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Life Science Journal

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Chemical Constituents and Biological Activity of *Forsskaolea viridis* Aerial parts

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Abstract: Chemical investigation of the chloroform and ethyl acetate extracts of the aerial parts of *Forsskaolea viridis* Ehrenb. ex Webb (Family: Urticaceae) led to isolation of eight compounds, for the first time, from this plant. Two phenolics and six flavonoid compounds were identified as *p*-coumaric and caffeic acids, 5-hydroxy-6,7,3',4'-tetramethoxy flavone, chrysoeriol, acacetin, chrysoeriol-7-O-*β*-D-glucopyranoside, kaempferol-3-O-(2''-O-E--*p*-coumaroyl)-*β*-D-glucopyranoside and isovetixin. The chemical structure of the isolated compounds was established by spectroscopic analysis UV, ^{1}H-NMR,^{13}C-NMR and MS. The antimicrobial, antioxidant and cytotoxic activities of the ethyl acetate and chloroform extracts were evaluated. The ethyl acetate extract exhibited strong antimicrobial activity (12-30 mm) against the tested strains. The ethyl acetate and chloroform extracts showed fair antioxidant and cytotoxicity.

[Fatma A. Ahmed, Amani M.D. El mesallamy, Taha A.I. El Bassossy, **Chemical Constituents and Biological Activity of *Forsskaolea viridis* Aerial parts**. *Life Sci J* 2024;21(1):18-25]. ISSN 1097-8135 (print); ISSN 2372-613X (online). http://www.lifesciencesite.com.02.doi:10.7537/marslsj210124.02.

Key words: *Forsskaolea viridis*, phenolics, flavonoids, antimicrobial, antioxidant, antitumor.

Introduction:

Now adays, man is looking for alternative medicine which extracted from wild medicinal plants due to its strong effectiveness in the treatment of many diseases without occurring side effects from them. Family *Urticaceae* (Nettle family) comprises 54 genera and more than 2000 species of herbs, shrubs, small trees, and a few vines (Changkyun *et al*., 2015). *Forsskaolea* is a small genus in the nettle family, represented by 6 species, distributed in Canary Isles and southeast Spain eastwards to Pakistan, Africa, and Arabia to Western India (Kitikar and Basu, 1975; Alfarhan *et al*., 2005). Reported activities for *Forsskalea* genus are diuretic, calculolitic, antiflu (Darias *et al*., 1986) and anti-septic (Darias *et al*., 2001).

Forsskaolea viridis Ehrenb. ex Webb is an annual or short–lived perennial herb distribute in Egypt (South east Egypt- wadi Kansisrob), Oman (Dhofar), Saudi Arabia, Yemen (Hadhramaut), Namibia, Sudan, Ethiopia, Eritrea, and Kenya (GBIF Secretariat).

The aim of this study is to investigate the chemical constituents of *F. viridis*, as well as its antimicrobial, antioxidant and antitumor activities, because there are no studies concerning isolation of the active constituents and screening biological activities for this plant.

Material and Methods:

Plant Material

Aerial parts of *F. viridis* Ehrenb. ex Webb (Family: *Urticaceae*) were collected from their wild habitat in wadi Kansisrob, Gebel Elba region, southeast corner of Egypt in January 2016. The plant specimens were identified and authenticated by Dr. Omran Ghaly, Desert Research Center. A voucher herbarium specimen was deposited in the herbarium of Desert Research Center (CAIH) with Code Number: CAIH-1000-R.

Chemicals and Instruments:

All chemicals used (butanol, ethyl acetate, methanol, toluene, chloroform …. etc) were India, Whitman 1mm, 3mm paper chromatography (Germany), silica gel 230-400 mesh (Merck) for column chromatography and Sephadex LH-20 (Merck) were used. TLC analysis was carried out using silica gel 60 F_{254} plates (Merck); chromatograms were visualized under UV light at 254 and 365 nm.

Extraction

The air-dried powdered of the *F. viridis* aerial parts (1.5kg) were extracted with 5L methanol. The combined extract was concentrated under reduced pressure to yield a sticky dark gum (170 g). The methanol extract was suspended in 500 ml water and

successively fractionated by separating funnels using pet. ether, chloroform, ethyl acetate, and methanol. Each extract was concentrated in vacuum to yield dry extracts (24g, 5g, 3.2g, 7g), respectively.

Isolation

The chloroform fraction (5g) was subjected to silica gel column chromatography (CC) eluted with hexane/ethyl acetate with increasing polarity to afford 13 sub-fractions (C1-C13). Collective fractions (C3-C5) were obtained and applied to preparative TLC with using system (CH_3Cl: MeOH 9:1) to give compound 1 (45mg) and compound 2 (39mg). The combined sub-fractions (C6-C12) were separated with preparative paper chromatography (PPC) using BuOH/AcOH/H_2O (BAW), 4:1:5 (the upper phase) and further purified with Sepahdex LH-20 column chromatography (CC) eluted with MeOH to give compound 3 (52mg) and compound 4 (37mg).

The ethyl acetate fraction (3.2g) was subjected to CC on silica gel and eluted with hexane/ethyl acetate with gradually increasing polarity to afford 14 sub-fractions (E1-E14). Similar fractions were collected according to paper chromatography (PC) manner using system (BAW 4:1:5). Sub-fractions (E2-E4) were collected and applied to TLC system (toluene: ethyl acetate: formic acid 5: 4: 1) and then subjected to Sepahdex LH-20 column chromatography (CC) to give compound 5 (21mg). Also, sub-fractions (E6-E9) were collected together and applied to PPC using BAW (4:1:5) which showed containing two major flavonoid compounds, so further purification occurred on Sepahdex LH-20 column chromatography eluted with MeOH to give compound **6** (33mg) and compound **7** (41mg). The combined sub-fractions (E11-E13) were subjected to (TLC) using system (Ethyl acetate: methanol: water 30: 5: 4) showed one major flavonoid compound, which purified on column Sephadex LH-20 using methanol as eluting system to afford compounds 8 (38mg).

Identification and structure elucidation of the isolated compounds were done by R_f values in PC, spectral data UV (Unicam UV-300 spectrophotometer) and nuclear magnetic resonance (NMR) spectra [Bruker AV-400 (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR]. The sugar moieties were identified after partial and complete acid hydrolysis using PC with authentic samples.

Antimicrobial activity

Antimicrobial activity was determined by diffusion agar technique in Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt according to CLSI (Clinical and Laboratory Standards Institute, 2004; 2012). Strains were obtained from the bacteria stock present at RCMB. Petri plates consisting of 20ml of nutrient (for bacteria) or malt extract (for fungi). Agar medium were seeded with 1-3 days cultures of microbial inoculums (standardized inoculums 1-2 X 10^7 cfu/ml 0.5 Mcfarland standard). Wells (6 mm in diameter) were cut off into agar and 100µl of plant extracts were tested in a concentration of 5mg/ml and incubated at 37˚C for 24hr. (bacterial strains) and at 25°C for 7 days (fungal strains). The calculation of antimicrobial activity was based on measurement of the diameter of the inhibition zone formed around the well. Positive control used for fungi was ketoconazole with MIC 100 mg/ml, while positive control used for bacteria strains was gentamycin with MIC 4 mg/ml.

Antioxidant activity (DPPH assay):

The free radical scavenging effect of plant extracts was assessed by the decolouration solution of DPPH radical according to (Letelier *et al.*, 2008) in Regional Center for Mycology and Biotechnology Al-Azhar University, Cairo, Egypt (RCMB). This assay was realized essentially by the method described by (Joyeux *et al.*, 1995) and its modification by (Viturro *et al.*, 1999). In a final volume of 1ml, the reaction mixture contained 20 µg/ml of DPPH (ethanol solution) and different concentrations of chloroform and ethyl acetate extracts. Blanks contained only ethanol and plant extract. DPPH bleaching activity of all mixtures was measured continuously at 37 ˚C for 20 min to 517 nm in a Unicam UV-300 UV-VIS Spectrophotometer. Rates of reaction were determined at conditions where product formation was linearly dependent to time and protein concentration. DPPH bleaching activity was expressed as Δ absorbance/20min, which corresponds to the difference between the initial (0 min) and final absorbance (20 min). Ascorbic acid was used as positive control.

Cytotoxic activity (Viability assay)

The human breast (MCF-7) and colon (Caco-2) carcinoma cell lines were obtained in frozen state under liquid nitrogen (-180ºC) from the American Type Culture Collection. The tumor cell lines were conserved by serial sub-culturing in the National Cancer Institute, Cairo, Egypt.

The cytotoxic effect of chloroform and ethyl acetate extracts were evaluated in the National Cancer Institute, Cairo University according to MTT assay method (Mosmann, 1983). Briefly, cells were seeded in 96 well plate at a density of 5000 cells/well in 100µl culture medium. Following 24h incubation, cells were treated with various concentrations of chloroform and ethyl acetate extracts and then incubated for 24h. at 37˚C with 5% CO_2. After incubation, medium was replaced with 100µl of MTT solution prepared fresh as 0.5mg/ml in Dulbecco's Modified Eagle's medium

(DMEM), filtered through a -0.22µm filter, then it was added to each well, and the plates were incubated in the dark for 4h at 37°C. Then, the media were removed and 200µl of dimethylsulphoxide (DMSO) was added to each well and absorbance was measured at 570 nm using a microplate reader. The results are expressed as the percentage of cell viability in comparison with the control cells (Cells without extracts). The cell viability of the control group without exposure to the extracts was defined as 100%.

Results and discussion:

Chloroform and ethyl acetate fractions of aerial parts of *F. viridis* revealed potent antimicrobial activity, so they were subjected for further phytochemical investigations on silica gel (230-400 mesh) column chromatography followed by preparative paper chromatography and Sephadex LH-20 column chromatography for isolation of bioactive secondary metabolites.

Identification and structure elucidation of the purified phenolic and flavonoid compounds were done by comparison, R_f values, UV,^{1}H-NMR,^{13}C-NMR spectral data with the reported data in literature.

Compound 1 was isolated from chloroform sub-fractions (C3-C5) as yellowish powder which exhibited *Mr* of 164.04 in ESI-MS analysis ($[M+H]^+$ at *m/z* = 165.04, $[M+H-H_2O]$ at *m/z* =147.04), R_f 0.84 (BAW), 0.42 (6% AcOH). UV λ_{max} (nm): (MeOH) 209, 220, 286. ^{1}H-NMR (DMSO-d_6): δ 7.63 (1H, d, *J* = 15 Hz, H-7), 7.35 (2H, d, *J* = 8 Hz, H-2 and H-6), 6.69 (2H, d, *J* = 8 Hz, H-3 and H-5), 6.15 (1H, d, *J* = 15 Hz, H-8). ^{13}C-NMR (DMSO-d_6): δ 170.95 (C-9), 160.90 (C-4), 146.45 (C-7), 131.09 (C-2 and C-6), 127.19 (C-1), 114.32 (C-3 and C-5). Compound 1 was identified as *ρ*-Coumaric acid. The spectroscopic data of compound 1 were harmony with physical and spectral data reported by (Si *et al*., 2011).

Compound 2 was isolated from collective chloroform sub-fractions (C3-C5) as yellow amorphous crystals which exhibited *Mr* of 180.04 in ESI-MS analysis ($[M+H]^+$ at *m/z* = 181.04 , $[M+H-H_2O]$ at *m/z* =163.04), R_f 0.81 (BAW), 0.45 (6% AcOH). UV λ_{max} (nm): (MeOH) 216, 245, 294, 326. ^{1}H-NMR (DMSO-d_6): δ 7.54 (1H, d, *J* = 15Hz, H-7), 7.08 (1H, s, H-2), 6.96 (1H, d, *J* = 8 Hz, H-6), 6.76 (1H, d, *J* = 8 Hz, H-5), 6.22 (1H, d, *J* = 15 Hz, H-8). ^{13}C-NMR (DMSO-d_6): δ 171.1 (C-9), 149.5 (C-4), 147.0 (C-7), 146.8 (C-3), 127.6 (C-1), 122.8 (C-6), 116.5 (C-5), 115.4 (C-2), 115.3 (C-8). Compound 2 was identified as Caffeic acid by comparison of the physical and spectral data with the reported data (Eun *et al*., 2010).

Compound 3 was isolated from collective chloroform sub-fractions (C6-C12) as yellow needles which exhibited *Mr* of 358.1 in ESI-MS analysis ($[M+H]^+$ at *m/z* = 359.1, $[M-CH_3]^+$ at *m/z* =343), R_f 0.78 (BAW), 0.04 (15% AcOH). UV λ_{max} (nm): (MeOH) 275, 337; ($AlCl_3$) 259 (sh), 285, 365; ($AlCl_3$/HCl) 257 (sh), 287, 359; (NaOAc) 280, 325; (NaOAc/H_3BO_3) 278, 337. ^{1}H-NMR (DMSO-d_6): δ 7.87 (1H, dd, *J* = 8.5, 2.2 Hz, H-6'), 7.61 (1H, d, *J* = 2.2 Hz, H-2'), 7.10 (1H, d, *J*= 8.5 Hz, H-5'), 6.91(1H, s, H-8), 6.77 (1H, s, H-3), 3.8-3.95 (12 H, OMe).^{13}C-NMR (DMSO-d_6): δ 182.88 (C-4), 165.10 (C-2), 160.01 (C-7), 154.49 (C-5), 154.35 (C-9), 154.20 (C-4'), 150.58 (C-3'), 133.24 (C-6), 124.54 (C-1'), 121.14 (C-6'), 111.95 (C-5'), 110.77 (C-2'), 106.55 (C-10), 104.58 (C-3), 91.90 (C-8), 60.33 (6-OMe), 57.10 (7-OMe), 56.55 (3'-OMe), 56.25 (4'-OMe). Compound 3 was characterized as 5-hydroxy-6,7,3',4'-tetramethoxyflavone by comparison of the physical and spectral data with the reported data (Seo *et al*., 2003; Zhao *et al*., 2012).

Compound 4 was isolated from collective chloroform fractions (C6-C12) as yellow powder exhibited *Mr* of 300 in ESI-MS analysis ($[M+H]^+$ at *m/z* = 301, $[M-CH_3]^+$ at *m/z* = 285.03), R_f 0.82 (BAW), 0.05 (15 % AcOH). UV λ_{max} (nm): (MeOH) 244, 349; (NaOMe) 266, 407; ($AlCl_3$) 263, 276, 390; ($AlCl_3$/HCl) 257, 275, 388; (NaOAc) 273, 323; (NaOAc/H_3BO_3) 268, 348; ^{1}H-NMR (DMSO-d_6) δ 7.76 (1H, d, *J* = 8.4, 2.2 Hz, H-2'), 7.61 (2H, dd, *J* = 8.4, 2.2 Hz, H-6'), 7.20 (1H, d, *J* = 8.4 Hz, H-5'), 6.98 (1H, s, H-3), 6.78 (1H, s, H-8), 6.54 (1H, s, H-6), 3.89 (3H, s, 4'-OCH_3); ^{13}C-NMR (DMSO-d_6) δ 181.9 (C-4), 166.1 (C-2), 162.8 (C-5),161.8 (C-7), 159.8 (C-9), 152.0 (C-4'), 149.1 (C-3'), 124.5 (C-1'), 121.4 (C-6'), 116.7 (C-5'), 110.9 (C-2'), 105.9 (C-10), 104.6 (C-3), 101.2 (C-6), 94.9 (C-8), 56.45 (OCH_3). Compound 4 was identified as Chrysoeriol by comparison of the physical and spectral data with the reported data (Jin *et al*., 2013).

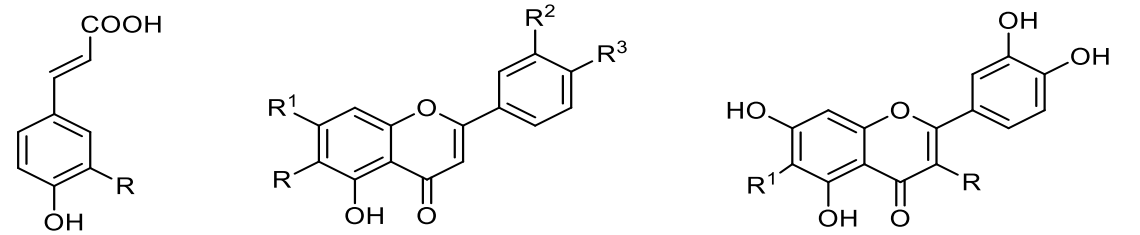
Compound 5 was isolated from ethyl acetate collective sub-fractions (E2-E4) as yellow crystals *Mr* of 284 in ESI-MS analysis ($[M+H]^+$ at *m/z* = 285, $[M-H_2O]^+$ at *m/z* = 267), R_f 0.91 (BAW), 0.12 (15% AcOH). UV λ_{max} (nm): (MeOH) 268, 326; (NaOMe) 274, 366; ($AlCl_3$) 257 (sh), 279, 380; ($AlCl_3$/HCl) 257 (sh), 287, 359; (NaOAc) 280, 325; (NaOAc/H_3BO_3) 278, 337. ^{1}H-NMR (DMSO-d_6) δ 8.11 (2H, d, *J* = 9.0, H-2' and H-6'), 7.20 (2H, d, *J* = 9.0, H-3' and H-5'), 6.80 (1H, d, *J* = 2.5, H-6), 6.72 (1H, s, H-3), 6.50 (1H, d, *J* = 2.5, H-8), 3.82 (3H, s, 4'-OCH_3). ^{13}C-NMR (DMSO-d_6) δ 181.7 (C-4), 163.8 (C-7), 162.8 (C-2), 162.3 (C-5), 161.0 (C-4'), 156.8 (C-9), 128.2 (C-2' and C-6'), 122.6 (C-1'), 114.5 (C-3' and C-5'), 104.9 (C-10), 103.6 (C-3), 100 (C-6), 94.7 (C-8), 56.8 (OCH_3). Compound 5 was identified as Acacetin by comparison of the physical and spectral data with the reported in

the literature (Mabry *et al.*, 1970; Agrawal and Rastogi, 1981; Harborne and Mabry, 1982).

Compound 6 was isolated from ethyl acetate collective sub-fractions (E6-E9) as yellow powder *Mr* of 462 in ESI-MS analysis ($[M-H]^-$ at *m/z* = 461, $[M-CH_3]^-$ at *m/z* = 446), R_f 0.42 (BAW), 0.12 (15% AcOH). UV λ_{max}; (MeOH) 254, 269 (sh), 345; (NaOMe) 245, 265, 305, 390; ($AlCl_3$) 272, 300 (sh), 353, 390; ($AlCl_3$/HCl) 276, 303, 352, 390; (NaOAc) 250, 266, 349; (NaOAc/H_3BO_3) 250, 266, 346. ^{1}H-NMR (DMSO-d_6); Chrysoeriol moiety: 7.76 (1H, d, *J* = 8.4, 2.2 Hz, H-2'), 7.61 (2H, dd, *J* = 8.4, 2.2 Hz, H-6'), 7.20 (1H, d, *J* = 8.4 Hz, H-5'), 6.98 (1H, s, H-3), 6.78 (1H, s, H-8), 6.54 (1H, s, H-6), 3.89 (3H, s, 4'-OCH_3); glucosyl moiety: δ 5.01 (1H, d, *J*= 8.2 Hz, H-1"), 3.2-3.8 (5H, m). ^{13}C-NMR (DMSO-d_6): Chrysoeriol moiety: δ 181.9 (C-4), 166.1 (C-2), 162.8 (C-5), 161.8 (C-7), 159.8 (C-9), 152.0 (C-4'), 149.1 (C-3'), 124.5 (C-1'), 121.4 (C-6'), 116.7 (C-5'), 110.9 (C-2'), 105.9 (C-10), 104.6 (C-3), 101.2 (C-6), 94.9 (C-8), 56.45 (OCH_3); glucosyl moiety: δ 98.21 (C-1"), 81.12 (C-5"), 75.96 (C-3"), 72.60 (C-2"), 68.84 (C-4"), 61.58 (C-6"). Compound 6 was identified as Chrysoeriol-7-O-*β*-D-glucopyranoside, the spectroscopic data were identical with the reported data in (Harput *et al*., 2006).

Compound 7 was isolated from ethyl acetate collective sub-fractions (E6-E9) as yellow powder exhibited *Mr* of 594.1 in ESI-MS analysis ($[M+H]^+$ at *m/z* = 595.1, $[M-H_2O]^+$ at *m/z* = 577.1), R_f 0.61 (BAW), 0.32 (15% AcOH). UV λ_{max} nm (MeOH): 245, 266, 296 (sh), 314, 360 (sh).^{1}H-NMR (DMSO- d_6) δ kaempferol moiety: 8.56 (2H, d, *J* = 9 Hz, H-2' and C-6'), 6.88 (2H, d, *J* = 9 Hz, H-3'and C-5'), 6.36 (1H, s, H-8), 6.20 (1H, s, H-6); *p*-coumaroyl moiety: 7.61 (1H, d, *J* = 16 Hz, H-7'"), 7.54 (2H, d, *J* = 9 Hz, H-2'", H-6'"), 6.83 (2H, d, *J* = 9 Hz, H-3'", H-5'"), 6.41 (1H, d, *J* = 16 Hz, H-8'") glucosyl moiety: 5.40 (1H, d, *J* = 9 Hz, H-1"), 4.90 (1H, d, H-2"), 3.22-3.79 (5H, m). ^{13}C-NMR (DMSO-d_6) kaempferol moiety: δ 177.2 (C-4), 165.0 (C-7), 160.3(C-4'), 159.9 (C-9), 156.2 (C-2), 156.0 (C-5), 133.1 (C-3), 131.1 (C-2', 6'), 121.0 (C-1'), 115.5 (C-3' and C-5'), 103.6 (C-10), 98.5(C-6), 93.5 (C-8); *p*-coumaroyl moiety: δ 165.8 (C-9'"), 159.9 (C-4'"), 145.0 (C-7'"), 130.2 (C-2'" and C-6'"), 125.1 (C-1'"), 115.2 (C-3'" and C-5'"), 114.3 (C-8'"); glucosyl moiety: δ 99.2 (C-1"), 76.9 (C-3"), 74.0 (C-2", 5"), 70.1 (C-4"), 60.6 (C-6"). Compound 7 was characterized as kaempferol-3-O-(2"-O-*E*-*p*-coumaroyl)-*β*-D-glucopyranoside. The spectroscopic data were harmony with the reported data (Hohmann *et al.*, 1997; Wei-Ku *et al.*, 2012).

Compound 8 was isolated from ethyl acetate collective sub-fractions (E11-E13) as yellow powder *Mr* of 432 in ESI-MS analysis ($[M+H]^+$ at *m/z* = 433.1, $[M+ Na^+]^+$ at *m/z* = 455.05), R_f 0.56 (BAW), 0.54 (15% AcOH). UV λ_{max} (nm): (MeOH) 273, 330; (NaOMe) 277, 331, 394; ($AlCl_3$) 266, 301, 348, 370; ($AlCl_3$/HCl) 262, 303, 343, 381; (NaOAc) 279, 389; (NaOAc/H_3BO_3) 271, 319, 346. ^{1}H-NMR (DMSO-d_6): apigenin moiety: δ 7.84 (2H, d, *J*= 8.6Hz; H-2' and H-6'), 6.82 (2H, d, *J*= 8.7 Hz; H-3' and H-5'), 6.65 (1H, s; H-3), 6.42 (1H, d, *J*= 2.0 Hz; H-8); glucosyl moiety: δ 4.53 (1H, d, *J*= 9.2 Hz; H-1"), 3.2-3.8 (5H, m). ^{13}C-NMR (DMSO-d_6): apigenin moiety: δ 183.01(C-4), 164.43 (C-2), 164.22 (C-7), 161.88 (C-4'), 161.32 (C-5), 156.88 (C-9), 129.34 (C-2' and C-6'), 122.05 (C-1'), 117.12 (C-3' and C-5'), 109.70 (C-6), 104.33 (C-3), 103.45 (C-10), 94.67 (C-8); glucosyl moiety: δ 83.12 (C-5"), 79.69 (C-3"), 74.21 (C-1"), 72.30 (C-2"), 71.28 (C-4"), 61.88 (C-6"). Compound 8 was characterized as Isovitexin by comparison of the physical and spectral data with the reported data (Mabry *et al.*, 1970; Luzzatto *et al*., 2007).



1 R = H
2 R = OH

	R	R^1	R^2	R^3
3	OCH_3	OCH_3	OCH_3	OCH_3
4	H	OH	OCH_3	OH
5	H	OH	H	OCH_3
6	H	*O*-Glu	OCH_3,	OH

7 R = *O*-(2"-*O*-*E*-*p*-coumaroyl)-*β*-D-glucopyranoside, R^1= H
8 R = H, R^1= Glu

Figure 1. Chemical structures of the isolated compounds (1-8) from *F. viridis* aerial parts.

Antimicrobial activity

The antibacterial and antifungal activities of chloroform and ethyl acetate extracts of *F. viridis* aerial parts were carried out by diffusion agar technique. Mean zone of inhibition in mm produced on a range of pathogenic microorganisms were measured and the results were recorded in table (1). It showed that chloroform extract has moderate antimicrobial activity against all tested strains exception *Candida albicans*, *Penicillium expansum* and *Aspergillus fumigatus* showed no activity. On the other hand, ethyl acetate extract exhibited higher potent antimicrobial activity than Gentamycin and Ketoconazole against all tested strains except *Micrococcus* sp., *Escherichia coli* and *Candida albicans* which showed ethyl acetate had good potent activity against these strains but less than Gentamycin and Ketoconazole.

These results were agreed with those obtained by (Assaf *et al.*, 2015) who reported that the ethyl acetate fraction of *Forsskaolea tenacissima* had potent antimicrobial activity against tested strains. Also, these results are agreed with antimicrobial studies occurred on the other plants belonging to Urticaceae family which proved the activity of chloroform, ethyl acetate and methanol extracts against viral, fungal and bacterial strains (Ibrahim *et al.*, 2018).

Table 1: The antimicrobial activity of chloroform and ethyl acetate extracts of *F. viridis* aerial parts against different bacterial and fungal strains.

Tested Organism	**Control**	**$CHCl_3$**	**EtOAc**
Gram Positive Bacteria	Gentamycin		
Micrococcus sp. (RCMB 028)s	22	10	19
Streptococcus mutants (RCMB 017) (ATCC 25175)	21	11	21
Methicillin-Resistant *Staphylococcus aureus*	15	8	20
Gram Negative Bacteria	Gentamycin		
Salmonella typhrimurium (RCMB 006) (ATCC 14028)	17	13	20
Escherichia coli (RCMB 010052) (ATCC 25955)	30	12	24
Klebsiella pneumonia (RCMB 003) (ATCC 13883)	21	11	22
Filamentous Fungi	ketoconazole		
Aspergillus fumigatus (RCMB 002008)	17	NA	20
Penicillium expansum (RCMB 001001)	17	NA	19
Yeasts	ketoconazole		
Candida albicans (RCMB 005003) (ATCC 10231)	20	4	16
Cryptococcus neoformans (RCMB 0049001)	25	16	30

NA: No activity; Positive control for fungi: ketoconazole (MIC) 100mg/ml. Positive control for bacteria: Gentamycin (MIC) 4mg/ml; RCMB: Regional Center for Mycology and Biotechnology in Cairo, Egypt, Al-Azhar University; ATCC: American Type Culture Collection.

Antioxidant activity:

DPPH scavenging method were utilized to evaluate the antioxidant activity of the chloroform and ethyl acetate extracts of *F. viridis* aerial parts using ascorbic acid as reference standard and the results were summarized in table (2). Chloroform and ethyl acetate extracts at concentrations (25, 50, 100, 200, 400, 800, 1200 and 3200µl) exhibited antioxidant capacity (17.80 - 92.75%) with IC_{50} 118.7µg/ml for chloroform and (45.49 - 93.30%) with IC_{50} 32.1µg/ml for ethyl acetate extract.

These results showed that ethyl acetate extracts showed moderate antioxidant activity. On the other hand, chloroform extract showed weak or no antioxidant activity.

Table 2: Antioxidant activity of chloroform and ethyl acetate extracts of *F. viridis* compared to ascorbic acid.

Conc (µg/ml)	Chloroform extract	Ethyl acetate extract	Conc (µg/ml)	Ascorbic acid
0	0	0	0	0
25	17.80	45.49	5	11.78
50	31.32	61.32	10	17.49
100	46.37	68.57	15	54.86
200	65.82	77.80	20	70.94
400	79.45	86.15	25	77.41
800	87.80	90.00	30	80.65
1600	91.32	91.76	35	87.53
3200	92.75	93.30	40	92.48
IC_{50}	118.7	32.1		14.2

Antitumor activity

The antitumor activity of chloroform and ethyl acetate extracts of *F. viridis* aerial parts was *in vitro* assessed against Caco-2 and MCF7 cell lines.

Table 3: Antitumor activity of chloroform and ethyl acetate of *F. viridis* aerial parts against breast (MCF7), colon (Caco2), hepatic (HepG2) and normal (Vero) cell lines.

Conc. µg/ml	Viability % of chloroform extract				Viability % of ethyl acetate extract			
	Vero	Caco-2	MCF7	HepG2	Vero	Caco-2	MCF7	HepG2
39.06	97.76	40.65	83.95	94.40	100.00	75.38	98.14	100.00
78.1	72.57	16.03	40.79	49.05	99.08	55.52	99.89	98.62
152.2	24.67	8.01	17.81	28.15	57.08	41.12	91.51	81.75
312.5	10.23	4.30	6.73	5.91	18.76	30.66	46.89	38.18
625	6.29	4.99	4.66	4.85	9.44	25.78	13.56	24.89
1250	6.29	4.30	4.35	4.53	6.69	11.27	6.32	7.27
2500	5.64	3.83	4.76	4.00	5.11	5.69	5.28	6.11
5000	5.11	3.83	4.04	3.69	4.98	3.95	4.76	4.43
10000	3.67	3.83	3.42	3.05	4.33	3.37	4.14	3.27
IC_{50}	115.22	40.6	69.95	105.82	205.92	368.63	148.9	271.84

The chloroform extract showed moderate antitumor activity for breast (MCF7) carcinoma cell line with IC_{50} 69.95µg/ml with effective about 39.2% compared to normal cell line, potent antitumor activity for colon (Caco-2) carcinoma cell line with IC_{50} 40.6µg/ml with effective about 64.7% and weak activity for hepatic (HepG2) carcinoma cell line with IC_{50} 105.82µg/ml with effective about 8.15%. On the other hand, the ethyl acetate extract showed no activity for colon (Caco-2) and hepatic (HepG2) carcinoma cell lines and weak activity for breast (MCF7) carcinoma cell line with IC_{50} 148.9µg/ml with effective about 27.6%.

American Cancer Institute (NCI) reported that, the criteria of cytotoxic activity for the crude extract is an IC_{50}< 20µg/ml. (Boik, 2001). So Only chloroform extract had moderate cytotoxic activity for both colon and breast carcinoma cell line.

These obtained results were agreed with some studies occurred on other plants of the family

Urticaceae which showed moderate cytotoxic activity of both chloroform and ethyl acetate fractions of *Urtica* species (El-Mokasabi, 2014).

Conclusion:

The ethyl acetate extracts exhibited strong potent for antimicrobial activity against all tested bacteria & fungi strains, moderate antioxidant activity and weak antitumor activity for breast carcinoma cell line. While the chloroform extract had moderate antimicrobial activity against all tested strains except *Candida albicans*, *Penicillium expansum* and *Aspergillus fumigatus* which showed no activity, weak antioxidant activity compared to ascorbic acid activity and exhibited potent antitumor activity for colon carcinoma cell line and moderate activity for breast carcinoma cell line.

The antimicrobial activity of *F. viridis* ethyl acetate extract may be due to the isolated compounds which represented as flavonoid nature that considered rich with antioxidant, antimicrobial and antitumor activity (Singh *et al.*, 2005 and He *et al.*, 2016). The biological activity for both chloroform and ethyl acetate extracts may be related to chemical constituents where they contained on flavonoids and phenolic compounds which considered to potent antioxidant, antimicrobial and cytotoxicity activities.

References:

[1]. Agrawal PK, Rastogi RP. ^{13}C-NMR Spectroscopy of flavonoids. Heterocycles (1981); 16(12): 2181-2236. DOI: 10.3987/R-1981-12-2181

[2]. Alfarhan AH, Al-Turki TA, Basahy AY. In "Flora of Jizan Region". Vol. 1, King Abdul Aziz City for Science and Technology (KACST). (2005)

[3]. Assaf HK, Nafady AM, Mohamed SA, Ahmed EA, Mohamed SK. Phytochemical and Biological Studies of Aerial Parts of *Forsskaolea tenacissima* Linn. (Urticaceae). Journal of Pharmacognosy and Phytochemistry (2015); 4(3): 282-290.

[4]. Boik J. Natural compounds in Cancer Therapy. Oregon Medical Press, Minnesota, USA. (2001): 96.

[5]. Changkyun K, Tao D, Mark C, Dai-Gui Z, Ze-Long N, Hang S. Generic phylogeny and character evolution in Urticeae (Urticaceae) inferred from nuclear and plastid DNA regions. Taxon (2015); 64(1): 65-78.

[6]. CLSI Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts, Approved Guideline CLSI document M44-A. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania, USA. (2004): 19087-1898.

[7]. CLSI Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed. CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania, 19087, USA. (2012).

[8]. Darias V, Bravo L, Barquin E, Martin HD, Fraile C. Contribution to the ethnophmacological study of the Canary Island. J. Ethnophamacol (1986); 15: 169-193.

[9]. Darias V, Martín-Herrera D, Abdala S, Fuente D. Plants used in urinary pathologies in the Canary Islands. Pharm. Biol. (2001); 39: 170-180.

[10]. El-Mokasabi F. Floristic composition and traditional uses of plant species at Wadi Alkuf, Al-Jabal Al-Akhder, Libya. American-Eurasian J. Agric. and Environ. Sci. (2014); 14(8): 685–697.

[11]. Eun JL, Kim JS, Kim HP, Lee JH, Kang SS. Phenolic constituents from the flower buds of *Lonicera japonica* and their 5-lipoxygenase inhibitory activities. Food Chem. (2010);120: 134–139.

[12]. GBIF Secretariat: GBIF Backbone Taxonomy. doi:10.15468/39omei Accessed via http://www.gbif.org/species/4101865 on 2016-06-09

[13]. Harborne JB and Mabry TJ In "The Flavonoids: Advances in Research". Springer. (1982). DOI: 10.1007/978-1-4899-2915-0

[14]. Harput US, Ihsan C, Saracoglu I, Ali AD, Nagatsu A. Secondary metabolites from *Phlomis syriaca* and their antioxidant activities. Turk. J. Chem. (2006); 30: 383-390.

[15]. He M, Min WJ, Kong LW, Xiao H, Jun L, Peng WA review on the pharmacological effects of vitexin and isovitexin. Fitoterapia (2016); 115: 74–85.

[16]. Hohmann J, Pall Z, Guenther G, Máthé I. Flavonol acyl glycosides of the aerial parts of Eryngium campestre. J. Planta Med. (1997); 63(1): 96-102. DOI: 10.1055/s-2006-957620.

[17]. Ibrahim M, Kanwal R, Aneeza R, Hussain I, Farooq T, Hussain H, Muhammad S. Investigations of phytochemical constituents and their pharmacological properties isolated from the genus *Urtica*: critical review and analysis. Critical Reviews in Eukaryotic Gene Expression (2018); 28(1): 25–66.

[18]. Jin HL, Park KH, Myoung HL, Hyun TK, Woo DS, Jun YK, Baek IY, Dae SJ, Tae JH. Identification, characterization and quantification of phenolic compounds in the antioxidant activity-containing fraction from the seeds of Korean perilla (*Perilla frutescens*)

cultivars. Food Chemistry (2013); 136: 843–852.

[19]. Joyeux M, Lobstein A, Anton R, Mortier F. Comparative antilipo peroxidant, antinecrotic and scavenging properties of terpenes and biflavones from Ginkgo and some flavonoids. Planta Med. (1995); 61(2): 126-129.

[20]. Kitikar KR, Basu BD. In "An ICS Indian Medical Plants", Bishen Singh & Mahendrapal Singh, New Delhi, 2nd edition, (1975); (3): 2291-2298.

[21]. Letelier ME, Molina BA, Cortés TJ, Jara SJ, Holst M, Palma K, Montoya M, Miranda D, González V. DPPH and oxygen free radicals as pro-oxidant of biomolecules. Toxicology in Vitro (2008); 22: 279-286.

[22]. Luzzatto T, Golan A, Yishay M, Bilkis I, Ben-Ari J, Yedidia I. Priming of antimicrobial phenolics during induced resistance response towards *Pectobacterium carotovorum* in the ornamental monocot calla lily. J. Agric. Food Chem. (2007); 55: 10315-10322. DOI: 10.1021/jf072037.

[23]. Mabry TJ, Markham KR, and Thomas MB. In " The Systematic Identification of Flavonoids". Berlin: Springer. (1970). DOI:10.1007/978-3-642-88458-0

[24]. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol Methods (1983); 65: 55-63.

[25]. Singh PR, Agrawal P, Yim D, Agarwal C, Agarwal R. Acacetin inhibits cell growth and cell cycle progression, and induces apoptosis in human prostate cancer cells: structure--activity relationship with linarin and linarin acetate. Carcinogenesis (2005); 26(4): 845-854.

[26]. Seo JM, Kang HM, Son KH, Kim JH, Lee CW, Kim HM, Chang SI, Kwon BM. Antitumor activity of flavones isolated from *Artemisia argyi*. Planta Med. (2003); 69(3): 218-222. DOI: 10.1055/s-2003-38486.

[27]. Si CL, Lu YY, Zhang Y, Xu J, Qin PP, Sun RC, Ni YH. Antioxidative low molecular weight extractives from triploid *Populus tomentosa* xylem. BioRes. (2011); 6(1): 232-242.

[28]. Viturro C, Molina A, Schmeda HG. Free radical scavengers from *Mutisia friesiana* (Asteraceae) and *Sanicula graveolens* (Apiaceae). Phytother. Res. (1999); 13(5): 422-424.

[29]. Wei KZ, Jie KU, LiZ K, Guan DU. Flavonoids from the bran of *Avena sativa*. Chinese Journal of Natural Medicines (2012); 10(2): 110-114. DOI: 10.3724/SP.J.1009.2012.00110

[30]. Zhao HY, Yang L, Wei J, Huang M, Jiang JG. Bioactivity evaluations of ingredients extracted from the flowers of *Citrus aurantium* L. var. *amara* Engl. Food Chem. (2012); 135(4): 2175-2181. DOI: 10.1016/j.foodchem.2012.07.018 .

1/12/2024