JCBPS; Section B; February 2018 – April 2018, Vol. 8, No. 2; 434- 443 E- ISSN: 2249 –1929

[DOI: 10.24214/jcbps.B.8.2.43443.]

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section B: Biological Sciences

CODEN (USA): JCBPAT Research Article

Phytochemical and Biological Activity of *Agastache foeniculum* (Pursh) Kuntze Cultivated in Egypt

Mostafa EM [1, 2], Abdelhady NM[3] and El-Hela AA[1].

[1]Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt.

[2] Pharmacognosy Department, College of Pharmacy, Jouf University, Sakaka, AJouf, KSA

[3] Pharmacognosy Department, Faculty of Pharmacy, Deraya University, El-Minia, Egypt.

Received: 19 March 2018**; Revised:** 03 April 2018; **Accepted:** 15 April 2018

Abstract: The present study was carried out aiming investigation of phenol and flavonoid contents of *Agastache foeniculum* (Pursh) Kuntze herb (Lamiaceae) and screening of their antioxidant potentials where chemical investigation resulted in isolation and identification of several terpenoid and flavonoid compounds viz., cholesterol, *β*-sitosterol, uvaol, ursolic acid and keampferol, quercetin, hyperoside, rutin respectively, their structural elucidation were established on the basis of NMR and MS spectral analyses. The total phenol and flavonoid contents were assessed using colourimetric methods as well as HPLC technique where their calculated values were 250 mg % and 48 µg % respectively meanwhile HPLC investigation revealed the existence of ninety phenolic acids and twenty-four flavonoids where their majors were as follows; coumarin (72.1 mg%), ferulic acid (22.79 mg%), rutin (3826.90 µg%) and luteolin-6-arabinose-8-glucose (628.20 µg%). The antioxidant activity of the total extract was evaluated by colourimetric method using 2, 2-diphenyl-1 picrylhydrazyl (DPPH) method where the gained results suggested *Agastache foeniculum* herb as one of the promising natural antioxidants.

Keywords: *Agastache foeniculum,* Phenol, Flavonoids, DPPH and Antioxidants

INTRODUCTION

Agastache is a genus of aromatic flowering herbaceous perennial plants in the family Lamiaceae. It contains 22 species native to eastern Asia (one species) and North America (the rest). The common names of the species are a variety of fairly ambiguous and confusing "hyssops" and "mints"; as a whole the genus is known as giant hyssops or hummingbird mints[1, 2]. Blue giant hyssop, *Agastache foeniculum* (Pursh) Kuntze, is a species of perennial plant of the mint family (Lamiaceae). It is native to the southwestern and eastern US and central Asia. This flowering plant is very attractive to bees and butterflies and commonly used as garnish for fruit salads, iced tea, desserts, and anise-flavored spices. This species is a candidate for large-scale, domestic cultivation as an aromatic plant with a wide variation in essential oil composition and content [3, 4].

In the herb and inflorescences of Agastache are found 0.9-1.2% (in dry matherial) volatile oils (limonene, anisole, linalool, methyl chavicol, estragole, Mentone, pulegona etc.), flavonoid derivatives, eugenol, cinnamic aldehyde, pogostol and others [5]. In therapeutic purposes is used for cardio-vascular, nervous, gastro-intestinal, colds, fever (in the form of infusion has been used frequently for this purpose by the Indians of North America), increased sweating (diaphoretic). It also used as anti-vomiting, antibacterial, antifungal and antacid [6]. It relieves symptoms of sore throats and colds. In Chinese tradition said that water processing of *Agastache* stimulates the spleen [6,7]. Based on the available literature there is no data concerning Phenolic Profile and /or the biological activity of *Agastache foeniculum*, so the study of the Phenolic Profile and /or the biological activity of *Agastache* cultivated in Egypt are very interesting.

MATERIAL AND METHODS

Plant material: *A. foeniculum* was collected at flowering stage from plants grown on the experimental farm of the Department of pharmacognosy, Al-Azhar University and were kindly authenticated by Dr. Abdo Marey, Asst. Prof. of taxonomy, Faculty of Science, University of Al- Azhar. Specimens are deposited in the herbarium, Pharmacognosy Department, Al-Azhar University, Cairo, Egypt. The dried collected herb was kept in tightly closed amber glass containers and protected in dark at low temperature as possible.

Preparation of extracts : 500 and 50 g finely-powdered sample were extracted separately in soxhlet systems with 2000 ml of 80% (v/v) aqueous methanol and 250 ml distilled water for one hour to yield the methanol and aqueous extracts respectively, they were filtered under vacuum through Whatmann No.1 filter paper, the residue was re-extracted following the same procedure two more times, extracts collected were vacuum dried at 40 ^{0}C to give 62 g and 7 g methanol and aqueous extracts respectively. 50 g of the methanol extract were suspended in water and successively fractionated using hexane, methylene chloride and ethyl acetate, each fraction was concentrated under reduced pressure to give 12 g, 8 g and 15 g respectively.

Separation of compounds from methylene chloride and ethyl acetate fractions : The methylene chloride (5g) was fractionated using on silica gel (column chromatography (150g, 5 x 120cm) with a step gradient of petroleum ether-ethyl acetate (5 % to 20% ethyl acetate), fractions of 50 ml were collected and monitored by TLC. The identical elutes were pooled together to give 5 fractions, as follows; A (0.25g), B (1.4g), C (2.7g) and D (2.5g) respectively, from fractions A and B compounds **1** (100mg) and **2** (150mg) were obtained by preparative TLC, the developing solvent system was (chloroform: methanol, 9:1) followed by chromatography on Sephadex LH- 20 (10g, chloroform:

methanol 1:1) while compound **3** (150 mg) was isolated from fraction C using silica gel column (10g ,10 x 0.5mm) and re-chromatography on Sephadex LH 20 (5g , 20x1mm) and chloroform: methanol as eluent meanwhile, compound **4** (84 mg) was obtained from fraction D through the same procedure.

5 g of ethyl acetate fraction was applied on silica gel column (200g, 5x120 cm) and eluted with chloroform with increasing amount of methanol 5-100% to give three fractions, where the elutes (50ml each) were collected and monitored by TLC using methylene chloride: methanol (85:15) as solvent system, the gained chromatograms were examined under UV light at 365nm before and after exposure to ammonia vapor where similar fractions were pooled and the solvents were separately evaporated under pressure to give three pooled fractions, they were as follows; fraction I (1.52 g) was further purified on sephadex LH-20 to give compound **5** (100mg), fraction II (0.2g) was chromatographed on Sephadex LH-20 using methanol to give compound **6** (10mg) and fraction III (0.5g) was purified by column chromatography on cellulose (60g, 3.5 x 120 cm) using 10% methanol in water. From fraction 1, 10ml were collected and monitored by TLC using as solvent system to give 2 subfractions, they were as follows; sub-fraction 1 which was further purified using Sephadex LH-20 to give compounds **7** (20mg) and **8** (30mg). UV spectral analysis for the isolated compounds were recorded on Unicam SP 1750 Pye spectrophotometer while ^{1}H-NMR and ^{13}C-NMR spectra were recorded on AMX-300 MSL instrument (Bruker, Karlsruhe) using TMS as internal standard and EI-MS was obtained on Joel 100 (70 eV).

Determination of total phenol compounds: Concentration of total phenolic in the methanol extract of *A. foeniculum* was determined colourimetrically using the Folin-Ciocalteu reagent [8, 9]. Standard curve was done using different concentrations of Gallic acid (1: 6 mg) in methanol. The concentrated extract of the tested plant was dissolved in least volume of methanol and completed to 10 ml, 100 µl of extract was diluted with 8 ml distilled water. 0.5 ml of 50 % Folin-Ciocalteu reagent was added and left 8 min, and 1.5 ml of 5% sodium carbonatewas added, mixed and allowed to stand for 60 min. protected from light. Their absorbance was measured using Genesys spectrophotometer (Milton Roy, INC., Rochester, NY) at 725 nm using methanol as blank and the concentration of the total phenolic content of the extract was calculated.

HPLC analysis of phenolic compounds : Phenolic compounds were determined by HPLC according to the method of Goupy *et al.*[10] trans-cinnamic, o-coumaric, p-coumaric, caffeic, ferulic, syringic, synapic, chlorogenic, syringic protcatechuic, gallic and ellagic) were obtained from Sigma Chemical Co. (St. Louis, Mo, USA), and (HPLC)-grade.

Determination of Flavonoid compounds: Flavonoid compounds were determined by HPLC according to the method of Mattila *et al.*, [11, 12].

Determination of antioxidant potentials: the test was carried out on 96 Micro-Well plate where a standard curve was done using different concentrations of BHT in methanol (7serial 2fold dilutions to give final range of 100 to 5 µm). 50 µl of a 0.022% DPPH solution in methanol was added to a range solution of different concentrations (7 serial -3 fold solutions to give final range of 1000 to 1.3 µg /ml) of the crude methanol and nanoparticulated extracts and (7 serial 2 fold dilutions to give final range of 100 to 5 µm) of compounds to be tested in methanol (230 µl) and their absorbance was measured at 515 nm after 30 min., the percent radical scavenging activities were calculated [13].

RESULTS AND DISSICUSION

Compound 1(100 mg): colorless crystals (MeOH), m.p. 148˚-150˚C, R_f 0.81 ($CHCl_3$-MeOH, 9:1). EI-MS *m/z* (% rel.int.): 386 (M^+, $C_{27}H_{46}O$, 5); 368 (M^+ - H_2O, 20); 353 (M+- (H2O- Me); 311 (15); 258 (100); 243 (9.9), 217 (7); 151 (5), 135 (5), 109 (17), 95 (22), 81(23) and 55 (65).

Compound 2 (150mg): white crystals (MeOH), m.p. 138˚- 148˚C, R_f 0.77 ($CHCl_3$-MeOH, 9:1). EI-MS *m/z* (% rel.int.): 414 (M^+, C $_{29}H_{50}O$, 100); 396 (M^+- H_2O, 50), 353 (M^+- (H_2O- Me), 255 (M^+ - side chain, 20), 243 (9.9); 217 (7); 151 (5), 135 (5), 109 (17), 95 (22), 81(23) and 55 (65).

Compound 3 (150 mg): colorless prisms (MeOH), m.p. 222˚- 225˚C, Rf 0.57 ($CHCl_3$-MeOH, 9:1). EI-MS *m/z* (% rel.int.): 442 (M+, $C_{27}H_{46}O$, 3); 427 (M+- CH_3, 5); 393 (3), 234 (25), 207 (10%), 203 (100%), 189 (9%), 175 (7%), 133 (10%).

Compound 4 (84 mg): white needle crystals (MeOH), m.p. 303˚ -305˚C, R_f 0.45 ($CHCl_3$-MeOH, 9:1). EI-MS *m/z* (% rel.int.): 456 (M^+, $C_{15}H_{10}O_7$, 5), 248 (100%), 219 (4), 203 (12), 133 (10).

Compound 5 (100mg): yellow amorphous (MeOH), m.p. 278-280˚C, R_f 0.92 ($CHCl_3$-MeOH, 9:1). UV λ_{max} (MeOH): 265, 364 nm, EI-MS *m/z* (% rel.int.): 268 (M^+, $C_{15}H_{10}O_6$, 100); The mass spectrum showed molecular ion peak at 268 *m/z*, which is likely for molecular formula $C_{15}H_{10}O_6$, was close to the published data for kaempferol.

Compound 6 (10 mg): yellow crystals (MeOH), m.p. 298 – 300 ˚C, R_f 0.52 ($CHCl_3$-MeOH, 9:1). UV λ_{max} (MeOH): 255, 269 nm, EI-MS *m/z* (% rel.int.): 302 (M^+, $C_{15}H_{10}O_7$, 100); *^{1}H-NMR* (500 MHz, DMSO-d_6): δ 7.72 (1H, d, *J* = 2.5 Hz, H-2'), 7.60 (1H, dd, *J* = 8.5 & 2.5 Hz, H-6`), 6.85 (1H, d, *J* = 8.5 Hz, H-5'), 6.35 (1H, d, *J* = 2.5 Hz, H-6) and 6.15 (1H, d, *J* = 2.5 Hz, H-8); *^{13}C-NMR* (DMSO-d_6): δ 177.2 (C- 4), 165.2 (C-7), 162.5 (C-5), 158.4 (C-2), 149.2 (C-9), 148.1 (C- 4'), 146.1 (C-3'), 138.3 (C-3), 124.3 (C-6'), 121.6 (C-1'), 116.2 (C-2'), 115.2 (C-5'), 104.6 (C-10), 99.2 (C-6) and 94.4 (C-8); MS: m/z 302, 281, 273, 207, 95, 81.

Compound 7 (20 mg): yellow crystals (MeOH) m.p. 217- 219 ^{o}C. EI-MS *m/z* (% rel.int.) 358 (0.9), 316 (1.1), 302 (100), 286 (4.7), 274 (4.6), 257 (1.9), 245 (3.3), 229 (3.5), 153 (5.2), 144 (1.9), 137 (5.9), 60 (8.0). *^{1}H-NMR* (500 MHz, DMSO-*d6*): [δ 6.5 (1H, d, *J* = 2 Hz, H-8) and 6.3 (1H, d, *J* = 2 Hz, H-6)] and 3 proton signals of a B ring at δ 7.98 (1H, d, *J* = 2.0 Hz, H-6'), 7.72 (1H, dd, *J* = 8.5, 2.5 Hz, H-5') and 6.99 (1H, d, *J* = 8.5 Hz, H-2'). *^{13}C-NMR* (300 MHz DMSO-*d6*)*:* (C2) 159,1, (C3) 136.6, (C4) 180.2, (C5) 163.7, (C6) 100.7, (C7) 167.1, (C8) 95,6, (C9) 159, (C10) 106.3, (C1') 123.1(C2') 169.9, (C3') 146.3, (C4') 150.2, (C5') 118.3, (C6') 132.7, (C1'') 106.3, (C2'') 73.4, (C3'') 75.8, (C4'') 70.8, (C5'') 78.8, (C6'') 62.8.

Compound 8 (30 mg): Yellow amorphous powder [MeOH]; ESI-MS: *m/z* 633 $[M+Na]^+$, UV λ_{max} (MeOH) nm: 260, 366, λ_{max} (MeONa) nm: 273, 315, 412, λ_{max} (AlCl3) nm: 276, 305, 439, λ_{max} ($AlCl_3$/HCl) nm: 275, 303, 409, λ_{max} (AcONa) nm: 277, 320, 395, λ_{max} (AcONa/boric acid) nm: 261, 389; *^{1}H NMR* (DMSO-*d6*, 500 MHz) δ12.5 (OH), 7.54 (1H, br s, H-2`), 7.52(1H, d, *J*=8.0 Hz, H-6`), 6.83 (1H, d, *J*=8.0 Hz, H-5`), 6.45 (1H, br s, H-8), 6.22 (1H, br s, H-6), 5.40 (1H, d, *J*=7.6 Hz, H-1``), 4.39 (1H, d, *J*=2.5 Hz, H-1```), 3.05- 3.38 (10H, m, H-2``-H-6`` of glc and H-2```-H-5``` of rha), 0.95 (1H, d, *J*=6.0 Hz, H-6``); *^{13}C NMR* (DMSO-*d6*, 125 MHz) δ177.74 (C-4), 164.72 (C-7), 161.49 (C-5), 157.17 (C-2), 156.75 (C-9), 148.87 (C-4`), 145.18 (C-3`), 133.68 (C-3), 122 (C-6`), 121.58 (C-1`),

116.60 (C-5`), 115.76 (C-2`), 105 (C-10), 101.57 (C-1``), 101.16 (C-1```), 99.2 (C-6), 94.14 (C-8), 76.83 (C-3``), 76.19 (C-5``), 74.43 (C-2``), 72.23 (C- 4```), 70.92 (C-3```), 70.69 (C-2```), 70.36 (C-4``), 68.61 (C-5```), 67.40 (C-6``), 18.11 (C-6```).

Table 1: Total Phenol and Flavonoids contents, Antioxidant potentials of *Agastache foeniculum:*

Plant	Total phenol mg %	Flavonoid µg %	Antioxidant %
Agastache foeniculum	250	48	98

Table 2: HPLC Analysis of Phenolic acids of *Agastache foeniculum:*

Phenolic Acid	R_t		% (mg/100g)
	St	Test	
Pyrogallol	6.92	6.90	4.67
Gallic	7.05	7.00	0.20
Protocatchuic	9.03	9.06	0.12
catechol	9.60	9.62	1.80
p-Hydroxy benzoic	9.84	9.81	2.29
Chlorogenic	10.01	10.10	3.09
Vanillic	10.21	10.23	1.16
Caffeic	10.20	10.31	1.96
p-Coumaric	11.58	11.60	3.18
Ferulic	11.80	11.80	22.79
Iso-ferulic	12.16	12.20	7.10
E-vanillic	12.24	12.30	1.18
O-Coumaric	13.27	13.10	1.76
Benzoic	13.30	13.30	1.44
Ellagic	13.18	13.40	1.07
Coumarin	14.44	14.41	72.10
Cinnamic	15.29	15.40	1.03
Salicylic	16.40	16.50	12.68
Total	**139.62**		

Table 3: HPLC Analysis of Flavonoid contents of *Agastache foeniculum*

Flavonoid	R_t		% (µg/100g)
	St	Test	
Luteolin-6-arabinose-8-glucose	9.48	9.44	628.20
Luteolin-6- glucose -8-arabinose	10.81	10.82	82.97
Apigenin-6-arabinose-8-galactose	11.37	11.36	27.30
Apigenin-6- rhamnose-8- glucose	11.81	11.83	310.47
Apigenin-6-glucose-8-rhamnose	12.19	12.18	79.77
Luteolin-7- glucose	12.30	12.28	39.31
Narengin	12.35	12.37	---
Hesperidin	12.48	12.48	99.76
Rutin	12.61	12.57	3826.90
Quercetin-3-*O*-glucoside	12.51	12.52	360.82
Apigenin-7-*O*-neohisperoside	13.14	13.41	---
Kaempferol-3,7-dirahmnoside	13.21	13.26	74.33
Quercetrin	13.45	13.42	54.75
Rosmarinic	13.88	13.92	127.11
Quercetin	14.90	14.91	7.91
Naringenin	15.03	15.05	92.98
Acacetin-neo rutinoside	15.10	15.12	15.14
Kaempferol-3-(3-*p*-coumaroyl) glucose	15.16	15.18	---
Hespertin	15.35	15.30	235.57
Kaempferol	16.24	16.26	110.88
Rhamnetin	16.44	16.49	141.28
Apigenin	16.56	16.64	129.45
Apigenin-7-glucose	17.24	17.26	100.02
Acacetin	18.82	18.86	50.84
Total	**6399.17**		

DISCUSSION

Chemical screening of *A. foeniculum* revealed the presence of phenols, flavonoids and terpenoid compounds[14]. The mass spectrum of compound **1** showed molecular ion peak at 386 *m/z*, which is likely for molecular formula $C_{27}H_{46}O$, was close to the published data for cholesterol [15,16]**,** the mass spectrum of compound **2** showed a molecular ion peak of 414 *m/z* ($C_{29}H_{50}O$), this mass values along with fragmentation pattern were found in conformity with those published for *β*-sitosterol in the literature [17], while the mass spectra of compounds **3** and **4** showed molecular ion peaks at *m/z* 442 and 456 , which are likely for molecular formula $C_{30}H_{50}O_2$ and $C_{30}H_{48}O_3$, both the mass spectra and fragmentation patterns were identical with the published data on uvaol [18] and ursolic acid [19]. Further confirmation for the previous compounds was carried out by direct comparison with Co-chromatography versus their authentic samples, determination of melting point and mixed melting point. The mass spectrum of compound **5** showed molecular ion peak at 268 *m/z*, which is likely for molecular formula $C_{15}H_{10}O_6$ was close to the published data for keampferol [20].

Compound **6** is soluble in mixture of chloroform and methanol with mp 312 ^oC , it gave negative Molisch's test and positive magnesium in concentrated hydrochloric acid test, it has yellow color under UV 365 nm changed to intensive yellow after spraying with 2 % aluminum chloride reagent this behavior indicates that it is of flavonoid aglycone nature, EI-MS spectrum showed a molecular ion peak at *m/z* 302 while UV spectral data, showed bathochromic shift in band I with aluminum chloride reagent which disappear on addition of hydrochloric acid that is an evidence of orthodihydroxy B ring [20]. ^{1}H-NMR spectrum showed an ABX coupling system of three proton signals as meta doublet (H-2`), ortho-meta dd (H-6`) and ortho doublet (H-5`) for 3`, 4` dihydroxy B-ring and AM-spin coupling system of two meta coupled protons at 6.38, 6.18 assigned H-8 and H-6 respectively while ^{13}C-NMR spectral data exhibited fifteen typical carbon resonances for quercetin moiety were assigned in the aromatic region, showed two key signals of quercetin aglycone were assigned at 149.15 (C-4`) and 145.49 (C-3`) ppm, accordingly, compound **6** was identified as 3, 5, 7, 3`, 4`, pentahydroxy –flavone (quercetin) [21].

EI-MS analysis of compound **7** exhibited a molecular weight 464, the UV spectrum in MeOH , indicated the possible flavonol skeleton The ^{1}H-NMR (500 MHz, MeOD) spectrum of **7** showed the presence of quercetin as aglycone including two proton signals of an A ring [δ 6.5 (1H, d, *J* = 2 Hz, H-8) and 6.3 (1H, d, *J* = 2 Hz, H-6)] and three proton signals of a B ring at δ 7.98 (1H, d, *J* = 2.0 Hz, H-6'), 7.72 (1H, dd, *J* = 8.5, 2.5 Hz, H-5') and 6.99 (1H, d, *J* = 8.5 Hz, H-2'). The ^{1}H-NMR (500 MHz, MeOD), therefore, the aglycone of **7** was assigned to quercetin, the coupling constants (*J*), signal splitting patterns and chemical shifts in the proton and carbon signals of the sugar moieties suggested that the sugars was galactose. Therefore, the compound was assigned as quercetin 3-*O*-*β*-D-galactopyranoside (hyperoside); that was confirmed with standard samples using Co-chromatography. ^{1}H-NMR and ^{13}C-NMR analyses of the hyperoside standard samples revealed similarities in the coupling constants, signal splitting patterns and chemical shifts in the proton and carbon signals with the isolated compounds. Based on UV-vis., MS, ^{1}H-NMR and ^{13}C-NMR analyses, the spectral characteristics of this compound was found to be identical with the literature [22, 23].

Compound **8** showed that Ring B contained three protons, H-5` appeared separately as doublet up filed at 6.79 (*J* = 8.4 4Hz) due to the shielding effect of oxygen substitution while both H-2` and H-6`appeared as multiplet downfield at δ 7.53 due to the deshielding effect of ring C meanwhile, ring A contained two protons, each of them appeared as doublet at δ 6.22 assigned for H-8 and 6.1 assigned for H-6. The coupling constant = 1.8 Hz in both protons indicating that they were meta-coupled.

Additional signals of sugar moiety, at 5.24 (H-1`` with *J* 1``* 2`` axial = 6.6 Hz) indicated anomeric glucose proton with *β*-linkage and this high value confirm 3-glycosidation rather 5 or 7-glycosidation), while those at 4.37 (H-1``` with *J* 1```* 2``` euqatorial = 1.5 Hz) and at 0.99 (Three protons of methyl) revealed rhamnose with *α*- linkage, these data indicate rutinosyl moiety, [20]. ^{13}C-NMR, showed 27 carbon atoms, 12 of them for signals representing carbon atoms of the rhamnoglucosly moiety at δ 101.81 (confirming *O*-glycosidation rather than *C*-glycosidation), 100.81, 76.57, 75.87, 74.17, 71.95, 70.61, 70.39, 69.99, 68,29, 67.07 and 17.80 (of rhamnosyl moiety), while the rest 15 carbon atoms were assigned to quercetin nucleus presented as follows: 9 aromatic non-oxygenated carbons viz. (156.75, 121.62, 115.92, 115.28, 102.57, 99.7 and 94.18), 5 oxyaromatic carbons viz. (167.95, 161.05, 149.27, 145.10 and 133.01) and one carbonyl carbon at 176.71 and so, compound **8** was identified as quercetin-3-*O*-*α*-L-rhamnosyl-*β*-glucopyranoside (Rutin).

The total phenol and flavonoids content values of *A. foeniculum* were determined using Folin–Ciocalteu's reagent and using $AlCl_3$ reagent [15] calculate (250 mg±05) as gallic acid GAE/g dry mg and (59±0.6) as qurectin, Q/g dry mg. In addition, to *in vivo* antioxidant activity was performed using DPPH assay and comparison with standards (Table 1). Forty three compounds were detected using HPLC technique. In addition, *in vivo* antioxidant activity was performed using DPPH assay and comparison with standards. They were detected as 19 phenolic acids and 23 flavonoids. Phenolic acids were detected as; Pyrogallol, gallic acid, protocatechuic, *p*-Hydroxy benzoic. Chlorogenic, Vanillic, Caffeic, *P*-coumaric, Ferulic, Iso-ferulic, *E*-vanillic, *O*-coumaric, Benzoic, ellagic 3,4,5-methoxy cinnamic acid, Coumarin, Cinnamic and salicylic acid (Table 2), ferulic acid (22%) was the major compound. The total flavonoids of plant extract detected by HPLC (6399.17µg %) (Table 2) and revealed the presence of 23 compounds of different class were represented as (aglycones and flavone glycosides, flavonol aglycones and 2, 3 dehydroflavone), the detected flavonol seem to be 3-*O*-glycosides; Rutin, Quercetin-3-*O*-glucoside, Kaempferol-3,7-dirahmnoside, Quercetrin the major compound were represented as Rutin (3826.90 mg/100g DW**)**. *C*-glycosyl flavones seem to be Luteolin-6-arabinose-8-glucose and Apigenin-6- rhamnose-8- glucose pattern of luteolin and apigenin; the major compounds were represented as Luteolin-6-arabino-8-glucose and Apigenin-6- rhamnose-8- glucose (628.20, and 310.47 mg/100g DW) The radical scavenging activity of the extract showed highly activities with 98 % inhibition for the rest investigated plants. The results are significant between the total phenolic and the antioxidant activity [23-25]. In the present work, phenolic acids, flavonoids and antioxidant of methanol extract were detected for the first time from the plant.

Spectrophotometric quantitative estimation using DPPH method of the antioxidant potential the crude methanol extract revealed that it possesses significant free radical scavenging potential when compared with the reference synthetic antioxidant butylated hydroxytoluene (BHT), which can be attributed to its high content of phenol and flavonoid compounds [26, 27].

REFERENCES

1. RHS A-Z, *Encyclopedia of garden plants*, United Kingdom, Dorling Kindersley, 2008, P. 1136.
2. Huo xiang, *Agastache rugosa*, Flora of China (Fischer & C. Meyer) Kuntze, 1891, 17: 106.
3. G.S. Ayers, and M.P. Widrlechner, *The genus Agastache as bee forage: a historical perspective*, American Bee Journal, 1994, 134: 341-348.
4. D.J. Charles, J.E. Simon, and M.P. Widrlechner, *Characterization of essential oil of Agastache species*, Journal of Agricultural and Food Chemistry ,1991,39:1946- 1949.

5. D. M. Marcel, D. I. Vârban, S. Muntean, C. Moldovan, M. Olar, *Use Of Species Agastache Foeniculum (Pursh) Kuntze*, Hop and medicinal plants, 2013, 2: (41-42).
6. R. E. Reyes, E. A. Hernandez, A. Garcia- Argaez, M. S. Hernandez, E. Linares, R. Bye, G. Heinze, M. M.Vazquez, *Comparative chemical composition of Agastache mexicana subsp. mexicana and A. mexicana subsp. Xolocotziana*, Biochemical Systematics and Ecology, 2004, 32: 685–694.
7. C.F. Matei, Research *on the biology and technology of cultivation of the species Agastache foeniculum (Pursh) Kuntze in Transylvanian Plain conditions*, Ph D Thesis, USAMV Cluj-Napoca, **2012**.
8. A. A. Hafez, *Physico-Chemical and Sensory Properties of Cakes Supplemented with Different Concentration of Marjoram*, Australian Journal of Basic & Applied Sciences, 6 (13): 463-470, **2012**.
9. K. Zhou, J. Hao, C. Griffey, H. Chung, S. F. O'Keefe, J. Chen, S. Hogan, *Antioxidant properties of Fusarium head blight-resistant and-susceptible soft red winter wheat grains grown in Virginia*, J Agric Food Chem, 2007, 55 (9): 3729-36.
10. A. El-Hela and A. Abdullah, *Antioxidant and Antimicrobial Activities of Methanol Extracts of some VerbenaSpecies: In Vitro Evaluation of Antioxidant and Antimicrobial Activity in Relation to Polyphenolic Content*, Journal of Applied Sciences Research, 2010, 6(6): 683-689,
11. P. Goupy, M. Hugues, P. Boivin, and M.J. Amiot, *Antioxidant Composition and Activity of Barley (Hordeum vulgare) and Malt Extracts and of Isolated Phenolic Compounds*, Journal of the Science of Food and Agriculture, 1999, 79: 1625-1634.
12. T. Mattila-Sandholm, P. Myllärinen and G. Mogensen, *Technological challenges for future probiotic foods*, International Dairy Journal, 12: (3), 173-182, **2002**.
13. M. Gálvez, J.M. Martín-Cordero, J. María, Antioxidant activity of *Plantago bellardii* All., Phytother. Res., 19(12), 2005, 1074-6.
14. N. Dasgupta and B. De, *Antioxidant Activity of Piper Betle L. Leaf Extract in Vitro*, Food Chemistry, 2004, 88: (2), 219-224.
15. C.T. Chang, C.Y. Yang, F.J. Tsai , S.Y. Lin and C.J. Chen, *Mass spectrometry-based proteomic study makes high-density lipoprotein a biomarker for atherosclerotic vascular disease*, BioMed Research International, 2015: ID 164846, 13.
16. P.J. Horn and K.D. Chapman, *Lipidomics in tissues, cells and subcellular compartments*, The Plant Journal, 2012, 70: 69–80.
17. M. Akimbo and A.K. Saluja, *Isolation of stigmasterol and β sitosterol from petroleum ether extract of aerial parts of Ageratum conyzoides (Asteraceae)*, Int J Pharm Sci, 2011,3: (1), 94-96.
18. N.A. Hatem and Z.M. Najah, *Isolation and elucidation of some chemical constituents of Lavandula officinalis*, J. Chem. Pharm. Res., 2016, 8 (3): 394-401.
19. A.R. Gohari, S. Saeidnia, A.Hadjiakhoondi, M.Abdoullahi and M.Nezafati, *Isolation and quantitative analysis of oleanolic acid from Satureja mutica Fisch. & C. A. Mey*., Journal of Medicinal Plants, 2009, 8: (5), 65-69.

20. P.K. Agrawal, *^{13}C NMR of Flavonoids*, Chapter 6, Elsevier, New York, 1989.
21. J.B. Harborne, Phytochemical *Methods: A Guide to Modern Techniques of Plant Analysis*, ^{3}rd Ed., Chapman and Hall: London, ISBN: 0-412-57270-2, 1998,302.
22. J.Y. Cho, S.H. ji , J.H. Moon, K.H. Lee, K.H. Jung and K.H. Park, *A novel benzoyl glucoside and phenolic compounds from the leaves of Camellia japonica*, Food Sci. biotechnol., 2008,17, 1060-1065.
23. H.H. Lee, J.Y. Cho, J.H. Moon and K.H.Park, *Isolation and Identification of Antioxidative phenolic acids and flavonoid glycosides from Camellia japonica flowers*, Hort. Environ. Biotechnol., 2011,52, 270-277.
24. A.A. Shahat, F. Hammouda, S.I. Ismail, S.A. Azzam, T. De Bruyne, B. Lasuvanpoel, L. Pieters, and A.J. Vlietinck, *Antiviral and antioxidant activity of flavonoids and proanthocyanidins from Crataegus sinaica*, J. Planta Medica,2002, 68: (8), 539- 41.
25. T.J. Mabry, K.R. Markham and M.B. Thomas, *The Systematic Identification of Flavonoids*, Springer Verlag; New York, **1970**.
26. R.R. Hafidh, A.A. Abdulamir, F. Abu Bakar, F.J. Abas, Z. Sekawi, *Antioxidant Research in Asia in the Period from 2000-2008*, American Journal of Pharmacology and Toxicology, 4(3), 2009, 48-66.
27. M.A. Ghareeb, A.H. Hussein, *Antioxidant and cytotoxic activities of Tectona grandis linn. Leaves*, Int. J. Phytopharm., 5 (2), 2014, 143e157.

Corresponding author: Ehab M Mostafa,

Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt.

Online publication Date:15.04.2018