Acta Poloniae Pharmaceutica – Drug Research, Vol. 76 No. 5 pp. 815–823, 2019
DOI: 10.32383/appdr/108519

ISSN 0001-6837
Polish Pharmaceutical Society

PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL EVALUATION OF *FORSSKAOLEA VIRIDIS* AERIAL PARTS

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Abstract: The chemical investigation of the chloroform and ethyl acetate extracts of the aerial parts of *Forsskaolea viridis* Ehrenb. ex Webb (Family: *Urticaceae*) led to isolation eight compounds for the first time, from the plant where two phenolic compounds, identified as *ρ*-coumaric and caffeic acids as well as six flavonoid compounds identified as 5-hydroxy-6,7,3',4'-tetramethoxy flavone, chrysoeriol, acacetin, chrysoeriol-7-O-*β*-D-glucoside, kaempferol-3-*O*-(2''-*O*-E—*p*-coumaroyl)-*β*-D-glucoside and isovetixin. The chemical structure of the isolated compounds was established by spectroscopic methods including UV, MS, ^{1}H-NMR, and ^{13}C-NMR. 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.

Keywords: *Forsskaolea viridis*, phenolics, flavonoids, antimicrobial, antitumor

Nowadays, man is looking for alternative medicine which extracted from wild medicinal plants due to its strong effect in the treatment of many diseases without any side effects. Family *Urticaceae* comprises 54 genera and more than 2000 species of herbs, shrubs, small trees, and a few vines (1). *Forsskaolea* is a small genus in *Urticaceae* family, represented by 6 species, distributed in Canary Isles and southeast Spain eastwards to Pakistan, Africa, and Arabia to Western India (2, 3). Reported activities for *Forsskaolea* genus are diuretic, cellulolytic, antiflu (4) and antiseptic (5).

Forsskaolea viridis Ehrenb. ex Webb is an annual or short–lived perennial herb distributed in Egypt (Southeast Egypt- wadi Kansisrob), Oman (Dhofar), Saudi Arabia, Yemen (Hadhramaut), Namibia, Sudan, Ethiopia, Eritrea, and Kenya (6).

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.

EXPERIMENTAL

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 samples were identified and authenticated by Dr. Omran Ghaly, Desert Research Center. A voucher herbarium sample was deposited in the herbarium of Desert Research Center (CAIH) with Code Number: CAIH-1000-R.

Extraction

The air-dried powdered of the *F. viridis* aerial parts (1.5 kg) were extracted by successive solvent extraction, beginning with using petroleum ether, chloroform, ethyl acetate and finally with methanol. Each extract was concentrated in vacuum to yield dry extracts (24 g, 8 g, 5.2 g, 13 g), respectively.

Isolation

The chloroform fraction (8 g) was separated by silica gel column chromatography (CC) eluted with

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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** (45 mg) and compound **2** (39 mg). 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 Sephadex LH-20 CC eluted with MeOH to give compound **3** (52 mg) and compound **4** (37 mg).

The ethyl acetate fraction (5.2 g) was subjected to CC on silica gel and eluted with chloroform/ethyl acetate with gradually increasing polarity till 100% MeOH to afford 14 sub-fractions (E1-E14), each fraction 100 ml. Similar fractions were collected according to paper chromatography (PC) manner using system (BAW 4 : 1 : 5). Sub-fractions (E2-E4) by eluting system (chloroform/ethyl acetate 2 : 8) were collected and applied to TLC system (toluene/ethyl acetate/formic acid 5 : 4 : 1) and then subjected to Sephadex LH-20 CC to give compound **5** (21 mg). Also, sub-fractions (E6-E9) by an eluting system (EtOAc/MeOH 4 : 6) were collected together and applied to PPC using BAW (4 : 1 : 5) which showed containing two major flavonoid compounds, so further purification occurred on Sephadex LH-20 CC eluted with MeOH to give compound **6** (33 mg) and compound **7** (41 mg). The combined sub-fractions (E11-E13) by eluting system (EtOAc/MeOH 2 : 8) 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 CC using methanol as eluting system to afford compounds **8** (38 mg).

Identification and structure elucidation of the purified 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 by partial and complete acid hydrolysis using PC with authentic samples. For column chromatography, silica gel 230-400 mesh (Merck) 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.

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 (7, 8). The bacterial (*Micrococcus* sp., *Streptococcus mutants* and *Methicillin-resistant Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumonia,* and *Salmonella typhimurium*) and the fungal strains: (*Cryptococcus neoformans*, *Candida albicans*, *Penicillium expansum,* and *Aspergillus fumigatus*) were obtained from the bacteria stock present at RCMB. Petri plates consisting of 20 ml of nutrient (for bacteria) or malt extract (for fungi). Agar medium was 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 100 µL of plant extracts were tested in a concentration of 5 mg/ml and incubated at 37^{o}C for 24 h. (bacterial strains) and at 25^{o}C for 7 days (fungal strains). The evaluation of antimicrobial activity was based on the measurement of the diameter of the inhibition zone formed around the well. The 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 decoloration solution of DPPH radical according to (9) in Regional Center for Mycology and Biotechnology Al-Azhar University, Cairo, Egypt (RCMB). This assay was realized essentially by the method described by (10) and its modification by (11). In a final volume of 1 ml, 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^{o}C for 20 min to 517 nm in a Unicam UV-300 UV-VIS Spectrophotometer. The rates of reaction were determined at conditions where product formation was linearly dependent on time and protein concentration. DPPH bleaching activity was expressed as Δ absorbance/20 min, which corresponds to the difference between the initial (0 min) and final absorbance (20 min). Ascorbic acid was used as the positive control.

Cytotoxic activity (Viability assay)

The human breast (MCF-7) and colon (Caco-2) carcinoma cell lines were obtained in a frozen state under liquid nitrogen (-180^{o}C) 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 the MTT assay method (12). Briefly, cells were seeded in 96 well plates at a density of 5000 cells/well in the 100 µl culture medium. Following 24 h incubation, cells were treated with various concentrations of chloroform and ethyl acetate extracts and then incubated for 24 h at 37°C with 5% CO_2. After incubation, the medium was replaced with 100 µL of MTT solution prepared fresh as 0.5 mg/ml in Dulbecco's Modified Eagle's medium (DMEM), filtered through 2 µm filter, then it was added to each well, and the plates were incubated in the dark for 4 h at 37°C. Then, the media were removed and 200 µl 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 CC 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 the literature.

Compound **1** was isolated from the 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 the physical and spectral data reported by (13).

Compound **2** was isolated from the 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 those reported data in (14).

Compound **3** was isolated from the collective chloroform sub-fractions (C6-C12) as yellow needles 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 (15, 16).

Compound **4** was isolated from the collective chloroform sub-fractions (C6-C12) as yellow powder which 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 (17).

Compound **5** was isolated from the ethyl acetate collective sub-fractions (E2-E4) as yellow crystals which exhibited *Mr* of 284 in ESI-MS

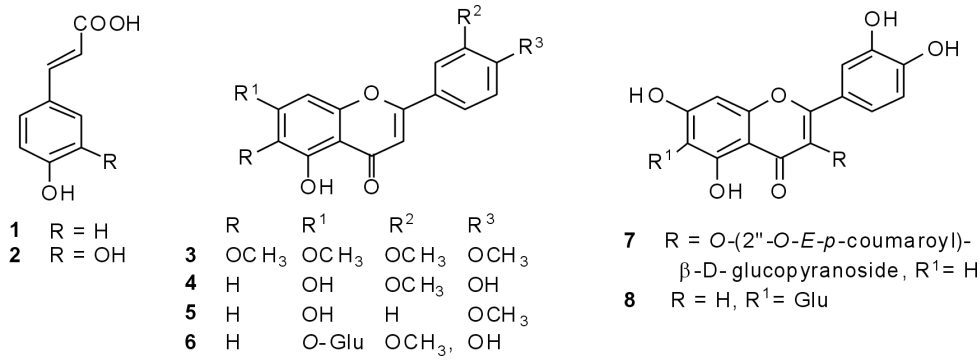
analysis ($[M + H]^+$ at m/z = 285, $[M - H_2O]^+$ at m/z = 267); R_f 0.91 (BAW), 0.12 (15% AcOH). UV λ_{max} (nm): (MeOH) 268, 326; (NaOMe) 274, 366; ($AlCl_3$) 257 (sh), 279, 380; ($AlCl_3$/HCl) 257 (sh), 287, 359; (NaOAc) 280, 325; (NaOAc/H_3BO_3) 278, 337. ^{1}H-NMR (DMSO-d_6) δ 8.11 (2H, d, *J* = 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 (18-20).

Compound **6** was isolated from ethyl acetate collective sub-fractions (E6-E9) as yellow powder exhibited *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 (21).

Compound **7** was isolated from the ethyl acetate collective sub-fractions (E6-E9) as yellow powder which 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); *ρ*-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); *ρ*-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*-*ρ*-coumaroyl)-*β*-D-glucopyranoside. The spectroscopic data were harmony with the reported data (22, 23).

Compound **8** was isolated from the ethyl acetate collective sub-fractions (E11-E13) as yellow powder exhibited *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*



	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

1 R = H
2 R = 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

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 typhimurium (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) 100 mg/ml. Positive control for bacteria: Gentamycin (MIC) 4 mg/ml; RCMB: Regional Center for Mycology and Biotechnology in Cairo, Egypt, Al-Azhar University; ATCC: American Type Culture Collection

= 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 (18, 24).

Antimicrobial activity

The incidence of microbial infections has increased in recent decades, especially mycoses which account for a high rate of death among patients with an immunosuppressed system. Opportunistic fungal infections are a serious threat to such patients and have been reported to occur at a worrying rate (25, 26).

The availability of a small number of antifungal drugs, the associated side effects of amphotericin B (i.e., nephrotoxicity), the high cost of the lipid formulations of amphotericin B, and the possible failure of some individuals to respond to the treatment with azoles may complicate the treatment of these mycoses. Hence, there is an urgent need to find new drugs or compounds possessing antifungal properties (27).

The plant-based antimicrobial compounds have marvelous therapeutic potential as they can serve the purpose without or with very little side effects that are often associated with synthetic drugs. Plants are an important source of possibly useful structures for the development of new chemotherapeutic agents.

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. Our results were agreed with those obtained by (28) 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 (29).

Also, the potent activity of ethyl acetate extract could be due to two reasons. Firstly, the bioactive constituents such as saponins, tannins, alkaloids, anthraquinones and vitamins in the plant extract may be enhanced in the presence of ethyl acetate extract. Secondly, the stronger extraction capacity of ethyl acetate may be responsible, such that more active ingredients may be present in the polar extracts. Some of the detected compounds in this extract may be responsible for the antibacterial activity observed and thus, justifying their traditional use as medicinal plants for the treatment of bacterial gastroenteritis (30).

Antioxidant activity

Free radicals are formed during the metabolism where bigger molecules are broken down into smaller molecules and smaller molecules are organized into bigger molecules. If these molecules lose an electron, they can turn into a free radical which unstable, electrically charged molecules in the cells, that can react with other molecules (like DNA) and damage them. During oxidative stress, important molecules in the body can be damaged, sometimes leading to cell death. So the role of antioxidants comes in where if a molecule loses an electron and turns into a free radical, the antioxidant molecule prevents the transfer of an electron from O_2 to organic molecules, neutralizes the free radicals and terminates free radical reactions (31).

DPPH scavenging method was utilized to evaluate the antioxidant activity of the chloroform and ethyl acetate extracts of *F. viridis* aerial parts using ascorbic acid as a 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 3200 µl) exhibited antioxidant capacity (17.80 – 92.75%) with IC_{50} 118.7 µg/ml for chloroform and (45.49 – 93.30%) with IC_{50} 32.1 µg/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.

The activity of ethyl acetate extract may be due to sufficient amount of flavonoid and phenolic acids besides other chemical constituents present in ethyl acetate extract as (tannins, polyphenols, terpenoids and saponins) which characterized by their powerful antioxidant properties where, they might be responsible for the antioxidant activity of ethyl acetate extract through breakdown the free radical reaction and neutralize the unpaired electron with donating electrons to eliminate the unpaired condition of the radical. The flavonoid and phenolic compounds may directly react with the reactive radicals and destroy them. Also, they may decrease the cellular level of free radicals either by inhibiting the activities or expressions of free radical generating enzymes (32, 33).

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.

Cancer diseases have been increasing in Egypt with a doubling in the incidence rate in the past 10 years especially the hepatocellular carcinoma due to

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

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.	Viability % of chloroform extract				Viability % of ethyl acetate extract			
µg/ml	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

several biological (hepatitis B and C virus infection) and environmental factors (aflatoxin). Other factors such as cigarette smoking, exposure to chemicals such as pesticides, and endemic infections in the community, such as schistosomiasis, may have additional roles in the etiology or development of the disease (34).

The outlined results presented in Table 3 showed that chloroform extract showed moderate antitumor activity for breast (MCF7) carcinoma cell line with IC_{50} 69.95 µg/ml with effective about 39.2% compared to normal cell line, potent antitumor activity for colon (Caco-2) carcinoma cell line with IC_{50} 40.6 µg/ml with effective about 64.7% and weak activity for hepatic (HepG2) carcinoma cell line with IC_{50} 105.82 µg/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} 148.9 µg/ml with effective about 27.6%.

The American Cancer Institute (NCI) stated that the criteria of cytotoxic activity for the crude extract is an $IC_{50} < 20$ µg/ml. (35). So only chloroform extract had a moderate cytotoxic activity for both colon and breast carcinoma cell line.

These obtained results were agreed with some studies performed on other plants of the family Urticaceae which showed a moderate cytotoxic activity of both chloroform and ethyl acetate fractions of *Urtica* species (36).

The activity of isolated phenolic and flavonoid compounds from the two extracts which have numerous hydroxy groups in the flavonoid structure, in combination with a highly conjugated ð-electron system, allow them to act as free radical scavengers via hydrogen atom or electron-donating activities. Furthermore, they can block the formation of ROS (reactive oxygen species), such as the hydroxyl radical, through chelation of redox-active transition metal ions (37) This can have a positive effect on cancer prevention by the minimization of phenomena such as oxidative damage to DNA. The relatively antitumor activity of both extracts was largely linked to their relatively isolated flavonoid simple structure where, the presence of conjugated electron systems and aromatic rings make them stable and reactive, whereas their overall structure allows them to act as substrates, inhibitors or agonists for numerous enzymes or molecules involved in the development and progression of cancer where they effect on estrogen production and signaling pathways has been linked to their role as aromatase inhibitors and their interaction with estrogen receptor and estrogen-metabolizing enzymes. They also act as breast cancer resistant protein inhibitors and interact with cytochrome P450 both as inhibitors and substrates. their ability to induce apoptosis and cell cycle arrest and to alter numerous signaling pathways involved in cancer-related phenomena such as inflammation and proliferation (38).

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 (39, 40). 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 too potent antioxidant, antimicrobial and cytotoxicity activities.

Conflict of interest

The authors declare no conflicts of interest.

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Received: 7.03.2019