Phenolics from *Schinus molle* stems and their anti-bacterial and anti-microalgal activity

Magda T. Ibrahim [1] and Eman G. Haggag [2]*

[1] Departement of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt
[2*] Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Cairo, Egypt
wemisr@hotmail.com

Abstract: **Objectives:** This work aimed to isolate phenolic compounds from the stems of *Schinus molle* and evaluate its antibacterial and anti-microalgae (phycotoxic) activity. **Methods:** 80% MeOH extract of stems of *Schinus molle* was subjected to chromatographic separation, structures of the isolated compounds were established by different chromatographic and spectral techniques UV, MS, ^{1}H and ^{13}C NMR. Anti-bacterial and anti-microalgal activity was evaluated by agar diffusion method. **Results:** Four known phenolic compounds were isolated for the first time from the genus *Schinus viz*, chlorogenic methyl ester **4**, quercitrin 2″-*O*-caffeate **6**, quercetin 3′-*O*-α-L-$^{1}C_{4}$-rhamnopyranoside **10** and rhamnetin-3-*O*-β-D-$^{4}C_{1}$-galactopyranoside **11**. Also four known quercetin glycosides *viz*, hyperin 6"-*O*-gallate **5**, quercetin 3-*O*-β-D-galacturonopyranoside **7**, isoquercitrin **8** and hyperin **9** together with gallic acid **1**, methyl gallate **2**, chlorogenic acid **3** and the free quercetin aglycone **12**, were obtained from the stem part of *Schinus molle* for the first time. Their structures were established on the basis of chromatographic properties, chemical, spectroscopic (UV, ^{1}H- & ^{13}C-NMR) and ESI-MS analysis. The four isolated phenolic compounds (**4, 6, 10** and **11**) showed antibacterial activity against both Gram+ve and Gram–ve bacteria. While compounds **10** and **11** showed phycotoxic activity against the tested microalgae, compounds **4** and **6** showed only high selective phycotoxic activity against *Scendensmus subspicatus*, when all compared to germanomycine antibiotic. **Conclusions:** *Schinus molle* contains phenolic compounds of a great potential as antibacterial drug and its phycotoxic activity giving it economic value as a natural herbicide (allelopathic) drug protecting medicinal crops and the environment from synthetic chemicals.

[Magda T. Ibrahim and Eman G. Haggag. **Phenolics from Schinus molle stems and their anti-bacterial and anti-microalgal activity**. *Life Sci J* 2013; 10(3): 1931-1937]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 286

Keywords: Anacardiaceae; California pepper; phenolics; antibacterial; phycotoxic activity allelopathy.

1.Introduction

Phycotoxins (from Greek, *phykos*, "seaweed"; and *toxikon*, "toxin") are complex allelopathic chemicals produced by photosynthetic organisms (like algae) as secondary metabolites, which are not harmful to the producer but may be toxic to others. Allelopathy is the mechanism by which plants directly or indirectly affects, inhibits or stimulates growth of others[1]. Therefore, exploration of plants and their effective allelopathic parts especially in the control of agricultural crops is always needed[2]. *Schinus molle* L. (Anacardiaceae), known as California pepper tree and native to South America, has been used in folk medicine as a hypotensive and diuretic drug[3]. Previous studies explored its activity as CNS depressor and analgesic[4] also as antispasmodic[5], anti-inflamatory[6] and as antioxidant[7]. The leaf extract showed antidepressant-like effect[8] and recently fruit and leaf extracts as well as their oils proven to have insecticidal[9,10] and antimicrobial activity[11]. The principle compounds that have been isolated from *Schinus molle* were sesquiterpenes[12], triterpenoidal-keto-acids[13,14], flavonoids[7], tritepenoids and biflavanones[6]. In our previous study[7], two novel acylated flavonol glycosides together with twelve phenolic compounds were isolated from the leaves of *Schinus moll* L., while this work aimed the isolation and identification of the constitutive phenolics from the stem part of this species growing in Egypt and evaluation of its antibacterial activity as well as its allopathic effect through evaluation of its antimicroalgal (phycotoxic) activity.

2. Materials and Methods

Apparatus

NMR (^{1}H- and ^{13}C-NMR) spectra were recorded at 300 & 500 MHz for ^{1}H and 75 & 125 MHz for ^{13}C on a Varian Mercury 500. The δ-values are reported as ppm relative to TMS in DMSO-d_6 and *J*-values are in Hz. ESI-MS spectra were measured on mass spectrometer connected to an ESI-II ion source (Finnigan, Lc-MSLCQ^{deca} Advantage MAX, Finnigan Surveyor LC pump). Shimadzu UV 240 spectrophotometer was used for UV analysis. Concentration of extracts was done at low temperature under vacuum using Rotatory evaporator (Bűchi G, Switzerland).

Plant materials

Stems of *Schinus molle* were collected from the plant grown in El-Maadi region, Cairo, Egypt in May 2002. Identification of the plant confirmed by Dr. Wafaa M. Amer, Prof. of Botany, Flora and

Taxonomy Department, Faculty of Science, Cairo University, Giza, Egypt. Voucher specimens (Reg. No.: S-2) are kept in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Cairo, Egypt

Chemicals

Polyamide 6S (Riedel-De Hän Ag, Seelze Hannover, Germany), cellulose (Pharmacia, Uppsala, Sweden) and Sephadex (Fluka, Switzerland), were used in chromatography. Sugars, reagents and solvents were of analytical grade.

Microorganisms

Bacterial strains; Escherichia coli (Strain ATCC 25922), *Bacillus subtilis* (Strain Bs 1091-1), *Streptomyces viridochromogenes* (Strain Tu 57), *Staphylococcus aurens* (Strain Sau 1091-5) and microalgae stains; *Chlorella vulgaris* (SAG 211-11b), *Chlorella sorokiniana* (SAG 211-8K) and *Scenedesmus subspicatus* (SAG 86-81) were all obtained from stocks maintained at the Department of Organic Chemistry, George-August-University, Gottingen, Germany.

Antimicrobial and antimicroalgal activity:

Antibacterial and antimicroalgal (phycotoxic) activities were estimated by the agar diffusion method[15] using paper discs of 8 mm diameter. Each paper disc impregnated with 50 µg of tested culture sample was placed on agar media suspended with tested microorganisms. Inhibition zones were observed after incubation at 37°C for 24 hours and results were compared to negative control (blank) and positive control (germanomycine antibiotic).

Extraction and isolation of phenolics

Powdered air dried stems of *S. molle* (1 kg) was extracted with hot 80% aqueous methanol under reflux (70°C) (4 × 5 L). The dried residue was extracted with chloroform under reflux (3 × 1 L) producing 20 g chloroform soluble portion and 80 g methanol soluble portion, which was then fractionated on polyamide column (Ø 5.5 × 115 cm), eluted with water followed by gradient increasing methanol proportions (H_2O/MeOH mixtures) for decreasing the solvent polarity. The obtained seven collective fractions (I-VII), were subjected to successive column chromatography on cellulose and/or Sephadex using different solvent system; MeOH, MeOH/H_2O mixtures, saturated *n*-butanol with water and/or *n*-butanol-isoprpanol-water (BIW) (4:1:5, v/v, upper layer), had afforded twelve phenolic compounds as illustrated in the flow chart (Figure 1). Separation processes were followed up by 2D-PC and CoPC using Whatmann No. 1 paper and the solvent systems; *n*-BuOH-AcOH-H_2O (4:1:5 v/v upper layer) (S_1) and 15% aqueous AcOH (S_2)[16], visualized under UV, exposed to ammonia and sprayed with $FeCl_3$ and Naturstoff reagent[17].

Acid hydrolysis for glycosides

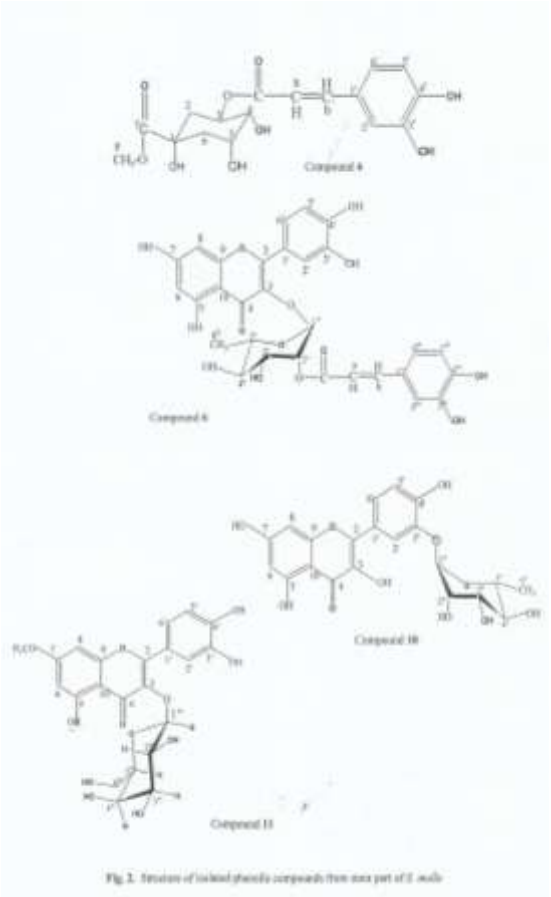
Complete acid hydrolysis was carried out by treating 4-5 mg of each compound with 1.5 N HCl in aqueous methanol (50%) for 2 hours at 100°C. Each hydrolysate was then extracted with ethyl acetate and the extract was subjected to CoPC investigation alongside with authentic aglycones. The mother liquor was neutralized with sodium carbonate and used for the identification of the sugars by CoPC against standard sugars[16].

3. Results

Compound **4**: white amorphous powder, (35 mg) of m.p 182°C showed single spot of R_f values: 0.44 (S_1) and 0.61 (S_2) on PC, blue fluorescence under UV changed to yellowish green on exposure to ammonia vapors and to blue color with $FeCl_3$ reagent; UV λ_{max} in MeOH: 236sh, 244, 300sh, 330 nm. ^{1}H-NMR (500 MHz, DMSO-d6) δ ppm: 2.03 (1H, m H_{ax}-2), 2.06 (1H, m, H_{eq}-2), 5.28 (1H, d, *J* = 3.5 Hz H-3), 3.72 (1H, dd, *J* = 3.5 & 7.6 Hz H-4), 4.13 (1H, m– H-5), 2.04 (1H, m H_{ax}-6), 2.22 (1H, m H_{eq}-6), 3.78 (3H, s, -MeO-8), 6.23 (1H, d, *J* = 15.5 Hz H_a-olefinic), 7.54 (1H, d, *J* = 15.5 Hz H_b-olefinic), 7.10 (1H, dd, *J* = 1.6 & 8.1 Hz H-6′), 6.70 (1H, d, *J* = 1.6 Hz H-2′), 6.84 (1H, d, *J* = 8.1 Hz H-5′). ^{13}C-NMR (125 MHz DMSO-d6) δ ppm 175.94 (C-7), 168.02 (CO-ester), 149.48 (C-4′), 147.91 (olefinic C_b), 146.69 (C-3′), 127.80 (C-1′), 122.98 (C-6′), 116.61 (C-5′), 115.90 (olefinic C_a), 115.22 (C-2′), 75.8 (C-1), 72.80 (C-4), 70.21 (C-5), 72.01 (C-3), 53.19 (C-8), 38.82 (C-6), 37.78 (C-2). ESI/MS spectrum showed a molecular ion peak at *m/z* 367 $[M-H]^-$.

Compound **6**: yellow amorphous powder, (45 mg), chromatographic properties showed dark purple spot of R_f values: 0.52 (S_1) 0.63 and (S_2) on PC, changed to yellowish green on exposure to ammonia vapors and turned to orange color with Naturstoff reagent and to green color with $FeCl_3$. UV-spectral data λ_{max} (nm) (MeOH): 260, 290sh, 355; (+NaOMe): 276, 320, 410; (+NaOAc): 220, 275, 375; (+NaOAc /H_3BO_3): 220, 263, 370; (+$AlCl_3$): 273, 310sh, 365, 405; (+AlCl3/HCl): 275, 300sh, 355, 395. ^{1} H NMR (300 MHz, DMSO-d_6):δ 12.99 (1H, s,OH-5 hydrogen bonded proton), 7.58 (1H, d, *J* = 15.5 Hz H-$_b$) 6.29 (1H, d, *J* = 15.5 Hz H-$_a$), 7.33 (1H, dd, *J* = 8.5 & 2.2 Hz H-6'), 7.30 (1H, d, *J* = 2.2 Hz H-2′), 6.88 (1H, d, *J* = 8.5 Hz H-5′), 7.05 (1H, dd, *J* = 8.5 & 2.2 Hz H-6'''), 7.02 (1H, d, *J* = 2.2 Hz H-2'''), 6.64 (1H, d, *J* = 8.5 Hz H-5'''), 6.41 (1H, d, *J* = 2.2 Hz H-8), 6.22 (1H, d, *J* = 2.2 Hz H-6), 4.28 (1H, d *J* = 1.7 Hz H-1″), 5.41 (1H, br H-2″), 3.21-3.60 (3H, m, H-3″, -4″ & -5″), 0.88 (3H, d, *J* = 6.23 CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): δ ppm 177.48 (C-4), 172.26 (C-6″), 165.86 (CO),

164.65 (C-7), 161.62 (C-5), 157.33 (C-2), 156.90 (C-9), 149.48 (C-4‴), 149.00 (C-4′), 147.89 (olefinic C_b), 146.69 (C-3‴), 145.37 (C-3′), 133.30 (C-3), 122.21 (C-1‴), 122.98 (C-6‴), 121.91 (C-6′), 120.22 (C-1′), 117.24 (C-5′), 115.95 (olefinic C_a), 115.93 (C-2′), 115.22 (C-2‴), 116.61 (C-5‴), 104.50 (C-10), 102.30 (C-1″), 99.29 (C-6), 94.25 (C-8), 70.01 (C-2″), 75.92 (C-4″), 74.55 (C-5″), 72.66 (C-3″). Negative ESI-MS: spectrum molecular ion peak at *m/z* 609 $[M-H]^-$ and at *m/z* 301.



Compound **10**: yellow amorphous powder (65 g) with yellow fluorescent spot by long UV light turned to yellow with Naturstoff reagent and faint blue with $FeCl_3$. R_f 0.31 (S1), 0.04 (S2) on PC. UV-spectral data λ_{max} (nm) (MeOH): 256, 296 sh, 370; (+NaOMe): 271, 322 sh, 402; (+NaOAc): 271, 318 sh, 389; (+NaOAc/H_3BO_3): 264, 389, (+$AlCl_3$): 260, 308, 432; (+$AlCl_3$/HCl): 262, 308, 430 nm. ^{1}H-NMR (300 MHz): 7.69 (1H, dd, *J* = 8.5 & 2.2 H-6'), 7.63 (1H, d, *J* = 2.2 H-2′), 6.87 (1H, d, *J* = 8.5 H-5′), 6.42 (1H, d, *J* = 2.2 H-8), 6.18 (1H, d, *J* = 2.2 H-6), 4.79 (1H, d, *J* = 1.7, H-1″), 3.85 (1H, br s, H-2″), 3.68-3.2 (m, remaining sugar protons), 1.09 (3H, d, *J* = 6.23 Hz CH_3-6″). ^{13}C-NMR (75 MHz): 175.90 (C- 4), 164.46 (C-7), 161.18 (C-5), 156.02 (C-9), 151.22 (C-4'), 146.23 (C-2), 140.80 (C-3′), , 136.21 (C-3), 124.90 (C-6′), 122.60 (C-1′), 122.43 (C-2′), 117.04 (CH-5′), 103.47 (C-10), 101.59 (C-1″), 98.72 (C-6), 94.53 (C-8), 72.87 (C-4″), 72.05 (C-2″), 70.89 (C-3″), 68.77 (C-5″), 18.41 (CH3-6″). Negative ESI-MS: spectrum molecular ion peak at *m/z* 447.09 $[M-H]^-$ and at *m/z* 301.04.

Compound **11**: yellow amorphous powder (75 mg), chromatographic properties: dark purple PC spot under UV R_f 0.50 (S_1), 0.26 (S_2), yellow fluorescent (UV/NH_3); turned to orange color with Naturstoff reagent and to green color with $FeCl_3$. UV λ_{max} (nm), MeOH: 256, 270sh, 300sh, 371; (+NaOMe): 242, 286, 331, 433; (+NaOAc): 255, 292sh, 387, 422sh; (+NaOAc/ H_3BO_3): 260, 389; (+$AlCl_3$): 273, 302sh, 330sh, 450; (+$AlCl_3$ /HCl) 268, 299sh, 363sh, 423. ^{1}H NMR (300 MHz, DMSO-d_6):δ 12.61(1H, s,OH-5 hydrogen bonded proton), 7.60 (1H, dd, *J* = 8.5 & 2.2 Hz H-6'), 7.57 (1H, d, *J* = 2.2 Hz H-2′), 6.84 (1H, d, *J* = 8.5 Hz H-5′), 6.39 (1H, d, *J* = 2.2 Hz H-8), 6.19 (1H, d, *J* = 2.2 Hz H-6), 5.44 (1H, d, *J* = 6.5 Hz, H-1″), 3.57 (1H, br d, *J* = 11.4 Hz, H-6″a), 3-3.5(4H, m, H-3″, -4″, -5″, -6″$_b$, hidden by H_2O-signal), 3.88 (3H, s, OMe-7). ^{13}C NMR (75 MHz, DMSO-d_6): δ_{ppm} 177.48 (C-4), 164.13 (C-7), 161.28 (C-5), 156.36 (C-

2), 156.22 (C-9), 148.48 (C-4′), 144.84 (C-3′), 133.39 (C-3), 121.63 (C-6′), 121.22 (C-1′), 116.26 (C-5′), 115.25 (C-2′), 104.04 (C-10), 100.96 (C-1″), 98.69 (C-6), 93.54 (C-8), 75.80 (C-5″), 73.40 (C-3″), 71.30 (C-2″), 68.01 (C-4″), 60.80 (C-6″), 56.49 (OCH_3). Negative ESI-MS: spectrum showed a molecular ion peak at *m/z* 477 $[M-H]^{-1}$ and at *m/z* 315.

4. Discussion

The defatted 80 % methanol stems extract of *S. molle* was chromatographed on polyamide column followed by successive separation on Sephadex LH-20 or cellulose columns affording pure samples of twelve known phenolic compounds; seven of which *viz* gallic acid **1**, methyl gallate **2**, chlorogenic acid **3**, hyperin 6"-O-gallate **5**, quercetin 3-*O*-β-D-galacturonopyranoside **7**, isoquercitrin **8**, hyperin **9**, and quercetin **12**, their data were in accordance with that reported before from the leaf part in our previous study[7], while chlorogenic methyl ester **4,** quercitrin 2″-*O*-caffeate **6**, quercetin 3′-*O*-α-L-$^1C_4$-rhamnopyranoside **10** and rhamnetin-3-*O*-β-D-$^4C_1$-galactopyranoside **11**, were obtained from the stem part of *Schinus molle* for the first time. Their structures (Figure 2), were established on the basis of chemical evidences, their chromatographic properties and an extensive spectroscopic study (UV, ^{1}H-, ^{13}C-NMR and ESI-MS).

Compound **4** was obtained as white amorphous powder, chromatographic properties showed single spot under UV spectrum exhibiting absorption band characteristic of phenyl propanoid[16]. The ethyl acetate extract obtained by acid hydrolysis contained caffeic acid (CoPC with authentic samples of different phenolic acids). ^{1}H-NMR showed signals of spin coupling system of typical *trans* doublets of the two protons characteristic of olefinic double bond at δ 7.54 and 6.23 ppm of (H-b and H-a, respectively) with large *J* value (*J* = 15.5 Hz), together with signals assignable to quinic acid moiety[18]. ^{13}C-NMR showed two carbonyl resonance signals at δ C 175.4 of the quinic acid and at δ 168.2 of ester group. Also it showed the typical resonances of quinic acid in aliphatic region and the two carbon resonance**s** of olifenic double bond at 147.1 (C_b) and 115.1 (C_a) in addition to other resonance of caffeoyl moiety[18]. Negative ESI/MS spectrum showed a molecular ion peak at m/z 367 $[M-H]^-$ along with two fragment ions at 353. $[M -Me]^-$ and [M -caffeoyl] , thus comparing with published data[18],.compound **4** was identified as: 3-*O*-*E* caffeoyl quinic acid methyl ester (Chlorogenic acid methyl ester).

Compound **6** was expected to be quercetin 3-*O*-glycoside-like structure as by complete acid hydrolysis as L-rhamnose was detected in the aqueous phase, while caffeic acid and quercetin were detected in the organic phase. UV methanol spectrum exhibited a characteristic absorption band I at ≈ 355 nm[16]. Band II was masked by an extra strong maximum at 262, 272 or 275 nm which was indicative to the presence of an acyl phenoyl radical (cinnamoyl or galloyl)[19]. ^{1}H NMR spectrum exhibited coupling system of a *trans*-caffeoyl moiety, which was detected through two *trans* olefinic doublets at δ 7.58 (*J* = 15.5 Hz) and 6.29 (*J* = 15.5 Hz) for H-$_b$ and H-$_a$, respectively, along with an AX splitting pattern of 5,7-dihydroxy A-ring at δ 6.41 (d, *J* = 2.2 Hz H-8) and at δ 6.22 (d, *J* = 2.2 Hz H-6). It also showed two ABX coupling system characteristic signals for two phenyl moieties: the 3', 4'-dihydroxy phenyl ring B and the phenyl caffeoyl moiety; at 7.33 (dd, *J* = 8.5 & 2.2 Hz), 7.30 (d, *J* = 2.2 Hz) and at δ 6.88 (d, *J* = 8.5 Hz) of H-6', H-2' and H-5', respectively of the phenyl ring B and at 7.05 (dd, *J* = 8.5 & 2.2 Hz), 7.02 (d, *J* = 2.2 Hz) and at δ 6.64 (d, *J* = 8.5 Hz) of H-6''', H-2''' and H-5''', respectively of the phenyl caffeoyl moiety. The presences of 3-*O*-α–rhamnopyranosyl moiety was concluded from its characteristic anomeric proton signal at δ 4.28 (*J* = 1.7 Hz) and its methyl group signal at δ 0.88 (*J* = 6.23 Hz). The sustitution of caffeoyl moiety on OH-2" of the rhamnosyl moiety was confirmed by the downfield shift of H-2″ to 5.41 (Δ + 1.4) relative to that of quercitrin (δ = 4.0 ppm)[20,21]. ^{13}C-NMR showed two carbonyl resonance signals at δ 177.48 of (C-4) and at δ 165.86 of C=O of caffeoyl moiety. Also it showed two carbon resonance**s** of olifenic double bond at 147.89 (C_b) and 115.95 (C_a) in addition to other resonance of caffeoyl moiety[18]. The glycosylation at 3-*O* position was confirmed by slight upfield shift of C-3 at δ 133.30 ppm compared to that of quercetin aglycone (138.90 C-3) and the downfield shift of both C-2 and C-4 at (157.33 and 177.48, respectively) compared to that of quercetin aglycone (148.30 C-2 and 176.7 C-4, respectively)[20,21] Also the substitution of the caffeoyl moiety on C-2" position was confirmed by slight upfield shift of C-2" at δ 70.01 ppm and the downfield shift of both C-1" and C-3" at 102.30 and 72.66, respectively compared to that of unsubstituted quercitrin (74.15 C-2", 101.90 C-1" and 70.60 C-3", respectively)[21,22]. ESI-MS spectrometric analysis showed a molecular weight established as 610 mu depending on its molecular ion peaks $[M-H]^-$ at *m/z* 609 in negative mode ionization. While at high CID voltage (120 V) two fragments were detected; one corresponding to the loss of an acyl moiety (quercitrin *m/z* 447) and the other one corresponding to the loss of acyldeoxy-rhamnoside residues (quercetin *m/z* 301). Additionally adduct molecular ion peaks with sodium ion were detected in positive ESI-MS. Thus, compound **6** was identified as quercetin 3-rhamnoside caffeate analogue ester.

Compound **10** showed a deep purple spot under UV-light of R_f values: 0.55 (S_3) and 0.13 (S_4), changed to yellow fluorescence with ammonia vapors, turned to orange color with Naturstoff reagent and to green color with $FeCl_3$. Acid hydrolysis resulted quercetin in the organic phase and rhamnose in the aqueous phase (CoPC). UV-spectrum in MeOH showed the two characteristic absorption at λ_{max} 256 (band II) and 370 (band I) for a quercetin aglycone. Bathochromic shift in band II after addition of NaOAc reagent indicated a free 7-OH group and the increase in the intensity of band I on addition of NaOMe suggested a free 4′-OH group, while the absence of bathochromic shift in both bands after the addition of H_3BO_3 confirmed the absence of two adjacent (ortho) free hydroxyl groups. The remained bathochromic shift in both bands I and II after the addition of HCl to the $AlCl_3$ cuvette indicating free 3- and 5-OH groups. Bathochromic shift in band I with gradual decomposition by addition of NaOMe indicated the presence of 3′-OH substitution[16]. The ^{1}H NMR exhibited ABX spin coupling system showing signals at δ 7.69 (dd, *J* = 8.5 & 2.2 Hz), 7.63 ppm (d, *J* = 2.2 Hz) and at δ 6.87 (d, *J* = 8.5 Hz) ppm for H-6`, H-2` and H-5`, respectively indicating 3′, 4′-substituted B- ring, while AM spin coupling system of two meta doublet (*J* = 2.0 Hz) signals at δ 6.42 and δ 6.18 ppm for H-8 and H-6, respectively indicating 5, 7-dihydroxy A-ring[16]. The appearance of doublet signals at δ 4.79 ppm (*J* = 1.7 Hz) indicating the presence of the characteristic anomeric proton of terminal α-rhamnose with its methyl protons at δ 1.09 (*J* = 6.23 Hz). The presences of rhamnose at 3′-OH was concluded by the slight downfield shift of H-2′ (δ 7.63 ppm) and by the upfield shift of H-1″ signal at δ 4.79 ppm compared to that of unsubstituted (free 3′-OH) compound[16]. ^{13}C NMR spectrum exhibited characteristic 15 carbon resonances of free 3-*O*-quercetin together with six carbons for $^1C_4$- α-rhamnose moiety[20], which was indicated by its signal at δ 101.59 ppm of C-1″ and its methyl at δ 18.41 ppm. The location of rhamnose moiety at 3'- OH was concluded from the slight upfield shift of C-3' at δ 140.80 ppm and downfield shift of both C-4' (δ 151.22 ppm) and C-2' (δ 122.43 ppm) compared with free 3'- OH flavonoids (145.39 C-3', 148.22 C-4' and 116.11 C-2')[21]. The free OH-3 was confirmed by the slight upfield shift of both C-2 (δ 146.23 ppm) and C-4 (δ 175.90 ppm) and by the downfield shift of C-3 (δ 136.21 ppm) compared with 3-*O*-glycosylated compounds (156.50 C-2, 177.50 C-4 and 133.80 C-3)[21,23]. Negative ESI-MS spectrum exhibited a molecular ion peak at *m/z* 447 corresponding to $[M-H]^{-1}$ molecular weight 448 and molecular formula of C_{21} $H_{20}O_{11}$ and at *m/z* 301 [quercetin-H]$^{-1}$, which was in complete accordance with a quercetin rhamnoside. Thus, compound **10** was established as quercetin 3'-*O*-α-L-$^1C_4$-rhamnopyranoside.

Compound **11** was expected to be rhamntin 3-*O*-glycoside-like structure, as by complete acid hydrolysis it gave rhamntin in the organic phase and galactose in the aqueous phase (CoPC). UV methanol spectrum showed characteristic absorption at λ_{max} 256 (band II) and 370 (band I) for a rhamntin aglycone. On addition of NaOMe, a bathochromic shift of the total spectrum was observed together with intrinsic shift of about 60 nm in band I accompanied with increase in its intensity indicating free 4′ –OH. The absence of the bathochromic shift in band II after addition of NaOAc reagent indicated the occupation of 7-OH group of ring A, while the bathochromic shift in band I after the addition of H_3BO_3 confirmed the presence of two adjacent (ortho) free hydroxyl groups in ring B[16]. ^{1}H NMR spectrum exhibited in the aromatic region the three characteristic protons resonances of 3′, 4′-dihydroxy B-ring as a doublet of doublet signal at δ 7.60 with two *J* values of 2.2 and 8.5 Hz of H-6' and two doublet signals at δ 7.57 (*J* = 2.2) and 6.84 (*J* = 8.5) of H-2' and 5', respectively. It also showed the splitting pattern of the two *meta* doublets at δ 6.19 (*J* = 2.2) and 6.39 (*J* = 2.2) assigned to H-6 and H-8, respectively of 5,7-dihydroxy A-ring[16]. The methoxylation on C-7 was indicating by the presence of a clear singlet (3 protons) at δ 3.88 ppm, which also appeared at δ 56.49 ppm in ^{13}C NMR spectrum and by the up-field shifts of C-7 (Δ = ~+4 ppm) and of C-6 (Δ = ~-2 ppm) relative to those of quercetin[20]. The multiplet peaks appeared in the range of δ 3-4 ppm and the doublet peak at δ 5.44 ppm (*J* = 6.5 Hz) indicated the presence of galactose moiety. The glycosylation at 3-*O* position was confirmed by the ^{13}C NMR spectrum showing slight upfield shift of C-3 at δ 133.39 ppm and the downfield shift of both C-2 and C-4 (156.36 and 177.48, respectively) of glycosylated rhamntin compared to that of free rhamntin (136.0 C-3, 147.30 C-2 and 164.90 C-4)[19,20]. Negative ESI-MS spectrum exhibits a molecular ion peak at *m/z* 477 $[M-H]^{-1}$ corresponding to molecular weight 478 and molecular formula of C_{22} $H_{22}O_{12}$ in addition to a peak at 315 [M-galactose]$^{-1}$ of rhamntin aglycone, which was in complete accordance with a rhamntin galactoside confirming the identity of compound **11** as rhamntin 3-*O*-β-D-$^4C_1$-galactopyranoside.

Antimicrobial and antimicroalgal activity:

Results showed that compounds **4**, **6**, **10** and **11**, obtained from the methanol stem extract have antibacterial activity against both Gram positive (*Streptomyces viridochromogene* and *Staphylococcus aurens*) and Gram negative bacteria (*Escherichia coli* and *Bacillus subtilis*), which is in accordance with data reported of the leaf essential oils having

antimicrobial activity[11]. However compounds **10** and **11** showed antimicroalgal activity (phycotoxic activity)[15] against all the tested microalgae (*Chlorella vulgaris, Chlorella sorokiniana, and Scenedesmus subspicatus*), while both compounds **4** and **6** showed only high selective phycotoxic activity against *Scenedesmus subspicatus* as shown by the inhibition zones and as compared to germanomycine antibiotic (Table 1).

Table 1. Antimicrobial activities of compounds isolated from *S. molle* stem part.

	EC	BS	SV	SA	CV	CS	SS
Compound **4**	14	13	12	13	0	0	22
Compound **6**	15	16	14	14	0	0	25
Compound **10**	12	13	11	11	10	11	15
Compound **11**	10.5	10.5	11	11	10	11	15
Germanomycine	25	28	22	19	7	6.5	0

Diameters of inhibition zones in mm. EC=Escherichia coli, BC=Bacillus subtilis, SV=Streptomyces viridochromogenes, SA=Staphylococcus aurens, CV= Chlorella vulgaris, CS=Chlorella sorokiniana, SS=Scenedesmus subspicatus.

Conclusion

In conclusion, the methanol stem extract of *S. molle* contain phenolic compounds some of which, were isolated for the first time from the genus *Schinus*, showed in addition to the antibacterial activity, a remarkably strong antimicroalgal activity (phycotoxicity) and as the production of toxins is one strategy that phytoplankton use to deal with broad range of parasites, thus *S. molle* has a great potential as antibacterial drug and its phycotoxic activity giving it economic value as a natural herbicide (allelopathic) drug protecting medicinal crops from parasites and the environment from synthetic chemicals.

Acknowledgement

The authors would like to thank Mrs. Fredricke Lissy, Dept. of Organic Chemistry, George-August University, Gottingen, Germany, for her technical assistance concerning the anti-microorganism activity.

Declaration of Interest

The authors report no declarations of interest.

Corresponding author

Dr. Eman G. Haggag
Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Cairo, Egypt. 11795
E-mail: wemisr@hotmail.com

References

1- Materechera SA, Hae ME (2008). Potential of aqueous extracts from parts of the pepper tree (*Schinus molle* L.) to affect emergence and seedling development of wheat (*Tritcum sativa* L) and weeds in a manure amended soil. *The Open Agriculture Journal* **2**: 99-104
2- Singh HP, Batish DR, Kohli PK (2003). Allelopathic interactions and phytotoxin: New possibilities for sustainable weed management. *Crit Rev Sci* **22**:239-311
3- Ambasata SP (1986). The useful plants of India. Publications & Information Directorate, CSIR, New Delhi, p. 556
4- Barrachina MD, Bello R, Martinez-Cuesta MA, Primo-Yúfera E, Esplugues J (1997). Analgesic and central depressor effects of the dichloromethanol extract from *Schinus molle* L. *Phytother Res* **11**:317-319
5- Bello R, Báltran B, Moreno L, Calatayud S, Primo-Yúfera E, Esplugues J (1998). *In vitro* pharmacological evaluation of the dichloromethanol extract from *Schinus molle* L. *Phytother Res* **12**:523-525
6- Yueqin Z, Recio MC, Manez S, Giner RM, Cerda-Nicolas M, Rios JL (2003). Isolation of two triterpenoids and a biflaavanone with anti-inflammatory activity from *Schinus molle* fruits. *Planta Med* **69**:893-898
7- Marzouk MS, Moharram FA, Haggag EG, Ibrahim MT, Badary OA (2006). Antioxidant flavonol glycosides from *Schinus molle*. *Phytotherapy Research* **20**:200-205
8- Machado DG, Kaster MP, Binfaré RW, Dias M, Santos AR, Pizzolatti MG, Brighente IM, Rodrigues AL (2007). Antidepressant like effect of the extract from leaves of *S. molle* in mice: Evidence for the involvement of the monoaminergic system. *Prog. Neuropsychopharmacol. Biol Psych* **31**:421-428
9- Ferrero AA, Chopa CS, Gonzalez JO, Alzogaray RA (2007). Repellence and toxicity of *Schinus molle* extracts on *Blattella germanica*. *Fitoterpia* **78**:311-314

10- Abdel-Sattar E, Zaitoun AA, Farag MA, El Gayed SH, Harraz FM (2010). Chemical composition, insecticidal and insect repellent activity of *Schinus moll*e L. leaf and fruit essential oils against *Trogoderma granarium* and *Triboloium castaneum*. *Natural Product Research* **24**:226-235
11- Gundidza M (1993). Antimicrobial activity of essential oil from *Schinus molle* Linn. *Cent Afr Med* **39**: 231-234.
12- Terhune SJ, Hogg JW, Lawrence BM (1974). *β*-spathulene: a new sesquiterpene in *Shinus molle* oil. *Phytochemistry* **13**: 865-866
13- Pozzo-Balbi T, Nobile L, Scapini G, Cini M (1978). The triterpenoid acids of *Shinus molle*. *Phytochemistry* **17**: 2107-2110
14- Dominguez XA, Carmona JF, De Venegas RB (1971). Lignoceric acid and other compounds of *Schinus molle*. *Phytochemistry* **10**: 1687-1688
15- Haggag EG, Laatsch H (2004). Biotransformation of indol alkaloid by terrestrial *Streptomyces* GW 14/587 strain into a selective phycotoxic agent. *Bull Pharm Sci Assiut University* **27** :113-122
16- Mabry TJ, Markham KR, Thomas MB (1970). The Systematic Identification of Flavonoids. Springer-Verlag New York Inc. USA
17- Brasseur T, Angento L (1986). Reagents for densitomeric determination of flavonoids. *J. Chromatography* **351**::351-355
18- Zhu X, Dong X, Wang Y, Ju P Luo S. (2005). Phenolic compounds from *Viburnum cylindricum*. *Helvetica Chemica Acta* **88**:339-342
19- Harborne JB (1994). Flavone and Flavonol Glycoside In: Textbook of The Flavonoids: Advances in research since 1986, Williams CA., Harborne, JB. eds. London: Chapman & Hall, Ltd, University Press Cambridge
20- Agrawal PK (1989). Carbon-13 NMR of flavonoids In: Textbook of Studies in Organic Chemistry 39, Agrawal PK, ed. New York: Elsevier Science.
21- Mahmoud II, Marzouk MS, Moharram FA, El-Gindi MR, Hassan A.M (2001). Acylated flavonol glycosides from *Eugenia jambolana* leaves. *Phytochemistry* **58**:1239-1244
22- Mahmoud II, Moharram FA, Marzouk MS, Linscheid MW, Saleh MI (2001). Polyphenolic constituent of *Callistemon lanceolatus* leaves. *Pharmazie* **57**:494-496
23- Marzouk MS., Moharram FA., El-Dib RA., El-Shenawy SM, Tawfike AF. (2009). Polyphenolic profile and bioactivity study of *Oenothera speciosa* Nutt aerial parts. *Molecules* **14**: 1456-1467.

8/12/2013