS analysis and biological activity tests.

GC–MS analysis

GC–MS analysis of the essential oils

GC Ultra/Mass spectrophotometer ISQ (Thermo Scientific), a trace instrument equipped with FID and DB-5 narrow bore column (length 10 m \times 0.1 mm ID, 0.17 μm film thickness; Agilent, Palo Alto, CA, USA), was used. Helium was used as the carrier gas with a flow rate of 1 mL/min, and the oven temperature program was: 45–165 °C (4 °C/min) and 165–280 °C (15 °C/min) with post run (off) at 280 °C. Samples (1 μL) were injected at 250 °C, with split/splitless injector (50:1 split ratio) in the splitless mode flow with 10 mL/min. The GC–MS was equipped with a ZB-5MS Zebron capillary column (length 30 m \times 0.25 mm ID, 0.25 μm film thickness; Agilent). Helium (average velocity 39 cm/s) was used as the carrier gas, and the oven temperature was held at 45 °C for 2 min, and then increased from 45 to 165 °C (4 °C/min), and 165 to 280 °C (15 °C/min).

All mass spectra were recorded in the electron impact ionization (EI) at 70 electron volts. The mass spectrometer was scanned from m/z 50–500 at five scans per second. Peak area percent was used for obtaining quantitative data with the GC with HP-ChemStation software (Agilent Technologies) without using correction factors. Identification of the constituents was performed based on MS library search (NIST and Wiley) [19–21]. Retention indices (RIs) were calculated using a generalized equation for all the components using a mixture of aliphatic hydrocarbons (C_8 – C_{32} , Sigma-Aldrich) that was 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.

GC–MS analysis of the extracts

Methanol, dichloromethane, and water extracts were analyzed for their chemical composition using Trace GC Ultra-ISQ Mass Spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 μm film thickness) apparatus at Atomic and Molecular Physics Unit, Experimental Nuclear Physics Department, Nuclear Research Centre, Egyptian Atomic Energy Authority, Inshas, Cairo, Egypt. The column oven temperature was initially held at 120 °C and then increased by 5 °C/min to 200 °C with holding 2 min and then increased to 280 °C (10 °C/min). Temperatures of the injector and detector (MS transfer line) were kept at 250 °C. Helium, the carrier gas, was kept in constant flow rate of 1 mL/min. The solvent delay was 2 min, and diluted samples of 1 μL were injected automatically using Auto-sampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–550 in full-scan mode. The ion source and transfer line temperatures were set at 200 and 250 °C, respectively. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database [20].

Determination of total phenolic and flavonoid contents

The total phenolic (TP) and flavonoid contents (TF) were determined by Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively [22]. For total phenolic contents, 1 mL of EOs, extracts, or standard solution of Tannic acid (10, 20, 40, 60, 80 and 100 mg/L) was added to 25-mL volumetric flask, containing 9 mL of distilled deionized water (dd H₂O). A reagent blank using dd H₂O was prepared. 1 mL of Folin–Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of 7 % Na₂CO₃ solution was added to the mixture. The solution was diluted to volume (25 mL) with dd H₂O and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with an UV/Vis Spectrophotometer (Unico® 1200, Alexandria, Egypt). The TP was expressed as milligrams of tannic acid equivalents (TAE) per 100 g extract (mg TAE/100 g dry extract).

For total flavonoid contents, an aliquot (1 mL) of EOs, extracts or standard solution of (+)-catechin (10, 20, 40, 60, 80 and 100 mg/L) was added to 10-mL volumetric flask containing 4 mL of dd H₂O, and 0.3 mL 5 % NaNO₂ was added to the flask. After 5 min, 0.3 mL 10 % AlCl₃ was added. At 6th min, 2 mL 1 M NaOH was added, and the total volume was made up to 10 mL with dd H₂O. The solution was mixed well, and the absorbance was measured

against prepared reagent blank at 510 nm (UV/Vis Spectrophotometer (Unico® 1200, Alexandria, Egypt). TF was expressed as milligrams of (+)-catechin equivalents (CE) per gram extract (mg CE/g dry extract).

Antioxidant activity of extracts

The total antioxidant activity (TAA %) of extracts and EOs was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [23]. Pure methanol (Sigma-Aldrich) was used to calibrate the spectrophotometer. An aliquot of 2 mL of stock solution of 0.1 mM DPPH (Sigma-Aldrich) reagent dissolved in pure methanol was added to a test tube containing 2 mL of the sample solution in methanol (200 μg/L). The mixture was mixed for approximately 10 s and left to stand in fiber box at room temperature in the dark for 30 min. The absorbance was measured at 517 nm, using a UV scanning spectrophotometer (Unico® 1200).

TAA % was expressed as the percentage inhibition of the DPPH radical using the following equation: $TAA (\%) = (A_0 - A_s / A_0) \times 100$, where TAA is the total antioxidant activity, A_0 (control) is the absorbance of DPPH solution in methanol and A_s is the absorbance of a DPPH solution with the tested sample (essential oils and extracts) or the positive controls (Tannic acid and (+)-catechin) solutions. The control contained 2 mL of DPPH solution and 2 mL of methanol. The average values of TAA % were carried out for three replicates, and are expressed as mean values ± standard deviation (SD). Also, the antioxidant activity of each extract was expressed in terms of IC₅₀ (the concentration required to inhibit DPPH radical formation by 50 %), calculated from the inhibition curve [24].

Antibacterial activity of essential oils and extracts

For preparation of the concentrated EOs, ME, DCME, and WE for the antibacterial assay, the respective amount of extracts were dissolved in 10 % dimethyl sulfoxide (DMSO, Sigma-Aldrich, 10 % DMSO was prepared by diluting the solvent in distilled water) to obtain the concentrations of 4, 8, 32, 64, 125, 250, 500, 1000, and 2000 μg/mL for each extract. We should mention that for the preparation of EOs concentration, 0.5 mL of Tween 80 was added to emulsify carrier oil in water.

Antibacterial activity of EOs and extracts from *Schinus molle* L. WB was assayed using the disk diffusion method [25], against the growth of the following human pathogenic bacteria: *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Sarcina lutea*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Micrococcus luteus* at the concentration of 200 μg/mL. All the bacterial strains were provided by Prof.

Yousry Gohar (Botany Department, Microbiology Section, Faculty of Science, Alexandria University, Egypt). Minimum inhibitory concentration (MICs) values were determined by serial dilution of the extracts and EOs (4, 8, 32, 64, 125, 250, 500, 1000, and 2000 µg/mL) in 96-well micro-plates [26]. Negative (DMSO) and positive (tetracycline 20 µg/disk) controls were used, and all tests were performed in duplicate.

Statistical analysis

All the values of TP, TF, TAA %, IC₅₀, and the antibacterial activity are expressed as mean values ± SD. Analysis of variance (ANOVA) was used to evaluate the significant difference among various treatments with the criterion of $p \leq 0.05$.

Results and discussion

GC/MS analysis of essential oils and extracts

Essential oils composition

The identified thirty components representing 99.42 % of the total essential oils (EOs) were presented in Table 1. The major chemical components of the EOs from *Schinus molle* L. wood branch (WB) were α -elemol (14.79 %), β -pinene (13.39 %), myrcene (12.26 %), α -phellandrene (10.41 %), caryophyllene (7.69 %), α -cadinol (5.18 %), cadinene (4.67 %), elixene (4.48 %), nerolidol (3.65 %), β -eudesmol (4.01 %), γ -eudesmol (2.5 %), and germacrene-D-4-ol (2.36 %).

Generally, monoterpene hydrocarbons (40.90 %) and sesquiterpenes (54.6 %) were the main chemical groups in the EOs from WB of *Schinus molle* L., and small amount of sesquiterpenoids (3.55 %) and polycyclic aromatic hydrocarbon as tetrahydronaphthalenes (0.37 %) were found (Table 1).

Previously, there were many studies related to the EOs composition from different parts (leaves, berries and aerial parts) of *Schinus molle* L. from different regions of the world [8, 16, 27–30]. For example, studies with Italian leaf EOs [29, 30], and Tunisian leaf EOs [30, 31], referred that the main components may be different either in the same region or in percent of the component. Moreover, differences or similarities on chemical compositions suggest the presence of different chemotypes of *Schinus molle* L. [15, 17]. The EOs composition of *Schinus molle* L. leaf grown in Italy contains α - and β -phellandrene and limonene [29], and the grown in southeast Portugal were reported to have α -phellandrene, limonene, β -myrcene, β -phellandrene and elemol [32].

Table 1 Essential oil constituents of *Schinus molle* L. wood branch analyzed by gas chromatography–mass spectrometry (GC–MS)

| Constituent | RI ^a | Percentage in oil ^b |
|--------------------------|-----------------|--------------------------------|
| α -pinene | 939 | 1.6 |
| Sabinene | 958 | 0.27 |
| β -pinene | 979 | 13.39 |
| Myrcene | 980 | 12.26 |
| Ocimene | 993 | 0.26 |
| α -phellandrene | 1007 | 10.41 |
| β -cymene | 1010 | 0.56 |
| Linalool | 1100 | 1.7 |
| Pinanediol | 1276 | 0.45 |
| (-)- β -elemene | 1364 | 1.72 |
| α -gurjunene | 1400 | 0.45 |
| Caryophyllene | 1425 | 7.69 |
| Elixene | 1430 | 4.48 |
| Aromadendrene | 1439 | 0.24 |
| Germacrene-D | 1444 | 0.48 |
| γ -muurolene | 1460 | 0.72 |
| γ -gurjunene | 1476 | 0.62 |
| β -selinene | 1484 | 0.37 |
| α -muurolene | 1485 | 0.55 |
| α -elemol | 1523 | 14.79 |
| Cadinene | 1529 | 4.67 |
| Nerolidol | 1540 | 3.65 |
| <i>Trans</i> -nerolidol | 1550 | 0.28 |
| Palustrol | 1551 | 0.21 |
| Germacrene-D-4-ol | 1557 | 2.36 |
| γ -eudesmol | 1606 | 2.5 |
| τ -muurolol | 1626 | 1.75 |
| Carotol | 1627 | 1.8 |
| α -cadinol | 1629 | 5.18 |
| β -eudesmol | 1647 | 4.01 |
| Monoterpene hydrocarbons | | 40.90 % |
| Sesquiterpenes | | 54.6 % |
| Sesquiterpenoids | | 3.55 % |
| PAHs | | 0.37 % |
| Total | | 99.42 % |
| Unidentified | | 0.58 % |

PAHs polycyclic aromatic hydrocarbons

^a Identification of the essential oil components was performed by comparison of mass spectra and retention index (RI) obtained in both columns with those of reference compounds and those reported in the literature or with those of mass spectra libraries [20–22]

^b Percentage of the total Flame Ionization Detector (FID) area obtained on Hewlett Packard (HP-5) capillary column

The major constituents of EOs from *Schinus molle* L. grown in Costa Rica were β -pinene and α -pinene [33], while p -cymene α -phellandrene, and sabinene were the major components in fruit and leaf EOs from Egypt [34, 35]. α -phellandrene, β -phellandrene, β -pinene, and

p-cymene were found as major compounds in EOs extracted from berries of *Schinus molle* L. grown in Tunisia [3]. The main EOs compounds from the aerial parts were limonene, (E)-caryophyllene, bicyclogermacrene, germacrene, sabinene and spathulenol from *Schinus molle* L. grown in Southern Brazil [18]. α -phellandrene was the main component of *Schinus molle* L. grown in Mexico [36].

The identified compounds in the EOs of WBs could be useful as a potential source in aromatherapy and pharmacy. The variation in the EOs compositions from aromatic and higher plants for the same species depends on several factors: genetic variability (chemotype); geographic location, ecologic factors, climatic conditions, the effect of intra-specific differences, soil-growth conditions, and extraction process [37]. The biological activities of the EOs are often attributed to their major compounds.

GC–MS analysis of methanol extract

Table 2 presents the chemical composition of ME of *Schinus molle* L. WB. The major components in ME were 6-(4-chlorophenyl)-3-cyano-4-(*N*-benzylpiperazino)-2H-

pyran-2-one (26.00 %), 2-naphthalene methanol, decahydro- $\alpha,\alpha,4a$ -trimethyl-8-methylene (19.95 %) and docosane (12.72 %), 3-(maleimido-2-yl)-1-methyl-2-(1-methylindol-2-yl)indole (6.37 %), phenyl 4-[bis(ethoxy carbonyl)but-3-ynyl]-2,3,4-trideoxy- α,L -glucero-pent-2-enopyranoside (6.26 %), 2H-Indeno[1,2-*b*]furan-2-one,3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl (6.16 %), hexadecanoic acid,1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester (5.95 %), and lupanol (5.39 %).

GC–MS analysis of dichloromethane extract

The chemical composition of the identified 40 compounds of DCME of *Schinus molle* L. WB is presented in Table 3. The main components in DCME were 12-methyl-*E,E*-3,13-octadecadien-1-ol (22.49 %), 1,2-benzenedicarboxylic acid, dioctyl ester (8.01 %), torreyol (6.46 %), acetic acid, (1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethylnaphth-2-yl) methyl ester (5.31 %), elemol (4.71 %), trans-caryophyllene (3.94 %), (1-butylheptyl)-benzene (3.12 %), and (1-pentylheptyl)-Benzene (3.09 %).

Table 2 The chemical compositions of methanol extract from *Schinus molle* L. branch wood analyzed by gas chromatography–mass spectrometry (GC–MS)

| Peak No. | RT | Compound name | Molecular formula | MW | Peak area % | SI | RSI | Most fragment ions with RI (%) |
|----------|-------|---|---|-----|-------------|-----|-----|---|
| 1 | 3.63 | Docosane | C ₂₂ H ₄₆ | 310 | 12.72 | 654 | 677 | 43 (88), 57 (100), 71 (34) and 99 (10) |
| 2 | 14.99 | 2H-Indeno[1,2- <i>b</i>]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl | C ₁₃ H ₁₈ O ₂ | 206 | 6.16 | 423 | 517 | 55 (46), 81 (47), 91 (48), 191 (95), 206 (3) |
| 3 | 22.23 | 2-Naphthalene methanol,decahydro- $\alpha,\alpha,4a$ -trimethyl-8-methylene | C ₁₅ H ₂₆ O | 222 | 19.95 | 495 | 659 | 59 (100), 81 (35), 108 (33), 164 (40), 164 (25) and 222 (15) |
| 4 | 34.51 | 3-(Maleimido-2-yl)-1-methyl-2-(1-methylindol-2-yl)indole | C ₂₂ H ₁₇ N ₃ O ₂ | 355 | 6.37 | 368 | 688 | 63 (20), 127 (25), 251 (28), 269 (45), 164 (25), 283 (100) and 355 (10) |
| 5 | 36.77 | Androst-5-en-3-one, 19-acetoxy-4,4-dimethyl-,oxime | C ₂₃ H ₃₅ NO ₃ | 377 | 1.74 | 392 | 424 | 95 (43), 135 (35), 283 (45) and 300 (100) |
| 6 | 37.34 | 6-(4-Chlorophenyl)-3-cyano-4-(<i>N</i> -benzylpiperazino)-2H-pyran-2-one | C ₂₃ H ₂₀ ClN ₃ O ₂ | 405 | 26.00 | 419 | 791 | 69 (45), 109 (25), 149 (30), and 405 (20) |
| 7 | 37.78 | Lupanol | C ₃₀ H ₅₂ O | 428 | 5.39 | 385 | 407 | 91 (100), 218 (25), and 428 (95) |
| 8 | 38.31 | Hexadecanoic acid,1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester | C ₃₆ H ₅₈ O ₆ | 586 | 5.95 | 427 | 431 | 57 (80), 266 (100), 330 (50), and 551 (3) |
| 9 | 40.24 | Lucenin 2 | C ₂₇ H ₃₀ O ₁₆ | 610 | 4.35 | 362 | 386 | 156 (25), 281 (100), 322 (25), and 354 (20) |
| 10 | 41.20 | Phenyl 4-[bis(ethoxycarbonyl)but-3-ynyl]-2,3,4-trideoxy- α,L -glucero-pent-2-enopyranoside | C ₂₁ H ₂₆ O ₆ | 374 | 6.26 | 481 | 968 | 281 (85) and 374(3) |

RT retention time (min), MW molecular weight (g/mol), SI standard index, RSI reverse standard index, RI relative intensities

Table 3 The chemical compositions of dichloromethane extract from *Schinus molle* L. branch wood analyzed by gas chromatography–mass spectrometry (GC–MS)

| Peak No. | RT | Compound name | Molecular formula | MW | Peak area % | SI | RSI | Most fragment ions with RI (%) |
|----------|-------|--|--|-----|-------------|-----|-----|---|
| 1 | 3.26 | Benzene, 1-ethyl-3-methyl | C ₉ H ₁₂ | 120 | 0.33 | 856 | 938 | 105 (100) and 120 (30) |
| 2 | 3.54 | α -Myrcene | C ₁₀ H ₁₆ | 136 | 1.88 | 869 | 894 | 69 (80), 93 (85) and 136 (15) |
| 3 | 3.88 | 1-Phellandrene | C ₁₀ H ₁₆ | 136 | 0.40 | 847 | 906 | 77 (45), 93 (100) and 136 (25) |
| 4 | 4.17 | Ethanone,1-(4-methylphenyl)- | C ₉ H ₁₀ O | 134 | 1.25 | 922 | 935 | 119 (100) and 134 (20) |
| 5 | 4.26 | Limonene | C ₁₀ H ₁₆ | 136 | 0.71 | 751 | 809 | 68 (100), 93 (60), 121 (25) and 136 (25) |
| 6 | 4.30 | α -Phellandrene | C ₁₀ H ₁₆ | 136 | 0.61 | 720 | 789 | 77 (30), 93 (100) and 136 (20) |
| 7 | 5.36 | 1,4-Benzenedicarboxylic acid, 4-(methoxycarbonylphenyl)methyl methyl ester | C ₁₈ H ₁₆ O ₆ | 328 | 0.21 | 548 | 607 | 77 (30), 105 (40) and 133 (25) |
| 8 | 10.65 | Exo-2-Hydroxy-5-ketobornane | C ₁₀ H ₁₆ O ₂ | 168 | 0.26 | 513 | 656 | 70 (45), 107 (40) and 168 (45) |
| 9 | 11.17 | 1-Methyl-4-(2-methyloxiranyl)-7-oxabicyclo[4.1.0]heptane | C ₁₀ H ₁₆ O ₂ | 168 | 0.17 | 551 | 670 | 67(35), 93 (30) and 168 (10) |
| 10 | 12.02 | (Z)-9-Octadecenoic acid | C ₁₈ H ₃₄ O ₂ | 282 | 0.20 | 512 | 544 | 55 (100), 69(55) and 282 (5) |
| 11 | 12.24 | Phenylmethyl ester-hexadecanoic acid | C ₂₃ H ₃₈ O ₂ | 346 | 0.25 | 483 | 557 | 57 (55), 91 (100) and 108 (80) |
| 12 | 12.89 | 2,4,5,6,7,8-Hexahydro-1,4, 9,9-tetramethyl-3H-3a,7-methanoazulene | C ₁₅ H ₂₄ | 204 | 0.32 | 644 | 784 | 91 (80), 105 (85) and 204 (90) |
| 13 | 13.16 | <i>trans</i> -Caryophyllene | C ₁₅ H ₂₄ | 204 | 3.94 | 792 | 810 | 69 (90), 93 (88), 133 (75) and 204 (10) |
| 14 | 13.98 | α -Caryophyllene | C ₁₅ H ₂₄ | 204 | 0.45 | 711 | 818 | 80 (40), 93 (100) and 204 (15) |
| 15 | 14.43 | 1H-Benzocycloheptene,2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis) | C ₁₅ H ₂₄ | 204 | 0.36 | 619 | 743 | 93 (80), 119 (75), 189 (60) and 204 (25) |
| 16 | 14.98 | α -Muurolene | C ₁₅ H ₂₄ | 204 | 1.76 | 704 | 771 | 93 (45), 105 (100), 161 (40) and 204 (20) |
| 17 | 15.36 | Seychellene | C ₁₅ H ₂₄ | 204 | 0.85 | 681 | 790 | 91 (95), 105 (80) and 204 (50) |
| 18 | 15.51 | α -Copaene | C ₁₅ H ₂₄ | 204 | 2.14 | 726 | 762 | 93 (45), 105 (90), 119 (95), 161 (100) and 204 (25) |
| 19 | 15.65 | (1-Butylhexyl)-benzene | C ₁₆ H ₂₆ | 218 | 0.91 | 823 | 868 | 91 (100), 147 (20), and 218 (10) |
| 20 | 16.12 | Elemol | C ₁₅ H ₂₆ O | 222 | 4.71 | 768 | 772 | 59 (100), 93 (80), 107 (45) and 204 (25) |
| 21 | 17.10 | Caryophyllene oxide | C ₁₅ H ₂₄ O | 220 | 2.22 | 760 | 797 | 79 (90), 93 (65), 109 (45) and 220 (15) |
| 22 | 17.29 | Epiglobulol | C ₁₅ H ₂₆ O | 222 | 0.48 | 655 | 707 | 55 (45), 82 (60), 161 (30) and 204 (30) |
| 23 | 17.56 | Palustrol | C ₁₅ H ₂₆ O | 222 | 0.23 | 589 | 831 | 55 (80), 69 (60), 111 (100) and 122 (80) |
| 24 | 17.84 | (1-Methyldecyl)-benzene | C ₁₇ H ₂₈ | 232 | 1.30 | 640 | 712 | 105 (100), and 232 (20) |
| 25 | 17.91 | (1-Butylheptyl)-benzene | C ₁₆ H ₂₆ | 232 | 3.12 | 775 | 802 | 91 (100), 147 (20), and 232 (20) |
| 26 | 18.14 | Acetic acid, (1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethylnaphth-2-yl) methyl ester | C ₁₆ H ₂₆ O ₂ | 250 | 5.31 | 571 | 580 | 105 (60), 175 (80), 190 (45) and 250 (30) |
| 27 | 18.31 | Junipene | C ₁₅ H ₂₄ | 204 | 2.57 | 676 | 703 | 91 (60), 105 (45), and 204 (30) |
| 28 | 18.62 | 12-Methyl-E,E-3,13-octadecadien-1-ol | C ₁₉ H ₃₆ O | 280 | 22.49 | 609 | 623 | 55 (100), 121 (40), and 248 (20) |
| 29 | 19.46 | 5-Ethyl-5-hydroxy-3-methyl-2-phenyltetrahydrofuran-2-carboxylic acid | C ₁₄ H ₁₈ O ₄ | 250 | 2.64 | 866 | 995 | 105 (100) |
| 30 | 20.00 | (1-pentylheptyl)-Benzene | C ₁₈ H ₃₀ | 246 | 3.09 | 667 | 686 | 91 (100), and 246 (15) |
| 31 | 20.11 | (1-butylloctyl)-Benzene | C ₁₈ H ₃₀ | 246 | 2.94 | 842 | 843 | 91 (100), 147 (20), and 246 (10) |
| 32 | 20.38 | (1-propylnonyl)-Benzene | C ₁₈ H ₃₀ | 246 | 2.07 | 745 | 755 | 91 (100), 133 (35), and 246 (10) |

Table 3 continued

| Peak No. | RT | Compound name | Molecular formula | MW | Peak area % | SI | RSI | Most fragment ions with RI (%) |
|----------|-------|---|--|-----|-------------|-----|-----|---|
| 33 | 20.48 | 1-Cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl) | C ₁₂ H ₂₀ O ₂ | 196 | 0.22 | 508 | 687 | 153 (100) and 196 (10) |
| 34 | 21.11 | Calarene epoxide | C ₁₅ H ₂₄ O | 220 | 0.22 | 564 | 706 | 69 (60), 93 (45), and 220 (25) |
| 35 | 21.29 | Isoaromadendrene epoxide | C ₁₅ H ₂₄ O | 220 | 1.31 | 673 | 739 | 55 (60), 107 (45), and 220 (20) |
| 36 | 21.72 | 2-Benzyl-3-heptyloxirane | C ₁₆ H ₂₂ O ₂ | 246 | 1.46 | 877 | 965 | 105 (100) |
| 37 | 22.21 | Torreyol | C ₁₅ H ₂₆ O | 222 | 6.46 | 617 | 643 | 119 (40), 161 (100), 204 (20) and 222 (5) |
| 38 | 23.22 | 11-phenyl-Heneicosane | C ₂₇ H ₄₈ | 372 | 0.86 | 549 | 608 | 91 (100), 231 (30), and 372 (10) |
| 39 | 23.31 | 17-Pentatriacontene | C ₃₅ H ₇₀ | 490 | 0.72 | 581 | 622 | 57 (90), 83 (55), and 490 (5) |
| 40 | 37.32 | 1,2-Benzenedicarboxylic acid, dioctyl ester | C ₂₄ H ₃₈ O ₄ | 390 | 8.01 | 566 | 661 | 57 (70), 149 (100), and 390 (5) |

RT retention time (min), MW molecular weight (g/mol), SI standard index, RSI reverse standard index, RI relative intensities

GC–MS analysis of water extract

The identified chemical composition of WE from *Schinus molle* L. WB is shown in Table 4 and represented 37 compounds. The main chemical compounds in WE were β -eudesmol (39.4 %), and (Z,Z,Z)-9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester (3.20 %).

In accordance with the previous findings, most of the identified compounds have been reported elsewhere in other studies on the same species and/or other species such as limonene, α -phellandrene, myrcene, elemol and caryophyllene in DCME of *Schinus molle* L. WB and these compounds were reported in the ME of leaves and fruits of *Schinus molle* L. [38]. 2-Naphthalene methanol, decahydro- $\alpha,\alpha,4a$ -trimethyl-8-methylene was identified as the major compound of hydro-distillation and methanol extracts of *Schinus molle* L. WB and was reported as a major compound in the hydro-distillation extract of the aerial parts of *Ferula assafoetida*. 12-methyl-E,E-3,13-octadecadien-1-ol was identified as the major (22.49 %) compound of DCME of *Schinus molle* L. WB as well as a major compound in the ethanolic seed extract of *Syzygium cumini* [39]. Docosane was presented as a major compound in steam distillation extract of the aerial part, flowers, leaves and stems of *Tamarix boveana* [40].

Some of the identified compounds have been reported to possess various biological activities. For instance, antioxidant, antimicrobial, anti-inflammatory, anticancer, antialdosteronic, antihepatotoxic, antinociceptive, antiaphidic, antiencephalopathic, antigestric, antiprosthetic, antipruritic and hypocholesterolemic activities as summarized in Table 5. Among the identified phytochemicals, hexadecanoic acid, phenylmethyl ester (palmitic acid ester) is reported to be an antioxidant, hypocholesterolemic, antiandrogenic, hemolytic [41], while 6,9,12-octadecatrienoic acid, methyl ester is said to be a cancer-preventive,

anti-inflammatory, hypocholesterole, and hepatoprotective [41]. Other antioxidants presented were stigmast-5-en-3-ol and myrcene. The presence of antioxidants in *Schinus molle* L. WB extracts justifies the observed antioxidant property [10, 14, 42].

Total phenolic, flavonoid contents and antioxidant activity

The contents of total phenolic (TP) equivalent to tannic acid (TA) and total flavonoid (TF) equivalent to (+)-catechin (CA) of ME, DCME, and WE from *Schinus molle* L. WB are presented in Table 6. In addition, the total antioxidant activity (TAA %) for ME, DCME, and WE, as well as the EOs was reported. The highest values of TP content (30.90 ± 1.90 mg TAE/g extract) was found in WE, and the highest TF content (50.70 ± 1.80 mg CAE/g extract) reported with ME (50.70 ± 1.80 mg CAE/g extract). The TAA % found with EOs (90 ± 1.23 %) was followed by WE (86.30 ± 1.40 %), and these values were higher than those measured by TA and CA (85 ± 5.12 , and 84.13 ± 1.90 , respectively). Also, the lowest IC₅₀ was found by WE and EOs with values of 13.11 ± 3.00 , and 12.66 ± 2.15 μ g/mL, respectively, and those values were lower than the IC₅₀ of TA and CA (24.70 ± 0.48 , and 30.75 ± 0.88 μ g/mL, respectively).

Pharmacologically, the extracts of leaves from *Schinus molle* L. had antioxidant and anticancer activities [43]. The concentration values >250 μ g/mL of the EOs had a good antioxidant activity [36]. EOs with a concentration of 16 mg/mL was promoted a free radical scavenging effect of 4.8 %; however, this is much lower than that which was observed for ascorbic acid as a standard one [32].

Previously, TAA % of leaves EOs was weak [33]. Also, EOs with ability to scavenge the DPPH radical are of main interest in food industry as the antioxidant capacity is

Table 4 The chemical compositions of water extract from *Schinus molle* L. wood branch analyzed by gas chromatography–mass spectrometry (GC-MS)

| Peak No. | RT | Compound name | Molecular formula | MW | Peak area % | SI | RSI | Most fragment ions with RI (%) |
|----------|-------|--|---|-----|-------------|-----|-----|--|
| 1 | 2.60 | 8-Octadecenal | C ₁₈ H ₃₄ O | 266 | 0.84 | 484 | 523 | 57 (100), 83 (80), and 97 (45) |
| 2 | 2.77 | 2-(1-Phenanthryl)benzaldehyde | C ₂₁ H ₁₄ O | 282 | 0.64 | 448 | 726 | 252 (60) and 282 (96) |
| 3 | 2.86 | Decane | C ₁₀ H ₂₂ | 142 | 2.22 | 688 | 869 | 57 (100), 71 (50), and 142 (20) |
| 4 | 3.49 | Docosane | C ₂₂ H ₄₆ | 310 | 0.63 | 427 | 442 | 57 (95), 71 (60), and 310 (5) |
| 5 | 4.69 | Deoxyspergualin | C ₁₇ H ₃₇ N ₇ O ₃ | 387 | 0.32 | 430 | 482 | 72 (60), 86 (35), and 387 (2) |
| 6 | 4.92 | 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | C ₆ H ₈ O ₄ | 144 | 2.16 | 545 | 858 | 55 (100), 101 (40), and 144 (45) |
| 7 | 5.42 | N2-(3-indolylmethylene)-furan-2-carbohydrazide | C ₁₄ H ₁₁ N ₃ O ₂ | 253 | 0.44 | 395 | 504 | 95 (90), 142 (100), and 253 (25) |
| 8 | 6.53 | Prednisone | C ₂₁ H ₂₆ O ₅ | 358 | 0.74 | 399 | 451 | 121 (100), 147 (65), 299 (45) and 358 (35) |
| 9 | 8.20 | 6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo [4.1.0]heptan-2-ol | C ₁₃ H ₂₂ O ₃ | 226 | 1.55 | 523 | 615 | 55 (35), 100 (40), 125 (45) and 226 (5) |
| 10 | 9.80 | 3,6-Epoxy-2H,8H-pyrimido[6,1-b][1,3]oxazocin-8-one, 3,4,5,6-tetrahydro-4-hydroxy-10-amino | C ₉ H ₁₁ N ₃ O ₄ | 225 | 1.48 | 511 | 617 | 81 (75), 111 (90), 152 (45) and 225 (25) |
| 11 | 10.73 | α-Cedrene | C ₁₅ H ₂₄ | 204 | 1.06 | 464 | 569 | 69 (80), 119 (100) and 204 (45) |
| 12 | 11.86 | 18,21-Didehydro-6,17-didemethoxy-18,21-dideoxo-18,21-dihydroxy-15-methoxy-6-methyl-11-O-methyl-geldanamycin | C ₃₀ H ₄₄ N ₂ O ₈ | 560 | 0.61 | 425 | 482 | 79 (90), 122 (92) 288 (25) and 560 (1) |
| 13 | 15.44 | 2-(7-Hydroxymethyl-3,11-dimethyl-dodeca-2,6,10-trienyl)-[1,4]benzoquinone | C ₂₁ H ₂₈ O ₃ | 328 | 0.89 | 469 | 521 | 69 (100), 123 (25), 161 (55) and 328 (5) |
| 14 | 15.92 | 6,9,12-Octadecatrienoic acid, methyl ester | C ₁₉ H ₃₂ O ₂ | 292 | 5.96 | 485 | 521 | 55 (50), 67 (55), 79 (45) and 292 (20) |
| 15 | 16.40 | 1-Propyl-2-methyl-7-methoxy-5H,6H-pyrido[3,4-b]indole | C ₁₆ H ₂₀ N ₂ O | 256 | 0.36 | 441 | 497 | 83 (100), 213 (60) and 256 (15) |
| 16 | 17.78 | (1,5,5,8-Tetramethyl-bicyclo[4.2.1]non-9-yl)-acetic acid | C ₁₅ H ₂₆ O ₂ | 238 | 1.19 | 450 | 503 | 95 (65), 153 (100) and 238 (15) |
| 17 | 19.48 | β-Eudesmol | C ₁₅ H ₂₆ O | 222 | 39.4 | 693 | 805 | 59 (100), 149 (45), 204 (20) and 222 (25) |
| 18 | 19.61 | Acetic acid, 17-(1-acetoxy-ethyl)-10,13 -dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a] phenanthren-11-yl | C ₂₅ H ₃₄ O ₅ | 414 | 0.23 | 429 | 453 | 159 (25), 279 (30), 294 (40) and 414 (5) |
| 19 | 19.70 | Murolan-3,9(11)-diene-10-peroxy | C ₁₅ H ₂₄ O ₂ | 236 | 1.16 | 471 | 605 | 69 (60), 91 (65), 159 (75) and 236 (15) |
| 20 | 20.24 | Ethyl iso-allocholate | C ₂₆ H ₄₄ O ₅ | 436 | 1.52 | 492 | 515 | 55 (95), 69 (75), 107 (45) and 436 (2) |
| 21 | 20.39 | Carotene | C ₄₀ H ₅₆ | 536 | 0.50 | 520 | 521 | 119 (45), 159 (75), and 536 (10) |
| 22 | 20.51 | 24,25-Dihydroxycholecalciferol | C ₂₇ H ₄₄ O ₃ | 416 | 1.30 | 494 | 550 | 55 (100), 118 (90), 136 (100) and 416 (2) |
| 23 | 20.79 | 12,12a-Dihydroxy-2,2,7,7,9,11-hexamethyl-7H-6,9a-methano-4H-cyclopenta[9,10]cyclopropa[5,6] cyclodeca[1,2-d]-1,3-dioxin-13-one,6,6a,7a,8,9,12,12a,12b-oct ahydro | C ₂₃ H ₃₂ O ₅ | 388 | 2.94 | 504 | 537 | 91 (90), 121 (100), 284 (65) and 388 (15) |
| 24 | 21.74 | (Z,Z,Z)-9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester | C ₂₁ H ₃₆ O ₄ | 352 | 3.20 | 530 | 574 | 79 (100), 95 (65), 15 (35) and 352 (5) |

Table 4 continued

| Peak No. | RT | Compound name | Molecular formula | MW | Peak area % | SI | RSI | Most fragment ions with RI (%) |
|----------|-------|--|---|-----|-------------|-----|-----|---|
| 25 | 22.14 | Stigmast-5-en-3-ol | C ₂₉ H ₅₀ O | 414 | 0.94 | 505 | 521 | 91 (100), 145 (65), 255 (35) and 414 (2) |
| 26 | 22.23 | 1,5-Diphenyl-1,4-pentadien-3-one | C ₂₄ H ₃₄ O ₃ | 370 | 0.80 | 439 | 494 | 55 (40), 161 (30) and 370 (45) |
| 27 | 22.50 | Cassamic acid methyl ester | C ₂₂ H ₃₂ O ₅ | 376 | 0.29 | 409 | 431 | 268 (45), 327 (90) and 376 (1) |
| 28 | 22.61 | Sophoracarpan B | C ₁₇ H ₁₄ O ₆ | 314 | 0.68 | 440 | 698 | 282 (100) and 314 (30) |
| 29 | 22.86 | 14-Hydroxy-13-methoxycodeinone-hydrazone | C ₁₈ H ₂₁ N ₃ O ₃ | 327 | 1.48 | 405 | 745 | 281 (80), 309 (40), 152 (96) and 327 (96) |
| 30 | 23.21 | 9-Desoxo-9- α -acetoxy-3,8,12-tri-O-acetylingol | C ₂₈ H ₄₀ O ₁₀ | 536 | 2.07 | 497 | 512 | 69 (25), 122 (300) and 536 (5) |
| 31 | 23.63 | Dodecanoic acid, 1 α ,2,5,5 α ,6,9,10,10 α -octahydro-5,5 α -dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetra methyl-11-oxo-1H-2,8 α -methanocyclopenta[a]cyclopropana[e]cyclodecen-6-yl ester | C ₃₂ H ₅₀ O ₆ | 530 | 0.70 | 417 | 421 | 122 (100), 312 (40) and 530 (2) |
| 32 | 24.75 | Olean-12-ene-3,15,16,21, 22,28-hexol | C ₃₀ H ₅₀ O ₆ | 506 | 0.33 | 388 | 407 | 249 (100), 280 (75), 334 (45) and 506 (2) |
| 33 | 26.38 | Stearic acid, 3-(octadecyloxy)propylester | C ₃₉ H ₇₈ O ₃ | 594 | 1.78 | 443 | 461 | 71 (100), 281 (70), 325 (60) and 594 (5) |
| 34 | 27.27 | 2-(3-Acetoxy-4,4,14-trimethylandro-8-en-17-yl)-propanoic acid | C ₂₇ H ₄₂ O ₄ | 430 | 0.60 | 426 | 438 | 83 (45), 355 (100), 415 (45) and 430 (25) |
| 35 | 31.09 | Colchicine | C ₂₁ H ₂₃ NO ₆ | 385 | 2.51 | 382 | 418 | 115 (40), 314 (60) and 385 (85) |
| 36 | 32.88 | Deoxyherqueinone | C ₂₀ H ₂₀ O ₆ | 356 | 0.43 | 385 | 769 | 341 (100), and 356 (80) |
| 37 | 32.95 | 1H-Pyrrole-3,4-diacetic acid, 2-acetoxymethyl-5-methoxycarbonyl-, dimethyl ester | C ₁₅ H ₁₉ NO ₈ | 341 | 0.89 | 360 | 429 | 190 (65), 281 (100) and 341 (25) |

RT retention time (min), MW molecular weight (g/mol), SI standard index, RSI reverse standard index, RI relative intensities

nowadays accepted as a criterion of high food quality [44]. On the other hand, oil of berries had a good antioxidant activity [2, 30]. Myrcene represented a percentage of the total 12.26 % in the oil of the WBs, and it was reported that the EOs of the chemotype rosemary rich in myrcene had the highest antioxidant activity and probably due to the high content of myrcene [44]. ME of *Schinus molle* L. bark and flowers exhibited remarkable antioxidant activity with EC₅₀ values of 8.6 ± 1.6 and 15.2 ± 4.0 µg/mL, respectively [7].

Antibacterial activity

Table 7 presents that the highest inhibition zone (IZ, mm) against the growth of *B. subtilis*, *B. cereus*, *E. coli*, *S. lutea*, *P. aeruginosa*, and *M. luteus* was observed by WE of *Schinus molle* L. WB with values of 18 ± 1.12, 20 ± 3.14, 15 ± 0.33, 15 ± 2.12, 16 ± 1.13, 14 ± 0.33 mm, respectively. On the other hand, EOs and ME showed the highest IZ values against the growth of *S. aureus* with values of 15 ± 0.33 and 15 ± 1.33 mm, respectively. Furthermore, most of IZ values are lower than those values obtained from the standard antibiotic.

According to the MIC values presented in Table 7, the EOs observed good antibacterial activity against the studied bacterial strains with MIC values of 250, 32, 64, 125, 125, 250, and 500 µg/mL, against the growth of *B. subtilis*, *B. cereus*, *S. aureus*, *E. coli*, *S. lutea*, *P. aeruginosa*, and *M. luteus*, respectively. However, these MIC values were lower than the value of positive control used (Tetracycline, 20 µg/disk) (Table 7).

The MIC values ranged from 32 to 1000 µg/mL for the ME and between 125 and 1000 µg/mL with DCME, and these values were higher than those from conventional antibiotic used (Tetracycline, 20 µg/disk) (Table 7). On the other hand, WE showed good antibacterial activity, where the MIC value for *B. subtilis* was 4 mg/mL, *B. cereus* 32 mg/mL, *S. aureus* 8 mg/mL, *E. coli* 8 mg/mL, *S. lutea* 4 mg/mL, *P. aeruginosa* 32 mg/mL and for *M. luteus* 125 mg/mL (Table 7). Clearly, the MIC values showed that the EOs and WE owned good antibacterial activity than either ME or DCME.

Previously, the EOs extracted from the fresh leaves of *Schinus molle* L. showed a significant activity against *Klebsiella pneumoniae*, *Alcaligenes faecalis*, *P. aeruginosa*, *Leuconostoc cremoris*, *Enterobacter aerogenes*,

Table 5 Summary of chemical compounds identified from the *Schinus molle* L. wood branch extracts and their general biological activities (Modified from Dr. Duke's: Phytochemical and Ethnobotanical Databases)

| Chemical compounds | Biological activities |
|---|---|
| 1- Stigmast-5-en-3-ol | Antihepatotoxic, anti-inflammatory, antinociceptive, antiophidic, antioxidant; antiviral, artemicide, cancer-preventive, estrogenic, hypocholesterolemic, ovulant; sedative |
| 2- Stearic acid, 3-(octadecyloxy)propylester | 5-alpha-reductase-inhibitor, cosmetic, flavor, hypocholesterolemic; lubricant, perfumery; propepic, suppository |
| 3- Dodecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropana[e]cyclodecen-6-yl ester | Flavor |
| 5- (Z,Z,Z)-9,12,15-octadecatrienoic acid -2,3- dihydroxypropyl ester | Analgesic, antipyretic, anticonvulsant, antiseptic |
| 6- Acetic acid, 17-(1-acetoxy-ethyl)-10,13 -dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a] phenanthren-11-yl | Acidulant, antibacterial, antitotic, antisalmonella, antivaginitic, expectorant, fungicide, keratitogenic, mucolytic, osteolytic, perfumery, pesticide, protisticide, spermicide, ulcerogenic, verrucolytic |
| 7- 2-Naphthalene methanol, decahydro- $\alpha,\alpha,4a$ -trimethyl-8-methylene | Cataractogenic |
| 8- 6,9,12-Octadecatrienoic acid, methyl ester | Anti-inflammatory, hypocholesterolemic, cancer-preventive, hepatoprotective, nematocide, insectifuge antihistaminic, antiarthritic, anticoronary, antieczemic, antiacne, 5- α reductase inhibitor antiandrogenic |
| 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | Antimicrobial, anti-inflammatory |
| 10- 2-(1-Phenanthryl)benzaldehyde | Allergenic, anesthetic, antibacterial, anticancer, antimutagenic, antipeptic, antiseptic, antisp, nematocide, insecticide, pesticide |
| 11- (1-Butylheptyl)-benzene | Antimicrobial |
| 12- 1,2-Benzenedicarboxylic acid, diisooctyl ester | Antimicrobial antifouling |
| 13- Torreyol | Pesticide, termiticide |
| 14- Benzene, (1-pentylheptyl) | Antimicrobial |
| 15- Caryophyllene oxide | Antiedemic, antifeedant, anti-inflammatory, antitumor, calcium-antagonist, fungicide, insecticide, pesticide |
| 16- Hexadecanoic acid, phenylmethyl ester | Antioxidant, hypocholesterolemic, antiandrogenic, hemolytic |
| 17- (1-Propylnonyl)-benzene | Antimicrobial |
| 18- (Z)-9-Octadecenoic acid | Cancer-preventive, insectifuge |
| 19- α -Phellandrene | Hyperthermic, Irritant, Spasmogenic, Tumor-Promoter; |
| 20- (1-methyldecyl)-benzene | Antimicrobial |
| 21- Limonene | AChE-inhibitor, acaricide, allelochemic, allergenic, antiacetylcholinesterase, antiadenomic, antialzheimeran, antiasthmatic, antibacterial; anticancer, antiesophagitic, antifeedant, antifu, anti-inflammatory, antilithic, antilymphomic, antimetastatic (stomach), antimutagenic, antiobesity, antiseptic, antispasmodic, antitumor, antitumor (breast), antitumor (colon), antitumor (pancreas), antitumor (prostate), antitumor (stomach), antiviral, apoptotic, bronchoprotectant, cancer-preventive, candidistat, chemopreventive, cholesterolytic, detoxicant, enterocontractant, expectorant, flavor, fungiphilic, fungistat, GST-inducer, herbicide, histaminic, immunomodulator, insecticide, insectifuge, interleukin-6-inhibitor, irritant, lipolytic, myorelaxant, nematocide, ornithine-decarboxylase-inhibitor, ozone-scavenger, peristaltic, pesticide, photosensitizer, sedative, transdermal |
| 22- Myrcene | ACE-inhibitor, aldose-reductase-inhibitor, allergenic, analgesic, antibacterial, anticonvulsant, antimutagenic, antinitrosaminic, antinociceptive.; antioxidant, antispasmodic, chemopreventive; flavor; fungicide, hypothermic, insectifuge, irritant, myorelaxant, perfumery, pesticide |
| 23- (1-Butyloctyl)-benzene | Antimicrobial |

Table 6 Total phenolic and flavonoid contents and antioxidant activity of different extracts from *Schinus molle* L. wood branch

| Extract | Total phenolic (mg TAE/g extract) | Total flavonoids (mg CAE/g extract) | TAA % | IC ₅₀ (µg/mL) ^a |
|----------------------|--------------------------------------|--|---------------------------|---------------------------------------|
| ME | 12.3 ± 2.00 ^c | 50.70 ± 1.80 ^a | 70 ± 1.90 ^d | 228.66 ± 1.12 ^b |
| Dichloromethane DCME | 25.1 ± 2.50 ^b | 19.33 ± 1.12 ^c | 30.02 ± 0.12 ^e | 334.11 ± 1.53 ^a |
| WE | 30.90 ± 1.90 ^a | 30.30 ± 1.13 ^b | 86.30 ± 1.40 ^b | 12.66 ± 2.15 ^e |
| EO | – | – | 90 ± 1.23 ^a | 13.11 ± 3.00 ^e |
| TA | – | – | 85 ± 5.12 ^c | 24.70 ± 0.48 ^d |
| CA | – | – | 84.13 ± 1.90 ^c | 30.75 ± 0.88 ^c |

(–) Not applicable

Values with different letters within the same column were significantly different at the level of $p \leq 0.05$

All values are mean ± standard deviation of three replicates

TAA % total antioxidant activity, TAE tannic acid equivalents, CAE (+)-catechin equivalents, TA tannic acid, CA (+)-catechin, ME methanol extract, DCME dichloromethane extract, WE water extract, EO essential oil

^a IC₅₀ data expressed as µg/mL. Lower IC₅₀ values indicated the highest radical scavenging activity

Table 7 Antibacterial activity of essential oil, methanol, dichloromethane, and water extracts from *Schinus molle* L. wood branch against some pathogenic bacteria

| Bacteria | Essential oil | | Methanol extract | | Dichloromethane extract | | Water extract | | Negative control ^a | Positive control ^b | |
|----------------------|---------------|-------------|------------------|-------------|-------------------------|-------------|---------------|-------------|-------------------------------|-------------------------------|-------------|
| | IZ (mm) | MIC (µg/mL) | IZ (mm) | MIC (µg/mL) | IZ (mm) | MIC (µg/mL) | IZ (mm) | MIC (µg/mL) | | IZ (mm) | MIC (µg/mL) |
| <i>B. subtilis</i> | 13 ± 0.12 | 250 | 15 ± 1.12 | 125 | 12 ± 0.12 | 125 | 18 ± 1.12 | 4 | NA | 25 ± 1.33 | 8 |
| <i>B. cereus</i> | 12 ± 0.00 | 32 | 13 ± 0.14 | 32 | 10 ± 1.33 | 500 | 20 ± 3.14 | 32 | NA | 20 ± 0.66 | 8 |
| <i>S. aureus</i> | 15 ± 0.33 | 64 | 15 ± 1.33 | 32 | 8 ± 0.33 | 1000 | 13 ± 0.66 | 8 | NA | 21 ± 0.00 | 16 |
| <i>E. coli</i> | 14 ± 0.00 | 125 | 10 ± 0.66 | 250 | 8 ± 0.00 | 1000 | 15 ± 0.33 | 8 | NA | 22 ± 0.00 | 16 |
| <i>S. lutea</i> | 12 ± 0.15 | 125 | 12 ± 0.16 | 1000 | 7 ± 0.00 | 1000 | 15 ± 2.12 | 4 | NA | 17 ± 1.12 | 8 |
| <i>P. aeruginosa</i> | 12 ± 0.00 | 250 | NA | NA | NA | NA | 16 ± 1.13 | 32 | NA | 26 ± 3.12 | 8 |
| <i>M. luteus</i> | 9 ± 0.00 | 500 | NA | NA | NA | NA | 14 ± 0.33 | 125 | NA | 20 ± 0.00 | 32 |

Values of inhibition zones (IZs) are presented at the concentration of 200 µg/mL

NA not active, MIC minimum inhibitory concentration (µg/mL)

^a Disks were impregnated with 10 % dimethyl sulfoxide (DMSO)

^b IZ of tetracycline (20 µg/disk)

Proteus vulgaris, *Clostridium sporogenes*, *Acinetobacter calcoacetica*, *E. coli*, *Beneckea natriegens*, *Citrobacter freundii*, *S. marcescens*, *B. subtilis* and *Brochothrix thermosphacata* [45]. Extracts from leaves, flowers and bark of *Schinus molle* L. displayed the strongest activity against both sensitive and resistant *S. aureus* strains (MIC between 62.5 and 250 µg/mL) and the flower extracts were the most active [7]. Hexane extract from fruits of *Schinus molle* L. showed good antimicrobial activity against *Streptococcus pneumoniae* with MIC = 62.5 µg/mL [46]. The aqueous extract of *Schinus molle* L. grown in Brazil, showed good antifungal activity against *Candida albicans* [47].

Elemol, a sesquiterpene, was isolated and identified from the EOs of *Amyris balsamifera* wood (Rutaceae) [48], and the oleoresin of *Canarium zeylanicum* bark and timber [49]. Elemol and *p*-cymene can be considered as

the principal antimicrobial components of the EOs [50]. β -selinene, α -elemol, and hinesol showed pronounced contact toxicity against *Drosophila melanogaster* adults [51].

Elemol was found to be as good, and, in some cases, better at repelling house flies (*Musca domestica* L.), and American cockroaches (*Periplaneta americana* L.), than *N,N*-diethyl-*m*-toluamide (DEET) or citronellal [52]. Phenolic compounds including simple phenolic such as EOs play a role in antioxidant activity in many species, consequently affecting the medicinal value of each plant part [53].

From all the above results, the identified chemical compounds from extracts of *Schinus molle* L. WB have been reported to possess various biological activities. *Schinus molle* L. is widely used in traditional medicine to

treat a variety of diseases in countries other than Egypt. To date, no information is available on medicinal uses of *Schinus molle* L. WB from Egypt, and our study is the first study to show the biological activities of *Schinus molle* L. WB from Egypt.

Conclusions

In the present study, chemical components of essential oils, methanol, dichloromethane and water extracts from wood branch supplied from the tree pruning wastes of *Schinus molle* L. were identified by GC–MS. The main constituents of the essential oils were α -elemol, β -pinene, myrcene, α -phellandrene, caryophyllene, α -cadinol, cadinene, elixene, β -eudesmol, nerolidol, γ -eudesmol, and germacrene-D-4-ol. The major components in methanol extract were 6-(4-chlorophenyl)-3-cyano-4-(*N*-benzylpiperazino)-2H-pyran-2-one, 2-naphthalene methanol, decahydro- $\alpha,\alpha,4a$ -trimethyl-8-methylene and docosane, in dichloromethane extract were 12-methyl-E,E-3,13-octadecadien-1-ol, 1,2-benzenedicarboxylic acid, dioctyl ester and acetic acid, (1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethylnaphth-2-yl) methyl ester, and in water extract were β -eudesmol, and (*Z,Z,Z*)-9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester.

The highest values of total phenolic contents were found in water extract, and the highest total flavonoid contents were found in methanol extract. The highest total antioxidant activity as measured by DPPH was found with essential oil (90 ± 1.23 %) followed by water extract (86.30 ± 1.40 %), and those values were higher than the values measured by tannic acid and (+)-catechin (85 ± 5.12 , and 84.13 ± 1.90 , respectively). The lowest IC₅₀ value was 13.11 ± 3.00 μ g/mL (water extract) and 12.66 ± 2.15 μ g/mL (essential oil), and those values were lower than the IC₅₀ of tannic acid and (+)-catechin (24.70 ± 0.48 and 30.75 ± 0.88 μ g/mL, respectively). The MICs values of essential oils and water extract observed good antibacterial activity against the growth of the studied bacteria. This suggests that the essential oil as well as water extract of WBs have a potential effect for use in food and/or pharmaceutical industries. Several compounds have been identified from essential oil, methanol, dichloromethane and water extracts of *Schinus molle* L. wood branch by GC/MS analysis. Some of the identified compounds have been reported to possess various biological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, antialdosteronic, antihepatotoxic, anti-inflammatory, antinociceptive, antiophidic, antiencephalopathic, antigastric, antiprosthetic, antipruritic, and hypocholesterolemic. The presence of various bioactive compounds justifies the use of

wood branches for various ailments by traditional practitioners. The yield of the essential oils from wood branch is 0.75 mL/100 g fresh weight, while in our previous works, it was 0.40 mL/100 g leaves fresh weight [35] and, from berries, was 4.26 mL/100 g berries fresh weight (unpublished data) or 0.49 % v/w from dried fruits (36 °C in a forced air drier with humidity control) [54]. Therefore, we recommend using the wood branch of *Schinus molle* L. as bioresource for phytopharmaceutical importance. However, further studies need to be undertaken to ascertain fully its bioactivity.

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