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Research Article

ACTIVE CONSTITUENTS AND BIOLOGICAL ACTIVITY OF METHANOLIC EXTRACT OF *FORSSKAOLEA VIRIDIS* AERIAL PARTS

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ABSTRACT

Objective: The objective of this study is to isolate and evaluate the antioxidant, antimicrobial, and antitumor activity of the methanolic constituents of *Forsskaolea viridis*.

Methods: The isolation and purification of phytochemical constituents were performed in the present study using various chromatographic methods (thin-layer [TLC], preparative paper [PPC], and column chromatography [CC]) where, the structures of isolated compounds were determined using different spectroscopic methods (Mass spectroscopy [Ms], ultraviolet [UV], proton and carbon nuclear magnetic resonance [^{1}H-NMR and ^{13}C-NMR]) and confirmed by data reported in the literature. The biological activity of the methanolic extract was performed as *in vitro* studies by diffusion agar technique, 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH), and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for antimicrobial, antioxidant, and antitumor activity, respectively.

Results: The chemical studies led to the isolation of five flavonoids and two phenolic compounds for the first time from this plant. The methanolic extract exhibited potent antimicrobial activity (13–26 mm) against some of the tested strains while it showed fair antioxidant and cytotoxicity activity.

Conclusion: It can be concluded from the results that the methanolic extract of *F. viridis* has potential antimicrobial activity while fair antioxidant and antitumor activity.

Keywords: *Forsskaolea viridis*, Phenolics, Flavonoids, Antimicrobial, Antioxidant, Antitumor.

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INTRODUCTION

Ever since ancient times, humans were looking for drugs to rescue their disease. At this time, there is no medicinal source help them in the treatment of their diseases other than medicinal plants which provided humankind with herbal remedies for several diseases for many centuries. Therefore, human beings tended to use therapeutic plants to treat many diseases where medicinal plants proved effective in treating diseases because they contain many active materials, proteins, vitamins, hormones, …etc. 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 the *Urticaceae* family, represented by six species, distributed in Canary Isles and Southeast Spain eastwards to Pakistan, Africa, and Arabia to West India [2]. Reported activities for the *Forsskaolea* genus are diuretic, calculolitic, antiflu [3], and antiseptic [4]. *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 [5]. The survey on the previous studies on the *F. viridis* plant showed no chemical and biological studies performed on it so; we aimed to investigate the active chemical constituents of its different extracts in addition to their biological activity [6]. In this study, we concerned to focus our study on the methanolic extract of *F. viridis* aerial parts and its biological activity of *F. viridis* aerial parts.

METHODS

Plant material

Aerial parts of *F. viridis* were collected from their natural habitat in Wadi Kansisrob, Gebel Elba region, Southeast corner of Egypt in January 2016. The plant specimens were identified, authenticated, and deposited in the herbarium of Desert Research Center (CAIH) with Code Number: CAIH-1000-R.

Chemicals

All chemicals used were of high quality and analytical grade purchased from Sigma-Aldrich.

Chemical studies

Extraction

About 1.5 kg of the air-dried powder of *F. viridis* aerial parts extracted by successive extraction by Soxhlet apparatus starting with petroleum ether to remove the lipoidal matters with increasing polarity then, filtered off, the marc lifted was re-extracted by the same way (this process repeated several times). The same combined extracts for each solvent were concentrated separately under reduced pressure at a temperature not exceeding 50°C till dryness. The petroleum ether, chloroform, ethyl acetate, 100% methanol, 50% methanol, and water were used [6]. The same combined extracts for each solvent were concentrated separately under reduced pressure at a temperature not exceeding 50°C till dryness.

Isolation

The aqueous methanolic extract (130 g) was dissolved in a little amount of water, treated with an excess of methanol and filtered to remove inorganic salts and non-phenolic compounds. The residual extract after removal of inorganic salts was extracted with increasing polarity according to scheme (Fig. 1) as 100% MeOH, 50% MeOH, and finally 100% water, then subjected to paper chromatography using butanol:acetic acid:water (BAW) upper layer (4:1:5) and 15% acetic acid:water (15% AcOH) which revealed similarity of two fractions 100% MeOH and 50% MeOH, so they were collected together and subjected to polyamide column starting with water with decreasing polarity to 100% MeOH afforded 22 fractions (M1-M22), each fraction was 250 ml. Similar fractions were collected together which finally give five main fractions that needed to further isolation on polyamide

Table 1: Column fractions of methanolic extract of *Forsskaolea viridis* aerial parts

Fraction No.	Solvent system	Pooled fractions	Weight (g)
I	H_2O	1–2	51.0
II	MeOH:H_2O (20:100)	3–10	4.3
III	MeOH:H_2O (40:100)	11–12	0.8
IV	MeOH:H_2O (60:100)	13–18	4.0
V	MeOH:H_2O (90:100)	19–22	1.7

column, preparative paper, and thin-layer chromatography, then applied to Sephadex LH-20 column for final purification of isolated compounds.

Methanolic extract (55 g dissolved in 50 ml aqueous methanol 3:1) over polyamide crude (250 g) column (150 cm × 5 cm) and elution with methanol/bi-distilled water mixture of decreasing polarities for gradient elution led to the desorption of five individual fractions (I-V) which were dried individually, in vacuum, and then subjected to two-dimensional paper chromatographic investigation.

Biological studies

Antimicrobial activity

Antimicrobial activity was determined by the diffusion agar technique in Regional Center for Mycology and Biotechnology Al-Azhar University, Cairo, Egypt (RCMB), according to CLSI [7,8]. Strains were obtained from the bacteria stock present at RCMB. Petri plates containing 20 ml 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 × 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°C for 24 h (bacterial strains) and 25°C for 7 days (fungal strains). The assessment 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 minimum inhibitory concentration (MIC) 100 mg/ml, while positive control used for bacteria strains was gentamycin with MIC 4 mg/ml.

Antioxidant activity (2,2-diphenyl-1-picrylhydrazyl hydrate [DPPH] assay)

The free radical scavenging effect of plant extract was assessed by the decoloration solution of DPPH radical, as described in Letelier *et al.* [9]. This assay was realized essentially by the method reported in Joyeux *et al.* [10] and its modification [11]. In a final volume of 1 ml, the reaction mixture contained 20 µg/ml of DPPH (ethanol solution) and different concentrations of methanol extract. Blanks contained only ethanol and plant extract. DPPH bleaching activity of all mixtures was measured continuously at 37°C for 20 min–517 nm in a ultraviolet (UV3) Unicam UV-visible spectrophotometer. Reaction rates 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 positive control.

Cytotoxic activity (viability assay)

The human breast (MCF-7), colon (Caco-2), and hepatic (HepG-2) carcinoma cell lines were obtained in the frozen state under liquid nitrogen (−180°C) from the American type culture collection. The tumor cell lines were maintained by serial subculturing in the National Cancer Institute, Cairo, Egypt. The cytotoxic effect of methanol extract was evaluated in the National Cancer Institute, according to the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method [12]. Briefly, cells were seeded in 96 well plates at a density of 5000 cells/well in 100 µl culture medium. Following 24 h incubation, cells were treated with various concentrations of methanol extract 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 Medium, filtered through a 0.22 µm filter, 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 dimethyl sulfoxide (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 extract). The cell viability of the control group without exposure to the extracts was defined as 100%.

Statistical analysis

Biological experimental results are presented as means scanning electron microscope, and all measurements and analyses were carried out in triplicate. SPSS statistical software was used. Statistical analysis was performed by one-way analysis of variance.

RESULTS AND DISCUSSION

Chemical studies

The isolation of active chemical constituents of the methanolic extract of *F. viridis* aerial parts was performed according to the scheme Fig. 1. The identification and structure elucidation of the purified phenolic and flavonoid compounds were done by comparison, R_f values, UV, ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR spectral data with the reported data in the literature.

Compound (1) appeared as a dark purple spot under UV light turning orange when fumed with ammonia, which exhibited a *Mr* of 610 in electrospray ionization mass spectrometry (ESI-MS) analysis ($[M+H]^-$ at *m/z*=611), R_f 0.43 (BAW), 0.57 (6% AcOH). UV λ_{max} (nm): MeOH: 258, 264, 295, 357; (NaOMe) 271, 325, 408; (NaOAc) 270, 324, 392; (NaOAc/H_3BO_3) 260, 296, 385; ($AlCl_3$) 275, 303, 433; ($AlCl_3$/HCl) 271, 299, 364, 402. ^{1}H-NMR (DMSO-d6): Quercetin moiety: δ 7.75 (1H, d, *J*=2.5 Hz, H-2'), 7.58 (1H, dd, *J*=2.5 and 8 Hz, H-6'), 6.93 (1H, d, *J*=8 Hz, H-5'), 6.85 (1H, d, *J*=8 Hz, H-5'), 6.43 (1H, d, *J*=2.5 Hz, H-8), 6.22 (1H, d, *J*=2.5 Hz, H-6). Glucose moiety: δ 5.14 (1H, d, *J*=8.5 Hz, H-1''), 3.15–3.85 (5H, *m*). Rhamnose moiety: δ 4.51 (1H, d, *J*=2.5 Hz, H-1'''), 3.2–3.85 (5H, *m*), 1.12 (3H, d, *J*=6.5 Hz, CH_3 rhamnose). ^{13}C-NMR (DMSO-d6): Quercetin moiety: δ 179.4 (C-4), 165.1 (C-7), 162.1 (C-9), 158.5 (C-5), 157.1 (C-2), 148.5 (C-4'), 145.6 (C-3'), 135.1 (C-3), 123.1 (C-6'), 121.5 (C-1'), 117.2 (C-5'), 115.8 (C-2'), 104.8 (C-10), 97.5 (C-6), 94.5 (C-8). Glucose moiety: δ 102.4 (C-1''), 79.3 (C-3''), 77.5 (C-5''), 73.9 (C-2''), 72.4 (C-4''), 66.9 (C-6''). Rhamnose moiety: δ 100.4 (C-1'''), 73.4 (C-4'''), 71.2 (C-3'''), 70.7 (C-2'''), 69.1 (C-5'''), 18.6 (CH_3-rhamnose). Compound (1) was identified as rutin. The spectroscopic data of compound (1) were similar to the physical and spectral data recorded in Wei *et al.* [13].

Compound (2) was purely isolated as deep purple spot under UV light turning yellowish green when fumed with ammonia, which exhibited a *Mr* of 610 in ESI-MS analysis ($[M+H]^-$ at *m/z*=611), R_f 0.14 (BAW), 0.39 (15% AcOH). UV λ_{max} (nm): MeOH: 256, 274, 345; (NaOMe) 268, 282, 342, 413; (NaOAc) 269, 283, 327, 407; (NaOAc/H_3BO_3) 277, 285, 362, 425; ($AlCl_3$) 279, 303, 332, 402; ($AlCl_3$/HCl) 261, 278, 295, 354, 386. ^{1}H-NMR (DMSO-d6): Aglycone moiety: δ 7.57 (1H, dd, *J*=8.5 Hz, 2.5Hz, H-6'), 7.39 (1H, d, *J*=2.0 Hz, H-2'), 6.88 (1H, d, *J*=8.5 Hz, H-5'), 6.46 (1H, s, H-3). Glucose moiety at position 6: δ 4.72 (1H, d, *J*=9.8 Hz, H-1``), 3.21–3.89 (5H, m sugar protons) glucose moiety at position 8: δ 4.95 (1H, d, *J*=8.6 Hz, H-1```), 3.21–3.89 (5H, m, overlapped protons). ^{13}C-NMR (DMSO-d6): δ 181.62 (C-4), 166.22 (C-2), 163.61 (C-7), 159.3 (C-5), 156.93 (C-9), 150.21 (C-4'), 147.54 (C-3'), 132.51 (C-1'), 129.16 (C-2', C-6'), 116.20 (C-5') 109.92 (C-6), 105.63 (C-8), 103.95 (C-10), 103.31 (C-3). Sugar moieties: δ 81.18 (C-5'', C-5'''-overlapped), 78.81 (C-3'', C-3'''-overlapped), 73.32 (C-1'', C-1'''- overlapped), 70.21 (C-2'', C-2'''–overlapped), 70.83 (C-4'', C-4'''–overlapped), 60.6 (C-6'', C-6'''-overlapped). Compound (2) was identified as lucenin-2. The spectroscopic data of compound (2) were harmony with those data recorded in Erel *et al*. [14].

Compound (3) was purely isolated as colorless needle crystals, appeared on PC as blue spot under UV light turning to intense blue when fumed with ammonia, it was found to exhibit *Mr* of 354 in ESI-MS analysis

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