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ISOLATION AND CHARACTERIZATION OF PHYTOCHEMICALS OF *PASTINACA UMBROSA*

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The *Pastinaca* genus belongs to *Umbelliferae* family. *Pastinaca umbrosa* grows widely in the north-west, west and center of Azerbaijan. In the present study, phytochemicals of the plants have been isolated and their structures have been elucidated. The mix of extractive substances was obtained by finely cutting the body of *Pastinaca umbrosa*, then drying in the room conditions (200 g) and extracting by acetone 3 times (3 days for each time). The acetone was filtered and evaporated on a water bath. The residue was a dark-brown resin (12 g, 6% yield). The resin (12 g) was dissolved in $CHCl_3$ (50 mL) and chromatographed over a column of neutral Al_2O_3 with elution by hexane, hexane + benzen, benzene, benzene +chloroform, chloroform and chloroform+ etanol, in different ratios. The volume of each fraction was 100 mL. The separation and isolation process was carried out using column (silicagel) and thin layer chromatographic (TLC) methods. Structure elucidation of thepurified compounds were based on IR, UV, ^{1}H and ^{13}C-NMR data, in comparison with those reported in theprevious literatures. The isolated compounds from the aceton extracts of *P. umbrosa* were identified as esculetin 2.5%, scopoletin 4.5%, and umbelliferon 3.5%.

Keywords: Apiaceae Lindl, Pastinaca umbrosa, biological activity, esculetin, scopoletin, umbelliferon.

Abbreviations: NMR – Nuclear Magnetic Resonance, IR – Intrared Spectroscopy, HNMR – Proton nuclear magnetic resonance, ^{13}CNMR – Carbon-13 nuclear magnetic resonance, DMSO – Dimethyl sulfoxide, UV – Ultraviolet.

Introduction

Of the 15 species of the genus *Pastinaca* L. (Chimi) belonging to the family *Apiaceae* Lindl, distributed in Eurasia and the Caucasus, 4 species are known in Azerbaijan: *P.umbrosa, P.armena*, *P.pimpinellifolia, P.glandulosa.* In the flora of Azerbaijan, these species are found in the middle mountain range to the alpine zone, in forests and shrubs, meadows, arid slopes [1].

Apiaceae Lindl species have an interesting coumarin composition [2]. According to the literature [Abishev, 2003; Pimenov, 2012] coumarin derivatives have been identified in more than 1626 plant species belonging to 134 genera and 568 genera in the world flora [3–9]. Current research is devoted to study the chemical composition of *Pastinaca umbrosa*.

Experimental part

Surface parts of *Pastinaca umbrosa* species collected in the flowering phase (3.VII. 2019) from the territory of Kalaman village of Gadabay region were used as the object of research. The mix of extractive substances was obtained by finely cutting the body of *Pastinaca umbrosa*, then drying in the room conditions (200 g) and extracting by acetone 3 times (3 days for each time). The acetone was filtered and evaporated on a water bath. The residue was a dark-brown resin (12 g, 6% yield).

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To obtain the extractive substances the mix was chromagraphed by the chromatography method in the glass column filled by Al_2O_3.

Column chromatography method was used to get the individual items. Thus, the sum of 12 g

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ingredients was chromatographed in the glass column (h=100; d=3 cm) filled with Al_2O_3 (neutral, with III–IV degrees of activity). The volume of each fraction was taken 100 ml. The column chromatography was eluated by Hexane (20 fractions), hexane + benzen (9 : 1; 8 : 2; 7 : 3; 6 : 4; 1 : 1; 1 : 2; 1 : 3; 1 : 4; 1 : 5 in proportions 35 fractions), benzene (40 fractions), benzene +chloroform (1 : 3 – 1 : 9 in proportions 30 fractions), chloroform (10 fractions) and chloroform+ etanol (95 : 5).

The individuality of the substances was defined using thin layer chromatography method (Silufol UV 254, solvent-benzol+chloroform 1 : 1), IR-spectrums were drawn in the Cary 630 FTIR Spectrometer.^{1}H, ^{13}C, ^{13}C Dept 135, Dept 90 spectrums were registered in the Bruker 300 MHz spectrometer for the isotope ^{1}H with frequency 300 MHz and for the ^{13}C isotope with 75 MHz frequency using DMSO-d_6 as a solvent. The obtained substances were compared based on the ^{1}H and ^{13}C NMR information provided in literature on the structure of the individual items.

Results and discussion

Natural products have begun to gain popularity worldwide for designing new and safe drugs or food additives (Raskin and others 2002; Veeresham 2012). In this sense, novel plant products or plant metabolites are today one of the wild plant's most interesting subjects in scientific explorations. Especially uninvestigated wild plants are considered as an important pool for new pharmaceuticals and bioactive food additives, often termed nutraceuticals.

Current research is devoted to studying the chemical composition of the wild plants *Pastinaca umbrosa*.

Three individual substances were obtained from the plant material mix using the chromatography method. The characterizations of the esculetin, scopoletin and umbelliferon, were defined on the basis of discovering the IR and NMR spectrums of the substances. The substance was obtained from the 126–127-th and 136–138 fractions by eluation by the mixture of the chromatographic column.

Esculetin may have the potential to slow down chronic inflammation in obesity [10]. In view of natural coumarin derivatives such as esculetin, fraxetin, and warfarin and their clinical use as anticoagulant agents [11, 12], esculetin may also have medicinal potential for the treatment of stroke.

Esculin is included in the drug esquit. Esculin is similar to hydroxycoumarin, which is a "blood thinner" (anticoagulant). It has been used to decrease swelling. It may increase the risk of bleeding and may be harmful if swallowed. In the combination medication is used to treat pain, itching, swelling, and discomfort caused by hemorrhoids and other problems of the anal area.

Esculetin is also a potent agent in protecting cells from ROS-mediated Abeta-damage [13]. In another study, esculetin is effective in protecting cells against DNA damage induced by oxidative stress [14]. Esculetin has the effect of promoting glucose metabolism and mediates adipocyte apoptosis by the mitochondrial pathway initiating the apoptotic process of 3T3-L1 adipocytes [15]. From simple coumarins in medicine they are used as anticoagulants of indirect action. *Umbelliferon* hasantibacterial [16, 17] and scopoletin has an spasmolitic effect [18, 19].

Three crystalline substances (esculetin 2.5%, scopoletin 4.5% and umbelliferon 3.5%) were obtained individually by chromatography in a glass column filled with neutral, grade III–IV activity Al_2O_3 from the total amount of extractives extracted from the surface part of the *Pastinaca umbrosa* plant.

Substances 1. (7-oksikumarin). *7-oksikumarin* – $C_9H_6O_3$, m.t. 233.0–234.0 °C In the field of characteristic absorption bands of the IR spectrum, absorption bands characterizing the carbonyl group of the δ-lactone cycle (1713, 1688 cm^{-1}) and the double bonds of the aromatic system (1622, 1613, 1575, 1512 cm^{-1}), ultraviolet (UV) λ_{max} 216 (lg ε 4.08), 244 (lg ε 3.45), 254 (lg ε 3.35), 300 (lg ε 3.90) 324 nm (lg ε 4.16), have been identified. The IR spectrum of umbelliferone has been identified with the known umbelliferon IR spectrum [20].

^{13}C-NMR ($CDCl_3$ 75 MHz) δ:2-C (C) 163.17; 3-C (CH) 112.34; 4-C (CH) 146.05; 5-C(CH) 130.66; 6-C (CH) 114.52; 7-C (C) 157.25 ; 8-C (C)103.40; 9-C(C) 133.59; 10-C(C) 113.14.

^{1}H-NMR ($CDCl_3$, 300MHz) δ:6.04 (1H, d, J = 9.4 Hz, 3-H), 7.54 (1H, d,J = 9.4 Hz, 4-H), 7.20 (1H, d,J = 8.2 Hz, 5-H), 6.65(1H, dd,J= 8.2 Hz/ 2.2, 6-H).

Substances 2. (Esculetin). Esculetin – $C_9H_6O_4$, m.t. 268–272.0 °C. There are absorption bands of carbonyl (1715, 1672 cm^{-1}) of the δ-lactone cycle and double bonds of the aromatic system (1625, 1570 sm^{-1}) in the IR spectrum and ultraviolet (UV) spectrum λ_{max} 230 (lg ε 4.11), 259 (lg ε 3.67), 300 (lg ε 3.74), 363 nm (lg ε 4.08). By directly comparing the IR spectrum of the compound with the IR spectrum of the esculet belonging to known simple coumarins [20], the substance under study was identified as an esculet.

^{13}C-NMR ($CDCl_3$ 75 MHz) δ:2-C (C) 161.55; 3-C (CH) 112.34; 4-C (CH) 145.16; 5-C(CH) 118.68; 6-C (CH) 113.65; 7-C (C) 156.15; 8-C (C)150.13; 9-C(C) 144.60; 10-C(C) 115.48.

^{1}H-NMR ($CDCl_3$, 300MHz) δ:6.32 (1H, d,J =9.5 Hz, 3-H), 7.61 (1H, d,J = 9.5 Hz, 4-H), 6.90 (1H, d,J = 8.6 Hz,5-H), 7.47 (1H, dd,J= 8.6 Hz/J = 2.2 Hz, 6-H)

Substances 3. (Scopoletin). Scopoletin – $C_{10}H_8O_4$, m.t. 204.0–205.0 °C. Absorption bands 1710 (C=O group of δ-lactone), 1631, 1613, 1570, 1520 cm^{-1} (double bonds of the aromatic system) revealed in the IR spectrum and UV spectrum λ_{max} 229 (lg ε 4.20), 254 (lg ε 3.72), 298 (lg ε 3.77), 346 nm (lg ε 4.12) indicate that it belongs to the group of simple coumarins. The IR spectrum of the studied substance is the same as the IR spectrum of the scopolet in the literature [20].

^{13}C-NMR ($CDCl_3$ 75 MHz) δ: 160 (C-2), 149.4 (C-3), 144.4 (C-4), 111.6(C-5), 145 (C-6), 110.4 (C-7), 102.7 (C-8), 151(C-9), 109.5 (C-10), 55.94 (-O-CH3), m/z = 192.

^{1}H-NMR ($CDCl_3$ 300 MHz) δ: 10.32 (s, 1H), 7.91 (d, J=9.3Hz, 1H), 7.22 (s, 1H), 6.78 (s, 1H), 6.23 (d, J=9.3Hz, 1H), 3.82 (s, 3H).

Obtained 3 substances were 7-oxicoumarin, esculetin, scopoletin. The ^{1}H-NMR spectra were recorded at 300 MHz on a Bruker AM300 NMR spectrometer. The ^{13}C-NMR spectra were recorded at 75 MHz onthe same instrument.

Regarding the valuable biological properties, esculetin may have the potential to slow down chronic inflammation in obesity, umbelliferon has antibacterial and scopoletin has an spasmolitic effect.

The obtained biologically active substances may be used in the medical practice. Further investigation needed for the isolation of other phytochemicals and also an investigation of biological properties.

Conclusion

Three compounds were isolated the first time from the extracts obtained from the aerial parts of *Pastinaca umbrosa*. The structures of the isolated compounds were identified as: *7-oksikumarin 3.5%*, *esculetin 2.5%* and *scopoletin 4.5%* on the basis of spectroscopic and by comparing their physical proprieties reported in the literature.

The coumarin species derived from the plant pastinaca umbrosa have been used in medicine.

Coumarin derivatives of the plant pastinaca umbrosa are expected to be used in medicine.

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