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Purification, Characterization, and Antioxidant Activity of Daucosterol and Stigmasterol from *Prangos ferulacea*

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Abstract: The genus *Prangos* is traditionally used for medicinal and food purposes. This genus contains a wide range of bioactive metabolites. In this work, phytochemical investigation and antioxidant activity evaluation of *Prangos ferulacea* were carried out. Chromatographic techniques were employed for the purification of extracts components. Spectroscopic techniques such as NMR, FT-IR, together with elemental analysis, were used for the structure elucidation of isolated compounds. Stigmasterol, daucosterol, and salicylic acid were purified and identified. Isolated compounds showed moderate to high antiradical activity in DPPH antioxidant assay. Results indicated the potential of *P. ferulacea* as a source of steroid and their glycosides and also its possible applications as antioxidant agents.

Keywords: *Prangos*; phytochemistry; stigmasterol; daucosterol; antioxidant.

1. Introduction

The genus *Prangos* L. is belongs to the Apioideae subfamily of the Apiaceae family, including about 72 herbaceous hemicryptophyte species in the world [1]. Most members of the genus are distributed in central and southeast Asia. *Prangos* has 15 species in Iran, of which 5 species are endemic to the country [2].

The genus has traditional uses such as fodder, food, spice, and instead of chemical drugs for the treatment of some diseases and health problems like seizures, bleeding, headache, leukoplakia, and digestive disorders [3-5]. The genus exerts several pharmacological effects such as a diuretic, antiflatulent, abortifacient, anti-hemorrhoid, anti-inflammatory, antispasmodic, anthelmintic, emollient, carminative, and tonic [6-8]. Furthermore, modern studies have shown some biological properties, including cytotoxic, allelopathic, antibacterial, antioxidant, and antifungal activities [9, 10]. *Prangos* species contain a large number of coumarin compounds, of which osthol, oxypeucedanin, and isoimperatorin are the most famous. In addition, terpenoids, alkaloids, and flavonoids have been reported from this genus [11-13]. Volatile compounds of the genus have important biological activities, too, such as antioxidant, antidiabetic, anti-Alzheimer's, skin-care, and insecticidal effects [14, 15]. The most abundant volatiles of the genus is α-pinene, β-pinene, carene, β-phellandrene, and α-bisabolol [16]. Besides biological properties, the chemical composition of essential oils is important for the classification of species in chemotaxonomic studies [17].

The local people use the oily exudate from freshly cut roots of *P. ferulacea* as a topical cream for wound healing agents [18]. Also, according to our observation, people apply sodden of dried leaves of *P. ferulacea* as anti-worm medicine for human and livestock at Kanizard, Grdaseh, and Snjaleh villages, Piranshahr, West Azerbaijan province, Iran. The local name of *P. ferulacea* is Halz in Kurdish, and it is called Jasher in Persian.

At the present work, we aimed to evaluate the chemical composition of *P. ferulacea* from West Azerbaijan for the first time. Also, radical scavenging properties of isolated natural products were estimated using the DPPH assay.

2. Materials and Methods

2.1. Chemicals and reagents.

The solvents were provided by Carlo Erba (Italy) and Daejung (South Korea). Silica gel for column chromatography and TLC Silica gel were purchased from Merck (Germany).

2.2. Plant material.

Fresh leaves and stems were collected in June 2015 from the mountains of Kanizard village, Piranshahr, West Azerbaijan province, Iran. Taxonomic identification of plant was performed by Mr. Shahram Bahadori at the herbarium of Faculty of Pharmacy, Urmia University of Medical Sciences, Urmia, Iran.

2.3. Preparation of extracts.

The extracts were achieved from aerial parts of *P. ferulacea* by the maceration method. For this, 750 g of powdered material was extracted by 3 L of *n*-hexane, dichloromethane, ethyl acetate, and methanol consecutively. The extraction was performed using a magnetic shaker at room temperature for 48 h. The extracts were filtered by a paper filter, and solutions were concentrated by a rotary vacuum evaporator at 40°C until obtaining crude dry extracts [19].

2.4. Purification of metabolites.

The ethyl acetate extract (8.8 g) was fractionated by a vacuum liquid chromatography (VLC) (silica gel 70-230 mesh), eluted with hexane-CH_2Cl_2 (10:0, 7:3, 1:1, 3:7, 0:10) and CH_2Cl_2-MeOH (7:3, 1:1, 3:7, 0:10) and MeOH-acetone (7:3, 1:1, 3:7, 0:10) to obtain 13 fractions (F1-F13). All fractions were monitored by TLC, and then similar fractions were combined. The new fraction F_{34} (2.57 g, obtained from the combination of F3 and F4) was loaded on four preparative TLC plates (silica gel 60 Gf_{254}) and eluted by $CHCl_3$-hexane (4:1) which afforded 2 fluorescent lines which were crushed from plates and washed by methanol and ethyl acetate. The result was two pure compounds, 1 (11 mg) and 2 (18 mg). F_{567} (3.37 g, obtained from the combination of F5-F7) was separated by silica gel column chromatography eluted by $CHCl_3$-MeOH (100:0, 98:2, 95:5, 90:10, 80:20, 0:100) to obtain 10 sub-fractions. After solvent evaporation of fractions at room temperature, cubic and white crystals were obtained from fraction 10 (compound 3, 20 mg).

2.5. NMR experiments.

NMR spectrum was obtained by recording on Bruker Avance 400 MHz spectrometer (Germany), acting at 400 MHz for ^{1}H and 100 MHz for ^{13}C. DMSO-d_6 and $CDCl_3$ were applied as deuterated solvents and TMS as an internal standard.

2.6. FT-IR analysis.

IR spectrum was recorded on a Shimadzu FTIR-8400S spectrophotometer (Japan) operating KBr pellets.

2.7. Melting point.

Measurement of melting points was done in open glass capillaries using the Electrothermal melting point apparatus.

2.8. Elemental analysis.

The elemental analysis for C, N, and H atoms was performed by using the Costech elemental analyzer [20].

2.9. Antioxidant activity assessment.

DPPH (2,2-Diphenyl-1-picrylhydrazyl), which has $C_{18}H_{12}N_5O_6$ molecular formula, was used to estimate the antioxidant properties of obtained compounds (Fluka Chemie AG, Bucks). As the reference compound, rutin was used. Several concentrations of isolated compounds (solved in methanol) were provided (7.8-500 µg/mL). Eight milligrams of DPPH was dissolved in methanol to achieve a stock with a concentration of 80 µg/mL. DPPH (1 mL) was added to dilute solutions (1 mL) and maintained 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm [21]. The experiments were performed in triplicate, and the average absorption was recorded for each concentration. IC_{50} values were calculated and expressed as mean ± SD [22].

3. Results and Discussion

The ethyl acetate extract of the aerial parts of *P. ferulacea* was subjected to vacuum liquid chromatography, column chromatography, and also preparative thin layer chromatography to yield compounds **1-3**. The structure elucidation of purified compounds was performed using ^{13}C NMR, ^{1}H NMR, and FT-IR spectra as well as elemental analysis. The obtained data were compared with those of the literature and were confirmed. Compound **1** was identified as a glycoside sterol due to its characteristic signals in NMR spectra. Elemental analysis showed the molecular formula of $C_{35}H_{60}O_6$, which is in accordance with a sterol aglycone and a six-carbon glycoside. IR spectra confirmed the presence of OH groups, olefinic hydrogen, and the C-O bond. Finally, the structure of 3-O-Gly-β-sitosterol, also called daucosterol, was determined for this compound [23]. The melting point of compound **1** was measured as 274-276°C, which is in accordance with those reported for daucosterol in the literature (Figure 1). This is the first report of daucosterol from the genus *Prangos*. It has wide pharmacological activities such as antimicrobial, neuroprotective, and anticoagulant [24]. In recent years, there is a focus on the evaluation of its anticancer potential.

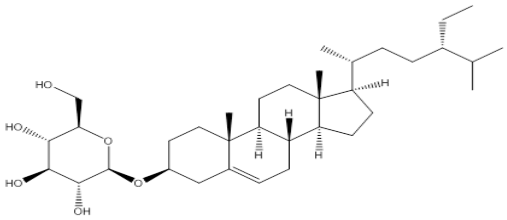

Figure 1. Chemical structure of daucosterol.

Compound **2** was identified as a phytosterol. Except for the glycoside region, it has a similar pattern in NMR signals with compound **1**. The presence of four olefinic carbon signals, and characteristic methyl groups of sterols in ^{1}H NMR, revealed that its chemical structure is a phytosterol with 29 carbon atoms, of which four atoms are involved in carbon-carbon double bonds. A comparison of obtained spectra with related herbal compounds showed that compound **2** could be stigmasterol. IR results, together with elemental analysis ($C_{29}H_{48}O$), confirmed the structure of stigmasterol (Figure 2). Moreover, its melting point (161-164.5 °C) was obtained similar to previous works which reported the isolation of stigmasterol from natural sources [25, 26]. To the best of our knowledge, this is the first time that stigmasterol is isolated from *Prangos* members. Stigmasterol has hypoglycemic, hypolipidemic, antibacterial, and memory-enhancing effects [27].

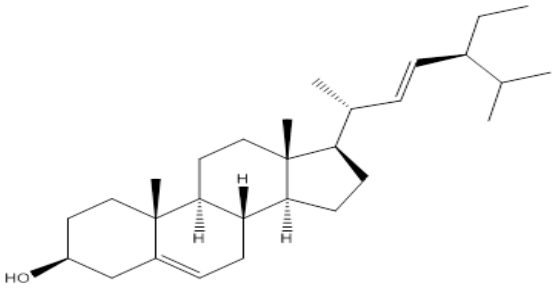

Figure 2. Chemical structure of stigmasterol.

Compound **3** has a simple ^{13}C NMR spectrum, including 6 aromatic carbons and one ketone signal. Also, ^{1}H NMR spectrum showed the presence of 4 aromatic protons together with one carboxylic acid proton. These data, in combination with elemental analysis ($C_7H_6O_3$) and melting point determination (157-158.5 °C), led to salicylic acid structure for compound **3** (Figure 3). Salicylic acid has important biological properties such as pain killer, anti-inflammatory, and antifungal [28]. Moreover, it is the starting agent for the synthesis of Aspirin.

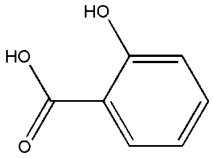

Figure 3. Chemical structure of salicylic acid.

Isolated compounds were also investigated for their radical scavenging activities through DPPH assay. Radical scavenging ability is a very important mechanism of antioxidant activity [29]. Purified compounds in this study, found to have moderate to strong antioxidant potential. Salicylic acid exerted the highest antioxidant ability with IC_{50} value of 48.2 ± 2.9 µg/mL followed by stigmasterol and daucosterol (Figure 4).

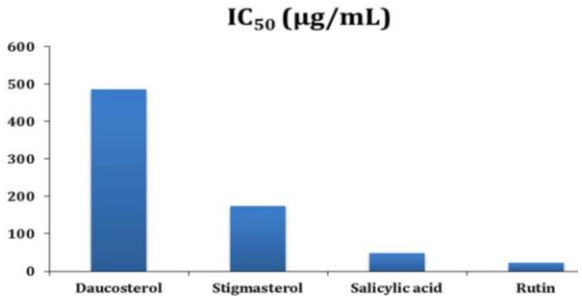

Figure 4. Antioxidant activity of isolated compounds.

4. Conclusions

Antioxidant, natural constituents were purified from the aerial parts of *P. ferulacea*. The chemical structure of the isolated metabolites was elucidated using spectroscopic techniques like NMR and FT-IR as well as elemental analysis. Daucosterol, stigmasterol, and salicylic acid were reported for the first time from the genus *Prangos*. Moreover, their radical scavenging activity was determined using DPPH assay. Findings showed that *P. ferulacea* contains natural steroids and their glycosides and could be considered for more phytochemical and pharmacological studies.

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Conflicts of Interest

The authors declare no conflict of interest.

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