INTRODUCTION

The genus Satureja L. belonging to the family Lamiaceae contains about 200 species of aromatic herbs and shrubs that are native to warm temperate regions and may be annual or perennial grow in the Middle East, Mediterranean region to Europe, West Asia, and North Africa. Over 30 species of this genus are distributed in eastern parts of the Mediterranean area [1]. Many of these species have different biological activities such as antibacterial, antifungal, antioxidant, cytotoxic, insecticidal, anti-diabetic, anti-lipase, wound healing, trypanocidal/anti-protozoal, enzyme inhibition, anti-spasmodic, vasodilatory-vasorelaxant, anti-tumor, and diuretic activities [2-9]. Previously, phytochemical analysis of Satureja species revealed the presence of volatile oils, phenolic acids, anthocyanins, flavones, diterpenes, triterpenes, and steroids [10-14].

The major constituents of hydrodistilled volatile oils from the aerial parts of Satureja montana and Sascifraga cuneifolia growing in Croatia were identified by gas chromatography–mass spectrometry (GC-MS) and found carvacrol (17.7%) and spathulenol (13.2%) as the major compounds [15]. Twenty one compounds in the oil of Satureja montana essential oil were identified by GC-FID analyses and carvacrol was the main (60 %) [9]. Many flavonoids were isolated from Satureja khaszitanica [16]. The identified flavonoids are aromadendrin, taxifolin, naringenin, 5,7,3’5’-tetrahydroxy flavanone, xanthomirolic acid, acacetin, cirsimaritin, 7-methoxyluteolin, apigenin, cirsilineol, diosmetin, and 6-hydroxyluteolin 7,3’-dimethyl ether, in addition to a new monoterpene–flavonoid known as saturejin, while Matloubi-Ahmadi et al., in 2005 [17], isolated thymol, oleanolic acid, ursolic acid, and these compounds were effective against Artemia salina larvae. While luteolin, oleuropein acid, β-sitosterol, and diosmetin were isolated from the ethyl acetate and methanol extracts of Satureja sahendica [13].

Ahamad et al., in 2005 [17], isolated thymol, oleuropein acid, ursolic acid, and caryophyllene oxide from the aerial parts of S. macrantha and these compounds were effective against Artemia salina larvae. While luteolin, oleuropein acid, β-sitosterol, and diosmetin were isolated from the ethyl acetate and methanol extracts of Satureja sahendica [13].

The surface flavonoids in Satureja thymbra and Satureja spinosa were studied [18] and identified the following compounds: Naringenin, aromadendrin, eriodictyol, taxifolin, apigenin, genkwanin, ladanene, cirsimaritin, thymusin, xanthomirolic acid, luteolin7-methyl ether, 6-hydroxyluteolin 7,3’-dimethyl ether, 6-hydroxyluteolin 7,3’4’-trimethyl ether, cirsilineol, thymonin, and 8-methoxycirsilineol.

METHODS

Plant material

S. montana L. was cultivated and grown in the farm of National Research Center (NRC), Giza, Egypt, and it was collected in May 2015.
The authentication of plant sample was achieved by Dr. Mohammed Algebly a taxonomist at NRC. A voucher specimen was deposited in the herbarium of the NRC. The aerial parts were air-dried for 2 weeks under laboratory conditions at 28 ± 2°C. The dried material was ground using a domestic blender to fine powder.

**Instruments and chemicals**

UV spectra were recorded on Shimadzu model UV-240 and 2401 PC spectrophotometer (Shimadzu Inc., Tokyo, Japan). Buchi apparatus was used to determine the melting points of the isolated pure compounds in open capillaries. Nuclear magnetic resonance (NMR) experiments were recorded on Bruker spectrometer (Switzerland) 600 (1H NMR spectra 600 MHz; 13C NMR spectra: 150 MHz). The chemical shifts are given in δ (ppm) relative to tetramethylsilane (Me_Si). Column chromatography (CC) was carried out on Polyamide S6 (Riedel-de Haen AG, Seelze Hanover, Germany) and Sephadex LH-20 (Pharma, Upssala, Sweden). Paper chromatography (PC, descending) Whatman No. 1 and 3 mm papers, using solvent systems: (1) H_2O, (2) 15% HOAc (H_2O: HOAc, 85:15), (3) BAW (n-BuOH: HOAc:H_2O, 4:1:5, upper layer), and (4) BPBW (C_6H_5_n-BuOH:Pyridine:H_2O, 1:5:3, upper layer).

**Isolation and purification of the chemical constituents**

The powder of the aerial parts (2 kg) was extracted 3 times at room temperature with 70% methanol. The aqueous-methanol extract was evaporated under reduced pressure and temperature to obtain a residue of 350 g. The residue was defatted by n-hexane giving (80 g) n-hexane extract and (270 g) methanol extract.

**Fractionation of lipid constituents**

A fraction (1 g) of the n-hexane extract was analyzed for volatile constituents by GC/MS and after the, the rest of the n-hexane extract was passed over fuller’s earth (to remove the colored pigments). The solvent was evaporated under reduced pressure at 35°C, the obtained residue (20 g) was saponified to give unsaponifiable matters and the fatty acid methyl esters [19], which were analyzed by GC/MS and/or gas-liquid chromatography (GLC) as follows.

**GC/MS analysis of volatile constituents of n-hexane extract**

The analysis of n-hexane fraction of S. montana was performed using a Thermo Scientific capillary gas chromatography (model Trace GC Ultra) directly coupled to ISQ Single Quadrupole MS and equipped with TG-5MS, non-polar 5% phenyl methylpolysiloxane capillary column (30 m × 0.25 mm ID × 0.25 µm). The operating condition of GC oven temperature was maintained as initial temperature 40°C for 3 min, programed rate 5°C/min up to final temperature 280°C with isotherm for 5 min. For GC/MS detection, an electron ionization (EI) system with ionization energy of 70 eV was used. Helium was used as a carrier gas at a constant flow rate of 1.0 ml/min. 1 µl of the extract was injected automatically in the splitless mode. The quantification of the components was based on the total number of fragments (total ion count) of the metabolites as detected by the mass spectrometer.

Identification of the constituents was carried out by comparison of their retention times and fragmentation patterns of mass with those of published data and/or those of the Wiley and NIST mass spectra libraries.

**GLC analysis of unsaponifiable matters and GC/MS of fatty acid methyl esters**

The GLC analysis was carried out for unsaponifiable matters using the following conditions; Instrument: Varian model 3700 GC. Column for unsap.: 10% OV-101 on chromosorb W/HP, 80/100, (2 m stainless steel, 0.25 mm id.), column for unsap.: Column: 70°C up to 270°C, 4°C/min., injector: 280°C., detector (FID): 290°C., temperature for unsap.: 10% OV-101 on chromosorb W/HP, 80/100, (2 m stainless steel, 0.25 mm id.), injector: 280°C., detector (FID): 290°C., temperature for unsap.: 10% OV-101 on chromosorb W/HP, 80/100, (2 m stainless steel, 0.25 mm id.), injector: 280°C., detector (FID): 290°C., temperature for unsap.: 10% OV-101 on chromosorb W/HP, 80/100, (2 m stainless steel, 0.25 mm id.), injector: 280°C., detector (FID): 290°C., temperature for unsap.: 10% OV-101 on chromosorb W/HP, 80/100, (2 m stainless steel, 0.25 mm id.), injector: 280°C., detector (FID): 290°C., temperature for unsap.: 10% OV-101 on chromosorb W/HP, 80/100, (2 m stainless steel, 0.25 mm id.), injector: 280°C., detector (FID): 290°C., temperature for unsap.: 10% OV-101 on chromosorb W/HP, 80/100, (2 m stainless steel, 0.25 mm id.), injector: 280°C., detector (FID): 290°C., temperature for unsap.: 10% OV-101 on chromosorb W/HP, 80/100, (2 m stainless steel, 0.25 mm id.), injector: 280°C., detector (FID): 290°C., temperature for unsap.: 10% OV-101 on chromosorb W/HP, 80/100, (2 m stainless steel, 0.25 mm id.), injector: 280°C., detector (FID): 290°C., temperature for unsap.: 10% OV-101 on chromosorb W/HP, 80/100, (2 m stainless steel, 0.25 mm id.), injector: 280°C., detector (FID): 290°C., temperature for unsap.: 10% OV-101 on chromosorb W/HP, 80/100, (2 m stainless steel, 0.25 mm id.), injector: 280°C., detector (FID): 290°C., temperature for unsap.: 10% OV-101 on chromosorb W/HP, 80/100, (2 m stainless steel, 0.25 mm id.), injector: 280°C., detector (FID): 290°C.

While the conditions for FAMEs are instrument: A TRACE GC ultragels chromatographs (THERMO Scientific Corp., USA) coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC–MS system was equipped with a TG-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 80°C for 1 min; rising at 4.0°C/min to 300°C and held for 1 min. The injector and detector were held at 240°C. Diluted samples (1:10 hexane, v/v) of 0.2 µl of the mixtures were always injected. Mass spectra were obtained by EI at 70 eV, using a spectral range of m/z 40–450.

**Isolation of the phenolic constituents**

The methanol extract was subjected on polyamide S6 CC, eluting with H_2O followed by MeOH/H_2O mixtures of decreasing polarity which yielded four fractions 40, 60, 80, and 100% methanol. Each fraction was subjected to further purification using columns chromatography (small polyamide and Sephadex LH-20) and preparative paper chromatography (Whatman 3MM, with different solvent systems). The fraction 40% MeOH/H_2O yielded compound 1 (14 mg), followed by the fraction 60% MeOH/H_2O which yielded compound 2 (16 mg), compound 3 (17 mg), compound 4 (13 mg), and compound 5 (15 mg). On the other hand, compound 6 (13 mg) and compound 7 (18 mg) were isolated from the fraction 80% MeOH/H_2O, while 100% MeOH fraction yielded compound 8 (19 mg).

**Acid hydrolysis**

It was carried out for 2 h at 100°C using 2N HCl; the hydrolyzed part was then extracted with ethyl acetate; the extract being subjected to PC investigation to detect the aglycones. Sugars were identified by PC using (benzene:n-butanol:pyridine:water, 1:5:3:3) with authentic sugars. The dried chromatograms were visualized by aniline phthalate reagent, the sugar spots were observed in daylight. The tested sugars were compared with reference sugars. Identification of the aglycones was based on direct PC using authentic samples and/or spectral analyses.

**Antimicrobial activity study**

The antimicrobial activity of the tested extracts and/or isolated compounds against some microorganisms was determined using a modified Kirby-Bauer disc diffusion method [20-22].

Plates inoculated with filamentous fungi as Aspergillus flavus at 25°C for 48 h; Gram (+) bacteria as Staphylococcus aureus and Bacillus subtilis; Gram (-) bacteria as Escherichia coli and Pseudomonas aeruginosa, they were incubated at 35–37°C for 24–48 h and yeast as Candida albicans incubated at 30°C for 24–48 h, and then, the diameters of the inhibition zones were measured in millimeters [20]. The diameter of the inhibition zone surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism. Standard discs of ampicillin (antibacterial agent), amphotericin B (antifungal agent) served as positive controls for antimicrobial activity.

**Calculations**

% inhibition = sample inhibition zone (cm)/plate diameter ×100. The filter discs impregnated with 10 µl of solvent (distilled water, chloroform, DMSO) were used as a negative control. The test was carried out in triplicates [23].

**Antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method**

DPPH scavenging activity of the tested extracts and/or isolated compounds was measured at 517 nm by spectrophotometer method [24]. A 1 ml solution of 0.004% DPPH solution was added to the tested extracts and/or isolated compounds. The solution in the test tubes was shaken well and incubated in the dark for 30 min at room temperature. The disappearance of violet color of methanolic DPPH solution indicates scavenging capacity of the extract considered as the positive reaction.

**RESULTS AND DISCUSSION**

The GC/MS analysis was used to identify the volatile constituents from n-hexane fraction of S. montana, 39 compounds belonging to many
classes such as monoterpenes (30.7%) with carvacrol as the major one (24.23%), sesquiterpenes (4.06%) in which farnesyl acetone is the main, diterpenes (17.67%), one saturated fatty acid (palmitic acid, 17.1%), esters of fatty acids (10.14%) with methyl hexadecanetrieno as a main ester, phthalates constituted about 2.9%, and a sterol fraction of about 2.7% were identified (Table 1). These data were in accordance with that reported [15], where they identified the major constituents of hydrodistilled volatiles from the aerial parts of S. montana growing in Croatia and they found carvacrol (17.7%) as the major compound. Furthermore, Hanene et al. 2013, identified carvacrol (53.17%) as a main in the winter S. montana [25].

The fatty acids (Table 2) were found to contain a mixture of nine fatty acids from which six acids were identified and constituted about 91.28%. The saturated fatty acids four acids with palmitic acid as major (40.19%) and the unsaturated acids constitute about 47.64%, in which linolenic acid is the main acid. These data are very near from that reported by Ahmet et al., in 2003 [19], where they studied the fatty acids in S. thymbra and S. crescentifolia and found both palmitic acid and linolenic acid are major in both species.

The GLC analysis (Table 3) proved that the unsaponifiable fraction consists of a mixture of hydrocarbons (89.97%) in which n-tricosane

| Table 1: GC/MS analysis of n-hexane fraction of S. montana |
|---------------------------------|
| Peak No. | Rt (min.) | Rel. % | Molecular weight | Molecular formula | Compounds |
| 1 | 19.93 | 0.41 | 164 | C₆H₁₂O₂ | Thymoquinone |
| 2 | 21.33 | 0.24 | 250 | C₆H₁₂O₂ | Carvacrol |
| 3 | 21.48 | 0.46 | 182 | C₆H₁₂O₂ | 1,3-Diisopropyl-2,2-dimethyl-4,5-di-1-propenyl |
| 4 | 21.99 | 0.6 | 182 | C₆H₁₂O₂ | 2-(1H)-Naphthalen-1-one, octahydro-8a-hydroxy-4a-methyl-1H-naphthalen-4-one |
| 5 | 23.38 | 0.62 | 192 | C₆H₁₂O₂ | 3-Isopropyl-2,5-pyridinedione |
| 6 | 25.62 | 0.56 | 180 | C₆H₁₂O₅ | α-Isobutyrolactone |
| 7 | 26.37 | 2.1 | 220 | C₂₁H₃₂O₂ | Caryophyllene oxide |
| 8 | 27.47 | 1.99 | 180 | C₁₆H₂₆O₂ | Dihydroactinidiolide |
| 9 | 28.37 | 0.78 | 220 | C₆H₁₂O₂ | 3H-Benzopyran-3-one, 4H-oxo-2H-pyran-3-one |
| 10 | 29.54 | 0.59 | 190 | C₁₀H₂₂O₂ | α-Pinene |
| 11 | 29.86 | 0.61 | 222 | C₁₂H₂₀O₂ | Caryophyllene oxide |
| 12 | 30.2 | 0.51 | 220 | C₂₀H₃₂O₂ | Caryophyllene oxide |
| 13 | 30.31 | 3.87 | 178 | C₆H₁₂O₂ | 5,5-dimethyl-2,3-dihydrofuran-4-one |
| 14 | 30.56 | 0.41 | 290 | C₂₁H₃₂O₂ | Methyl 2,5-octadecadiynate |
| 15 | 30.64 | 0.48 | 220 | C₁₂H₂₀O₂ | Apisol |
| 16 | 31.75 | 0.78 | 192 | C₁₂H₂₀O₂ | (4R, 5S)-4(2-butyl) cyclohexene |
| 17 | 31.91 | 2.89 | 108 | C₁₀H₁₄O₂ | 2-(2-Propenoyl) furan |
| 18 | 32.63 | 0.42 | 191 | C₁₂H₂₀O₂ | 1,5-Dihydro-1-propeno-4-yl-2,5-dimethylfuran |
| 19 | 32.81 | 0.74 | 212 | C₁₂H₂₀O₂ | 3,4,5-Trimethoxybenzoic acid |
| 20 | 33.89 | 1.84 | 268 | C₁₀H₂₀O₂ | Hexahydrofarnesylacetone |
| 21 | 35.35 | 1.11 | 238 | C₂₁H₃₂O₂ | 4,4,8,10-Tetramethyl-2,6-cycloheptatriene-3,5-dione |
| 22 | 35.62 | 12.5 | 270 | C₁₂H₂₀O₂ | Butyl phthalate |
| 23 | 36.43 | 1.08 | 278 | C₂₁H₃₂O₂ | 5,5-dimethyl-2,3-dihydrofuran-4-one |
| 24 | 37.33 | 17.1 | 256 | C₂₁H₃₂O₂ | Palmitic acid |
| 25 | 37.57 | 0.47 | 208 | C₁₂H₂₀O₂ | 2,5,5,8a-tetramethyl-1,2,3,5,6,7,8,8a-octahydro-1H-naphthalen-1-one |
| 26 | 38.89 | 2.08 | 294 | C₁₂H₂₀O₂ | Methyl linoleate |
| 27 | 39.04 | 7.64 | 364 | C₁₂H₂₀O₂ | Methylhexadecanetrienoate |
| 28 | 39.19 | 2.68 | 296 | C₁₂H₂₀O₂ | Phytol |
| 29 | 39.46 | 0.42 | 298 | C₁₂H₂₀O₂ | Methyl isosertarate |
| 30 | 39.68 | 0.76 | 300 | C₂₀H₂₈O₂ | Retinoic acid |
| 31 | 39.76 | 1.73 | 318 | C₁₂H₂₀O₂ | Pregnanolone |
| 32 | 40.02 | 0.6 | 300 | C₁₂H₂₀O₂ | 4-Androst-4-one-3, 6,17-trione |
| 33 | 40.13 | 0.43 | 408 | C₁₂H₂₀O₂ | 8,16,16-trimethyl-β-dihydroxylactone |
| 34 | 40.6 | 2.06 | 256 | C₁₂H₂₀O₂ | 3-(2-chlorophenyl) quinazolin-4 (3H)-one |
| 35 | 40.96 | 0.95 | 414 | C₁₂H₂₀O₂ | Cholesterol |
| 36 | 42.29 | 0.45 | 412 | C₁₂H₂₀O₂ | Stigmasterol |
| 37 | 42.42 | 0.64 | 334 | C₁₂H₂₀O₂ | 3,17-dihydroxy pregnen-20-one |
| 38 | 44.8 | 0.6 | 306 | C₁₂H₂₀O₂ | 3,11-Androstan-3-one |
| 39 | 46.33 | 1.81 | 390 | C₁₂H₂₀O₂ | Di (2-ethylhexyl) phthalate |

| Table 2: GC/MS data of FAME of S. montana |
|---------------------------------|
| Peak No. | Rt (min.) | Rel. % | Mol. Formula | Mol. Wt. | Compounds |
| 1 | 15.86 | 40.19 | C₁₀H₁₈O₂ | 270 | Methyl Palmitate |
| 2 | 19.74 | 5.68 | C₁₀H₁₈O₂ | 294 | Methyl Linoleate |
| 3 | 19.98 | 40.59 | C₁₀H₁₈O₂ | 292 | Methyl Linolenate |
| 4 | 20.44 | 3.01 | C₁₀H₁₈O₂ | 298 | Methyl Stearate |
| 5 | 24.77 | 1.37 | C₁₀H₁₈O₂ | 326 | Methyl Arachidate |
| 6 | 28.78 | 0.44 | C₁₀H₁₈O₂ | 354 | Methyl Behenate |
| 7 | 8.72 | Unknowns | C₁₀H₁₈O₂ | | |

GC-MS: Gas chromatography-mass spectrometry, S. montana: Satureja montana
Compound (1): Luteolin-7-rhamnoside-4'-O-β-glucopyranoside: It was obtained as yellow amorphous powder (14 mg), m.p. 320-324°C. UV spectral data λ <sub>max</sub> (nm) MeOH: 270, 340; NaOMe: 270, 377; Aliq: 276, 303sh, 348, 388; Aliq/C: 269, 337, 348; NaAc/H <sub>2</sub>O BO <sub>2</sub>, 269, 237, 339. <sup>1</sup>H-NMR (600 MHz, DMSO-d <sub>6</sub>) δ ppm at 7.49, (1H, dd, J=8 Hz, H-6), 7.51 (1H, d, J=2 Hz, H-2), 7.25 (1H, d, J=8 Hz, H-5). 6.58 (1H, s, H-3), 6.87 (1H, d, J=2.5 Hz, H-8), 6.55 (1H, d, J=2.5 Hz, H-6). Sugar moiety, at 100.8 (C-1'''), 72.4 (C-2''), 77.3 (C-3''), 77.1 (C-4''), 77.4 (C-5''), 60.7 (C-6''), 7.0 (C-7''), 7.07 (C-7''), 7.01 (C-4''), 6.97 (C-5''), and 1.8 (C-6'').

Compound (2): Quercetin-3-O-α-L-rhamnopyranoside: Yellow amorphous powder (16 mg), m.p. 316-318°C. UV spectral data λ <sub>max</sub> (nm) MeOH: 258, 269sh, 360; NaOMe: 272, 328sh, 405; Aliq: 275, 305sh, 332sh, 435; Aliq/HCl: 275, 305sh, 36, 403; NaAc: 269, 323sh, 380. NaOAc/H <sub>2</sub>O BO, 262, 300sh, 387; <sup>1</sup>H-NMR (600 MHz, DMSO-d <sub>6</sub>): Aglycone moiety: δ ppm: 7.6 (1H, d, J=2.5 Hz, H-2'), 7.5 (1H, dd, J=2.5 Hz and J=8 Hz, H-6'), 6.77 (1H, d, J=8 Hz, H-5), 6.24 (1H, d, J=2.0 Hz, H-8), 6.04 (1H, d, J=2.0 Hz, H-6) ppm. Sugar moiety: 5.5 (1H, d, J=2.0 Hz, H-1'' of rhamnose), 3.1-3.5, (3H, sugar protons). 0.98 (3H, d, J=6 Hz, CH, of rhamnopyranosyl) ppm. <sup>1</sup>C-NMR (150 MHz, DMSO-d <sub>6</sub>): 157.04 (C-2'), 13.45 (C-3'), 17.79 (C-4'), 161.7 (C-5'), 98.5 (C-6'), 151.2 (C-7'), 145.76 (C-3'), 149.1 (C-4'), 115.99 (C-5'), 121.52 (C-6'). Sugar moiety: 101.1 (C-1''), 70.6 (C-2''), 70.81 (C-3''), 71.66 (C-4''), 70.5 (C-5''), and 179 (C-6'). The acid hydrolysis of compound 2 yielded both quercitin and rhamnose, which was identified by Co-PC using authentic samples. Thus, the structure of compound 2 was proved as quercetin-3-O-α-L-rhamnopyranoside.

Compound (3): Quercetin-7-O-glucopyranoside: The compound was obtained as yellow amorphous powder (17 mg), m.p. 318-320°C. UV spectral data λ <sub>max</sub> (nm) MeOH: 257, 294 sh, 357; NaOMe: 266, 314, 406; Aliq: 273, 301sh, 335sh, 433; Aliq/HCl: 269, 300sh, 358, 402; NaAc: 259, 396sh, 367, 415; NaOAc/H <sub>2</sub>O BO, 261, 295sh, 378. <sup>1</sup>H-NMR (600 MHz, DMSO-d <sub>6</sub>): δ ppm at 12.60 (1H, s, OH-5), 7.61 (1H, d, J=2.2 Hz, H-2'), 7.58 (1H, dd, J=2.2, 8.0 Hz, H-6'), 6.86 (1H, d, J=8 Hz, H-5), 6.7 (1H, d, J=2.0 Hz, H-6), 6.48 (1H, d, J=2.0 Hz, H-6). The sugar proton at 5.5 (1H, d, J=7.5 Hz, H-1''). 3.1-3.6, (3H, complex signal, due to rest sugar proton).

Table 3: GLC data of unsaponifiable of <i>S. montana</i>

| Peak No. | Rt (min.) | Rel. % | Molecular formula | Compounds |
|----------|-----------|--------|-------------------|-----------|
| 1        | 6.48      | 2.87   | C<sub>2</sub>H<sub>4</sub>O | n-nonane   |
| 2        | 9.72      | 1.58   | C<sub>2</sub>H<sub>4</sub>O | n-undecane |
| 3        | 11.96     | 0.84   | C<sub>2</sub>H<sub>4</sub>O | n-pentadecane |
| 4        | 13.29     | 4.98   | C<sub>2</sub>H<sub>4</sub>O | n-heptadecane |
| 5        | 15.42     | 11.27  | C<sub>2</sub>H<sub>4</sub>O | n-octadecane |
| 6        | 16.46     | 0.9    | C<sub>2</sub>H<sub>4</sub>O | n-nonadecane |
| 7        | 17.36     | 12.32  | C<sub>2</sub>H<sub>4</sub>O | n-eicosane |
| 8        | 18.45     | 9.46   | C<sub>2</sub>H<sub>4</sub>O | n-heneicosane |
| 9        | 19.82     | 4.53   | C<sub>2</sub>H<sub>4</sub>O | n-docosane |
| 10       | 20.03     | 27.7   | C<sub>2</sub>H<sub>4</sub>O | n-tricosane |
| 11       | 21.39     | 4.99   | C<sub>2</sub>H<sub>4</sub>O | n-tetracosane |
| 12       | 22.71     | 1.26   | C<sub>2</sub>H<sub>4</sub>O | n-pentacosane |
| 13       | 23.47     | 6.12   | C<sub>2</sub>H<sub>4</sub>O | n-hexacosane |
| 14       | 24.41     | 1.15   | C<sub>2</sub>H<sub>4</sub>O | n-heptacosane |
| 15       | 26.01     | 0.98   | C<sub>2</sub>H<sub>4</sub>O | Cholesterol |
| 16       | 27.51     | 1.18   | C<sub>2</sub>H<sub>4</sub>O | Campesterol |
| 17       | 28.04     | 5.64   | C<sub>2</sub>H<sub>4</sub>O | Stigmasterol |
| 18       | 29.95     | 2.23   | C<sub>2</sub>H<sub>4</sub>O | β-Sitosterol |
Acid hydrolysis of compound 4 led to identification of both luteolin and glucose by Co-PC using authentic markers in different solvents. The compound 4 could be identified as luteolin-7-O-glucopyranoside.

Compound (5): 5-Hydroxy-6,7,8,4’-tetramethoxy flavone: It was obtained as white yellowish amorphous powder (15 mg), m.p. 224-226°C UV spectral data λ\_{max} (nm) MeOH: 254, 277, 294, NaOMe: 268, 406; AICI₃: 272, 287, 309, 362, 410, NaI: 262, 278, 310, 360, 408; NaOAc: 259, 276, 342, 406; NaOAc/H₂O: 254, 277, 339; 1-H-NMR (600 MHz, DMSO-d₆): δ (ppm) at 7.9 (2H, d, J=2.5 Hz, H, 8 Hz, H-2’ and H-6’), 7.01 (2H, d, J=2.5 Hz, 8 Hz for H-3’ and H-5’), 6.9 (1H, s, H-3), 4.1 (1H, s, OCH₃-4), 4.02 (2H, s, OCH₂-6), 3.95 (1H, s, OCH₃-7), 3.85 (1H, s, OCH₃-8). 13C-NMR: 162 (C-2), 103.1 (C-3), 183.02 (C-4), 148.54 (C-5), 136.30 (C-6), 152.92 (C-7), 133.07 (C-8), 145.66 (C-9), 106.66 (C-10), 121.50 (C-11), 110.48 (C-12), 116.4 (C-13), 154.6 (C-15), 128.98 (C-16), 62.31 (OCH₃-6), 62.37 (OCH₃-7), 61.95 (OCH₃-8), 55.24 (OCH₃-4). The above data showed that compound 5 is 5-Hydroxy-7,8,4’-tetramethoxy flavone.

Compound (6): Gallic acid: White amorphous powder; m.p. 258-260°C UV spectral data λ\_{max} (nm) MeOH: 272 nm. 1-H-NMR (600 MHz, DMSO-d₆): 6 (ppm) at 6.03 (2H, s, H-2, and H-6). 13C-NMR at 120.6 (C-1), 108.6 (C-2, and C-6). C-NMR at 147.25 (C-2), 136.18 (C-3), 176.29 (C-4). H-NMR (600 MHz, DMSO-d₆): δ (ppm) at 7.9 (2H, s, H-2, and H-6). 13C-NMR: 147.25 (C-2), 136.18 (C-3), 176.29 (C-4). H-NMR: 7.1 (2H, s, H-2, and H-6) of galloyl proton, 6.8 (1H, s, H-5 of hexahydroxydiphenoyl portion (HHDP) ring B), 6.74 (1H, s, HHDP H-5 ring B), 6.4 (1H, d, J=8 Hz, Glc, H-1), 5.7 (1H, d, J=8 Hz, Glc, H-2), 5.6 (1H, d, J=8 Hz, Glc, H-3), 4.3 (1H, d, J=8 Hz, Glc, H-4), 4.2 (1H, t, J=9.4 Hz, Glc, H-5), 4.1 (2H, s, H-2. Glc), 3.85 (1H, s, OCH₃-4), 3.82 (1H, s, OCH₃-6), 3.75 (1H, s, OCH₃-7), 3.74 (1H, s, OCH₃-8). The observed results in this study might be due to the presence of flavonoid compounds in methanol extract and some volatile compounds.

The antimicrobial activity of different extracts and isolated compounds

The antimicrobial activity of different extracts and isolated compounds was summarized in Table 4, which proved that the methanolic extract showed significant antimicrobial activity against S. aureus (85.7%), while, it showed a moderate antifungal activity against A. flavus (62.5%) and C. albicans (63.2%). On the other hand, the n-hexane extract showed a relatively low activity against Gram-positive B. subtilis (46.2%), while, it showed a low antifungal activity against A. flavus (31.3%).

Compound 2 showed the highest activity against Gram-positive B. subtilis and S. aureus (84.6% and 90.5%), respectively. Compound 8 exhibited a significant inhibition against the tested Gram-positive B. subtilis and S. aureus (80.8% and 81%), respectively. Gram-negative E. coli (76%) and P. aeruginosa (77%), while it showed a moderate antifungal activity against A. flavus and C. albicans (56.3% and 52.6%), respectively. Compound 4 exhibited a significant inhibition against B. subtilis (77%), Gram-negative E. coli and P. aeruginosa (76% and 77%), respectively, and relatively moderate antifungal activity against A. flavus and C. albicans (50% and 47.4%).

Furthermore, compound 3 exhibited a moderate activity against Gram-negative E. coli and P. aeruginosa (68% and 69.2%), respectively. Compounds 1 and 5 showed a significant inhibition against Gram-positive S. aureus (76.2% and 71.4%), respectively, and B. subtilis (57.7% and 53.8%), respectively. Gram-negative P. aeruginosa (65.4% and 57.7%), respectively, and E. coli (6-8% and 56%), respectively. On the other hand, both compounds showed a low antifungal activity against A. flavus (43.7% and 31.2%), respectively, and C. albicans (42.1% and 36.8%), respectively.

Compounds 6 and 7 showed a moderate activity against Gram-positive S. aureus (57.1% and 61.9%), respectively, B. subtilis (50% and 53.8%), respectively, Gram-negative P. aeruginosa (53.9%) both and E. coli (52%) both.

The observed results in this study might be due to the presence of flavonoid compounds in methanol extract and some volatile compounds.
The antioxidant activity study (Figs. 2-3) proved that the compound 2 and the methanolic extract exhibited the highest activity (inhibition % = 96.27 and 87.7, respectively). On the other hand, n-hexane extract and compounds 5-7 showed low activity, while compounds 2-4 gave moderate activity.

These data can be explained in light of the structure of different flavonoids play an important role in their activity as antioxidant where the structure of the flavonoids is the most significant determinant of radical scavenging [33,34]. The OH groups on the ring-B give hydrogen and an electron to OH and peroxyl radicals, which led to more flavonoidal radical stability and the activity increases linearly, according to the total number of OH groups [35]. When 3',4'-dihydroxy structure in ring-B, it increases lipid peroxidation inhibition effectively [36]. Hence, the peroxyl radical scavenging ability of luteolin substantially exceeds kaempferol [37]. Free radical scavenging of flavonoids is strongly dependent on the presence of a free OH group at C-3 in the flavonoid nucleus [38]. Flavonoids with a C-3-OH and 3',4'-dihydroxy are reported to be 10-fold more potent against peroxynitrite. The superiority of quercetin in inhibiting both metal and non-metal-induced oxidative damage is partially attributed to its free 3-OH substituent, which is thought to increase the stability of the flavonoid radical [39].

CONCLUSION
The findings of this study showed that the hydroalcoholic extract of S. montana showed significant antimicrobial and antioxidant activity. On the other hand, compound 2 showed the highest antibacterial activity against all the tested microorganisms, and compound 8 showed the highest antioxidant activity (96.27%) in DPPH assay.

CONFLICT OF INTEREST
The authors declare that there are no conflicts of interests regarding the publication of this article.
AUTHOR CONTRIBUTIONS

Ali M. El-Hagrassi, designed the experiments, extraction, separate pure compounds and lipid constituents and collect results, make the laboratory and store experiments and writing the manuscript. Walid E. Abdallah, share in isolation and separate pure compounds, volatile and lipid constituents constituents, and writing the manuscript. Abeer F. Osman, share in extraction, isolation, separate pure compounds, volatile and lipid constituents, antioxidant and antimicrobial activity and writing the manuscript. Khaled A. Abdelshafek, share in compounds identification, auditing writing the research and reviewed the manuscript.

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