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Original article

Identification of non-alkaloid natural compounds of *Angelica purpurascens* (Avé-Lall.) Gilli. (Apiaceae) with cholinesterase and carbonic anhydrase inhibition potential

Songul Karakaya [a], Zeynebe Bingol [b], Mehmet Koca [c], Sena Dagoglu [a], Nur Münevver Pınar [d], Betül Demirci [e], İlhami Gulcin [b], Marian Brestic [f,g], Oksana Sytar [f,h,*]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey
[b] Department of Chemistry, Faculty of Science, Ataturk University, Erzurum, Turkey
[c] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey
[d] Department of Biology, Faculty of Science, Ankara University, Ankara, Turkey
[e] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey
[f] Department of Plant Physiology, Slovak University of Agriculture, Nitra, Slovak Republic
[g] Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources Czech University of Life Sciences, 16500 Prague, Czech Republic
[h] Department of Plant Biology, Institute of Biology, Kiev National University of Taras Shevchenko, Volodymyrska, 64, Kyiv, Ukraine

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ABSTRACT

In current study is done antioxidant, anticholinesterase, and carbonic anhydrase isoenzymes I and II inhibition assays, screening of biological active compounds and electronic microscopy analysis of secretory canals of fruits, flowers, roots, and aerial parts extracts and essential oils of *Angelica purpurascens*. Phenolic constituents, antioxidant, and anti-lipid peroxidation potentials of variants were estimated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and thiobarbituric acid (TBA) processes. Cholinesterase inhibition effect was detected through Ellman's method. The GC/ Mass Spectrometry (MS) and gas chromatography (GC)-flame Ionization Detector (FID) was used for essential oils analysis. NMR techniques was used for identification of the isolated compounds. The fruit hexane and dichloromethane fractions exhibited a greater antioxidant capacity and total phenolic content. The dichloromethane fraction of fruit demonstrated the most higher acetylcholinesterase inhibition (39.86 ± 2.63%), while the fruit hexane fraction displayed the best inhibition towards butyrylcholinesterase (84.02 ± 1.28%). Cytosolic isoenzymes of human carbonic anhydrase (hCA) I, and II isoenzymes were influentially suppressed by flower and fruit dichloromethane fractions with 1.650 and 2.020 μM IC_{50} values, respectively. The electronic microscopy analysis of secretory canals found that the small number of secretory canals were at leaf while the largest shape of secretory canals was at the fruit. The secretory canals of roots, aerial parts, and fruits include more monoterpene hydrocarbons, while the canals, existing in the flowers are qualified by a higher presence of sesquiterpenes β-caryophyllene (12.1%), germacrene D (4.5%) and ether octyl acetate (11.9%). The highest level of monoterpene β-phellandrene (47.6%) and limonene (8.2%) were found in the fruit essential oil. The next isolated compounds from fruits of *A. purpurascens* like stigmasterol, β-sitosterol, bergapten, and oxypeucedanin have shown high anticholinesterase and antioxidant activities.

1. Introduction

The studies from essential oils and extracts obtained of various medicinal plants against different pathogens and their health effects are actually from the middle age time (Ahmed and Beg, 2001). Medicinal plant extracts and their essential oils are confirmed to get diversified biological acitivities like antiflatulent, antipathogenic, antispasmodic, cytotoxic, and hepatoprotective, etc. (Ložienė et al., 2018; Furtado et al., 2018). Furthermore, biolog-

* Corresponding author at: Department of Plant Biology, Institute of Biology, Kiev national university of Taras Shevchenko, Volodymyrska, 64, Kyiv, Ukraine.
E-mail addresses: oksana.sytar@gmail.com, oksana.sytar@uniag.sk (O. Sytar).
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ically active compounds with antioxidant effects are known to assist in the inclusion of lipid peroxidative destruction, which has been infected in specific medical maladies, such as coronary atherosclerosis, carcinogenesis, Alzheimer's disease (AD) and other age-related diseases (Kurutas, 2016). The efficiency and utilization of medicinal plants regarding their therapeutic properties are well described, but their chemical composition is not always completely known. Secondary metabolites of medicinal and dietary herbs comprise phenolic acids, quinones, lignans, tannins, stilbenes, flavonoids, coumarins, alkaloids, terpenes, etc. Natural components of phenolic nature, some esters and terpenes exhibit a prominent role in acetylcholinesterase (AChE) inhibitory activity (Huane et al., 2009; Anand et al., 2012; Topal et al., 2016; Tetali et al., 2019). Diversified secondary metabolites bioactivities are responsible for their capacities to inhibitory activity against AChE (Karakaya et al., 2019). Two alkaloids-related synthetic compounds (galantamine and rivastigmine) proved to have a high prevention effect for cholinesterase (Kandiah et al., 2017; Bayındır et al., 2019; Boztas et al., 2019). At the same time, antiacetylcholinesterase effect of pyrrolizidine alkaloids and their possible toxicity are under discussion (Benamar et al., 2017; Moreira et al., 2018). Latterly, other secondary metabolites like coumarins, terpenoids, and sesquiterpenes have been investigated as novel inhibitors of AChE, with the purpose to explore lesser toxic natural plant compounds compared to the pyrrolizidine alkaloids (Dall'Acqua et al., 2010). The coumarin derivatives as potential inhibitors of acetylcholinesterase have been started to study via biological and molecular docking investigations as well (Singla et al., 2016; Sonmez et al., 2017; Senol et al., 2011).

The components such as bicarbonate (HCO_3^-) and carbon dioxide (CO_2) are necessary in live organisms. The enzyme carbonic anhydrase (CAs, EC 4.2.1.1) which contains a ring with zinc, mobilizes a shifting reaction between dehydration of HCO_3^- and hydration of CO_2 (Bayındır et al., 2019). CAs exhibit in a lot of organisms and possess a major act in physiological and pathological cases containing bone resorption, pH regulation, cancer, osteoporosis, calcification, glaucoma, carboxylation reactions, synthesis of HCO3−, and neurological diseases (Yamali et al., 2018; Burmaoğlu et al., 2019; Küçükoğlu et al., 2019).

The chemical structure of coumarin compound is 2H-1-benzopyran-2-one or 1,2-benzopyrone. The coumarin and derivatives of it are majorly found through plant diversity and many of them display beneficial and various effects (Musiliyu et al., 2008). Coumarin and thiocoumarins were in these days reported to behave as classical CA inhibitors (CAIs), and inhibition mechanism of them was figured out (Supuran, 2010). A coumarin derivative from the *Leionema ellipticum* (Rutaceae) with molecular formula $C_{15}H_{18}O_4$ demonstrated CA inhibitor (CAI) effect. Coumarins display a new structural motif comparatively entire CAIs researched to date (Maresca et al., 2009).

The representative of the Apiaceae family has been qualified by a great content of monoterpenes, sesquiterpene hydrocarbons, and phenolic compounds (Mimica et al., 2004; Dall'Acqua et al., 2010) and shown promising influences on the central nervous system (Sadaoui et al., 2018). Last year have been developed studies about the usage of plant extracts and essential oils from the Apiaceae family as powerful biopesticides (Evergetis et al., 2013). Some species belonging to the Apiaceae displayed inhibitory activity against acetylcholinesterase (AChE) (Adsersen et al., 2006). *Angelica* is a big genus of the Apiaceae, which nowadays comprising near 100–120 species, which grown at the regions of North America, Asia, Africa and Europe. But in eastern Asia is the most significant location of *Angelica* species with great biodiversity (Güner, 2012). Three species of *Angelica* genus which are *A. archangelica* L., *A. sylvestris* L. and *A. purpurascens* (Avé-Lall.) Gilli, grow in Turkey. *Xanthogalum purpurascens* Avé-Lall. is the synonym of *A. purpurascens* and *A. purpurascens* is known as 'melekotu' in Turkey (Nikonov and Baranauskaite, 1965). It was estimated that coumarins imperatorin, isoimperatorin, xanthotoxin, and bergapten from *Angelica officinalis* L. (Apiaceae) fruits were displayed strong inhibition towards butyrylcholinesterase (BChE) (Ferreira et al., 2006). Anticholinesterase and antioxidant activity parameters are still thought as a part of prophylaxis for the treatment of AD neurological ailments (Vasll'eva and Pimenov, 1991). Prior biochemical researches on *Angelica* sp. has indicated in plant tissue sterols such as ostruthol, xanthogalin, xanthalin, xanthogalol, xanthogalol acetate, agasyllin, isooxypeucedanin and β-sitosterol and coumarins (Sokolova and Nikonov, 1969; Ozek et al., 2006). It was reported that acyl- and pyrano coumarins were defined in the root and rhizomes ethanol extracts and also, *A. purpurascens* essential oil had antimicrobial activity (Mahboubeh et al., 2013). The data regarding biochemical composition in the different plant parts of *A. purpurascens* are not complex characterized by regarding the use of different solvents withal variegated polarities. The complex analysis of biochemical composition with the anatomical background (connected to the different plant parts), antioxidant potential and inhibitory activity against acetylcholinesterase and butyrylcholinesterase of different plant extracts and essential oils of *A. purpurascens* is missed. Therefore, the present study reports the anti-lipid peroxidation, antioxidant, anticholinesterase, and suppression of isoenzymes I and II of carbonic anhydrase of the methanol (MeOH) extract, dichloromethane (CH_2Cl_2), butanol (BUOH), *n*-hexane, ethyl acetate (EtOAc) and aqueous fractions and essential oils from different experimental plant parts, and isolated compounds (stigmasterol (1), β-sitosterol (2), bergapten (3) and oxypeucedanin (4)) of *A. purpurascens*. Moreover, the total phenolic content and essential oil composition of specimens were assessed. Also, secretory canals structures were investigated. In conclusion, BChE and AChE inhibitory activities of the most active compound oxypeucedanin were done via molecular docking.

2. Material and methods

2.1. Plant specimens

The author Songul Karakaya composed *Angelica purpurascens* (Avé-Lall.) Gilli. (Apiaceae) from Palandoken Mountains at fruity and flowering stages in 2017 and 2018 from Erzurum. Prof. Dr. Hayri Duman identified *A. purpurascens*. The voucher samples of *A. purpurascens* were put at Atatürk University Herbarium, Faculty of Pharmacy with the herbarium number of AUEF 1276. GPS Coordinates: 39°53′23N, 41°17′12E. The plant materials were dried in the press apparatus in an airy environment under the shade and sun. Until they dry the cardboard papers were changed every day.

2.2. Extraction and isolation

The samples of fruits (450 g), roots (100 g), flowers (100 g), and aerial parts (100 g) of *Angelica purpurascens* were dried in an airy environment under the shade and sun. The dry powdered mass of experimental samples were liquefied with methanol (3 × 200 mL) (3 times × 8 h) at room temperature with assistance of mechanical mixer (350 rpm). Farther steps are filtration of extracts and evaporation of solution via rotary evaporator. Then, extracts were dissolved in solution methanol: water (1:9) and were fractioned with 200 mL of CH_2Cl_2, EtOAc, BUOH and *n*-hexane, in turn. On the other hand, 50 g of each studied part of the plant were milled and saturated with 250 mL of distilled water at 30–35 °C for 8 h/3 days, individually. Farther was done filtration of received aqueous extracts. Also, aqueous extracts were frozen and

lyophilized. The amounts of the milled parts and extracts of *A. purpurascens* are displayed in Table 1.

The identification of isolated compounds from fruit hexane fraction was performed regarding Karakaya et al., 2018a. The effective hexane fraction of fruit was performed on a silica gel columns. The elution gradient was Hexane: EtOAc (100:0 → 0:100, v/v). The fractions A-C were attained. For fraction A was used next solvent systems for chromatography with Hexane: EtOAc (95:15 and 90:10). Fr. A was subjected to the Sephadex column LH-20 eluting with EtOAc to contribute compound 1 and compound 2 in replications. For fraction B was used solvent Hexane: EtOAc (85:15) on SiO_2 column and gave compound 3. Fr. C was finalized on the SiO_2 column with solution Hexane: EtOAc (80:20) and gave compound 4. Fig. 1 is presenting a chemical structure of compounds 1–4.

2.3. Identification of the isolated compounds

The nuclear magnetic resonance (NMR) techniques were saved on a Varian Mercury Plus at 100 MHz for ^{13}C NMR and 400 MHz for ^{1}H NMR through utilizing as the internal standard tetramethylsilane (TMS). The solvents were deuterated chloroform ($CDCl_3$). The high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was carried through Agilent 6530 Accurate-Mass Quadrupole Time-Of-Flight (Q-TOF) liquid chromatography (LC)/MS. UV spectra were quantified utilized cuvette spectrophotometer and Thermo Scientific Multiskan GO microplate. The infrared (IR) spectra were gone ahead of a Bruker VERTEX 70v Fourier Transform Infrared (FTIR) Spectrophotometer.

2.4. Essential oil isolation via GC-FID and GC/MS methods

The isolation of phases of essential oils was done by methods GC-FID and GC/MS (Karakaya et al., 2016). The dried and powdered fruits, flowers, roots and aerial parts, essential oil % yields of *Angelica purpurascens* and essential oils colors were displayed in Table 2.

2.5. Evaluation of total phenolic

The evaluation of total phenolic was accomplished with Folin-Ciocalteau reagent in some modifications (Sytar et al., 2018). The powdered freeze-dried samples (20 mg) were blended at 70 °C with 500 μL of 70% methanol (HPLC-Gradient grade, Sigma–Aldrich, Darmstadt, Germany) during 10 min. Farther step was done with extracts centrifugation for 10 min (3500*g*). The collection of supernatants was done in individual tubes. Under the same conditions pellets were re-extracted. The 20 μL combined supernatants was dissolved into 2 mL of distilled water for the total phenolic assay. To the 200 μL of dissolved extract was added 0.8 mL of sodium bicarbonate solution (75 g L^{-1}) and 1 mL of Folin–Ciocalteu reagent. The mixture was left at 25 °C for 60 min. The absorbance for total phenolic was detected at 765 nm with a Jenway UV/Vis 6405 spectrophotometer (Jenway, Chelmsford, UK). The results are described as gallic acid equivalents (GAE/g sample).

2.6. Estimation of antioxidant activity

The DPPH radical scavenging capacity of the examples were accomplished using the DPPH assay. First, 20 mg samples were processed for extraction, which was achieved in two steps. First, 1 mL of H_2O_{dist} was combined with the dried material in an Eppendorf tube. Specimens for 15 min at 95 °C were heated and then centrifuged for a further 5 min (12,000 rpm, 25 °C). The supernatant was relocated into the novel tube. Farther step was done with supernatant dilution with 1 mL of $H_2O_{dist.}$ The same heating and centrifugation procedures were done. Reagent stock solution (1×10^{-3} M) was made via dissolving of 22 mg of DPPH in 50 mL of methanol. The stock solution was kept until utilization at 20 °C. The DPPH reagent study solution (6×10^{-5} M) was made via a commixture of 6 mL of the stock solution with 100 mL of MeOH. Then, sample preparation was taken from 0.1 mL specimens and for 30 s vortexed with 3.9 mL of DPPH working solution. Further, the admixture was stabilized in the dark for 30 min at room temperature. The absorbance of the admixture was carried out at 515 nm. The solution of DPPH without specimen was as the control example. The percentile of DPPH radical-scavenging activity was estimated as follows: $[(A_{control} - A_{specimen})/A_{control}] \times 100$, where $A_{control}$ is the absorbance of control reaction (presentment whole reagents except the test compounds), and $A_{specimen}$ is the same absorbance.

2.7. Estimation of anti-lipid peroxidation capacity

The anti-lipid peroxidation capacity was realized following the procedure outlined in (Karakaya et al., 2018b). The analysis of linear regression was handled to specify IC_{50}values.

2.8. Analysis of AChE and BChE inhibition activities

The AChE and BChE inhibition activities were estimated following the methodology described in papers of Karakaya et al., 2018b; Kuzu et al., 2019. The current method was recapped 3 times for each plate. Entire data were expressed as the mean ± SE of 3 biological replications. Donepezil was used as a cholinesterase inhibitory standard. Acetylcholinesterase (AChE, E.C.1.1.7), butyrylcholinesterase (BChE, EC3.1.1.8), 5,5-dithiobis (2-Nitrobenzoic acid) (DTNB), acetylcholine iodide (AChI), butrylcholine iodide (BChI), and donepezil hydrochloride were supplied from Sigma-Aldrich. Inhibitory activities of the test compounds against AChE and BChE were estimated colorimetrically by the Ellman's method with some modifications using as the reference compound commercially available donepezil hydrochloride. The method is dependently on the evaluation of alterations at 405 nm of absorbance. In the Ellman method instead of the oxy ester of acetylcholine was utilized the thioester acetylthiocholine. Acetylthiocholine hydrolysis goes through the AChE enzyme as thiocholine and acetate. The resultant thiocholine transforms the DTNB utilized as the reagent to the nitrobenzoate, which had maximum absorbances at 405 nm. The specimens were fastened in

Table 1
Amounts of the yield of the crushing and gained extract of Angelica purpurascens (w/w, %).

Species	Extracts/Fractions (g)	Root	Aerial part	Flower	Fruit
Angelica purpurascens	MeOH	27.83	24.92	25.43	18.72
	Hexane	4.02	3.71	3.48	3.61
	CH_2Cl_2	9.91	9.67	9.89	6.08
	EtOAc	2.54	1.25	1.62	0.95
	BuOH	5.78	4.78	4.65	3.90
	Aqueous residue	5.09	4.92	5.55	4.01
	Lyophilized aqueous	8.04	7.50	7.96	7.30

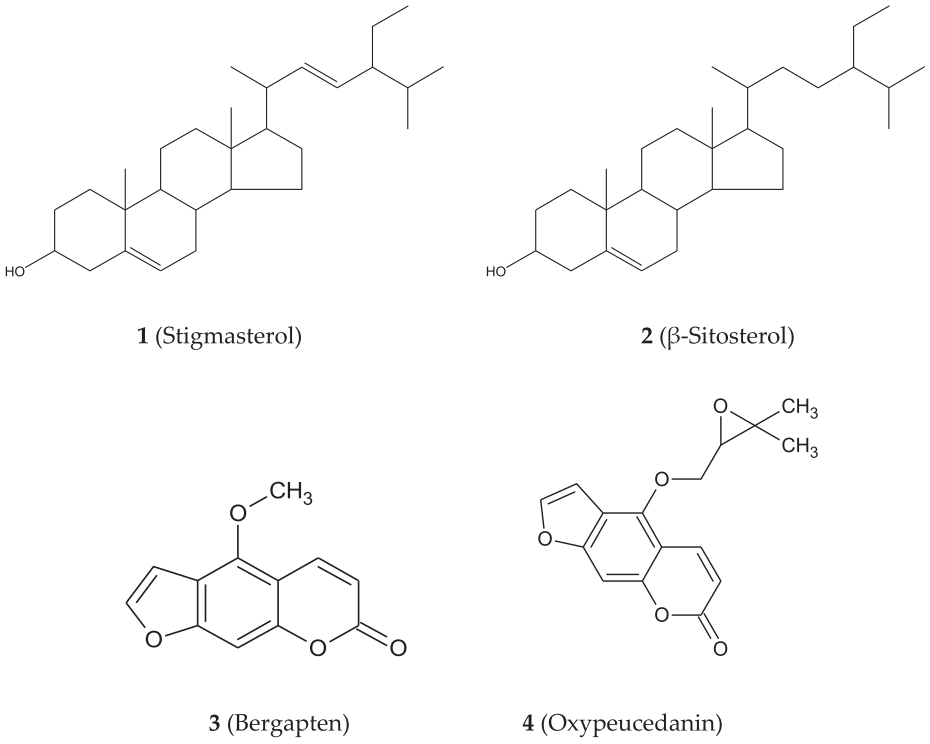

Fig. 1. Chemical structures of compounds 1–4 isolated from *Angelica purpurascens*.

Table 2
Anti-lipid peroxidation activities of *Angelica purpurascens* (TBA test).

Tested samples	IC_{50} values (μg/mL) ± SD*			
	Root	Aerial part	Flower	Fruit
MeOH	265.57 ± 3.46	322.54 ± 3.28	478.66 ± 3.02	145.26 ± 2.67
Hexane	390.16 ± 2.27	389.21 ± 2.67	500>	51.93 ± 1.86
CH_2Cl_2	410.18 ± 3.02	269.87 ± 3.25	187.48 ± 3.66	87.24 ± 1.91
EtOAc	318.96 ± 3.32	209.44 ± 2.47	500>	209.26 ± 3.65
BuOH	278.65 ± 3.09	500>	377.35 ± 4.19	398.43 ± 2.17
Aqueous residue	500>	467.55 ± 1.88	500>	487.33 ± 2.86
Lyophilized aqueous	500>	500>	500>	500>
Essential oils	89.37 ± 1.78	298.16 ± 2.66	189.19 ± 3.26	224.62 ± 1.84
Stigmasterol	500>			
β-Sitosterol	492.30 ± 2.03			
Bergapten	56.99 ± 3.87			
Oxypeucedanin	91.27 ± 2.76			
Chlorogenic acid	12.98 ± 4.89			
Propyl gallate	3.44 ± 2.05			
Rutin	9.65 ± 3.09			

* Standard deviation.

DMSO and then diluted in the solution of Tris buffer (50 mM, pH 8.0) to diversified concentrations. 25 μL of diversified four concentration of the compound inhibitor, (2.5, 5, 10 and 20 μg/mL) were stirred in 50 μL of the solution of Tris buffer, 125 μL solution of DTNB (3 mM), AChE or BChE enzymes at the concentration of 0.2 U/mL. 25 μL of such admixture was annexed to the wells and the admixture was permitted at 37 °C to incubate for 15 min. Incubation during 15 min was done that substrate solution of AChI or BChI (concentration of 15 mM in a volume of 25 μL) were take over to each of the wells. The absorbance of the admixtures reaction was measured at 405 nm with a microplate reader (Bio-Tek ELx800, USA) getting an evaluation each 45 s. This processing was recapped for each plate 3 times. Entire data were stated as mean ± SE of three autonomous analysis.

2.9. Molecular docking studies

Schrödinger's software suite (Maestro 11.8) was employed for docking simulations. The three-dimensional structure of AChE (PDB ID: 1-EVE) and BChE (PDB ID: 1P0I) were received from the Protein Data Bank. The grid box was defined by binding sites of inhibitors (In 1EVE: binding site of donepezil, centers of grid box: X = 2.8, Y = 64.5, Z = 67.9; In 1P0I: binding site of butanoic acid, centers of grid box: X = 139.4, Y = 113, Z = 41.71). The enzyme structures were prepared using the PROPKA software in which water molecules present were removed from the structure part, atoms of hydrogen were combined to the PDB structures. The pH was 7. Finally, reasonable minimization was performed with optimization of force field liquid simulations (OPLS3e). The structure of

the compound (oxypeucedanin) was established using module of Macro model in the Schrödinger software suite (Maestro 11.8). Then the structure of compounds minimized using the minimization method of Ploak-Ribiere conjugates gradient (PRCG). All compounds were docked to the target enzymes using Glide/XP docking protocols. Glide score was used as higher criteria for the best-docked ligands.

2.10. Protein preparation

The protein preparation of the examples was analyzed following the procedure outlined in (Karakaya et al., 2019). Protein Data Bank contributed the three-dimensional complex AChE structures (PDB ID: 1EVE) and BuChE (PDB ID: 1P0I) (Kryger et al., 1999; Nicolet et al., 2003). The protein structures were made regarding to the Protein Preparation Wizard panel tool of Scrödinger süite (Maestro 11.8). The first water molecules (>5A radius) and other small molecules were derived from crystal structures. The hydrogen atoms were annexed. The physiological PH was regulated at 7. After all, the reasonable minimization was carried out with the annexed hydrogen atoms to OPLS3e.

2.11. Ligand preparation

The preparation of ligand of the examples was analyzed following the procedure outlined in (Karakaya et al., 2019). Ligand preparation panel in the Ligprep programme was used for ligands docking. The receptor grids generation panel was used for the grid files. After all, Glide Ligand Docking panel was utilized for docking studies.

2.12. Carbonic anhydrase isoenzymes purification and inhibition assays

In this research, isoenzymes hCA I and II were isolated from erythrocytes of fresh human blood using via Sepharose-4B-L-Tyro sine-sulfanilamide affinity chromatography. To control of the hCA I and II isoenzymes purity, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used (Biçer et al., 2019). The analysis of isoenzymes hCA I and II was searched through the Verpoorte method (Verpoorte et al., 1967). The enzyme unit of CA esterase activity is determined in 1 min as hydrolysis of 1 μmol PNA to p-nitrophenol and acetate (Okten et al., 2019). The method is established upon the p-nitrophenyl acetate (PNA) hydrolysis to p-nitrophenol via CA isoenzymes. A maximum absorbance at 348 nm with use a spectrophotometer (UV-1800 Shimadzu, Kyoto, Japan) possesses p-nitrophenol. The protein content was measured at 595 nm by Bradford method (Huseynova et al., 2018). As a reference protein was handled bovine serum albumin (BSA). As a positive control for isoenzymes hCA I, and II was utilised acetazolamide (AZA). The values of IC_{50} were estimated from versus compounds plots activity (%). The three various concentrations were used to acquire Ki values. The graphs of Lineweaver-Burk were made and calculations was done as well (Lineweaver and Burk, 1934).

2.13. Microscopic analysis

Analysis of the examples used the light microscope (Zeiss 51425 camera connected to the Zeiss 415500-1800-000 light microscope) with use also electron microscopy (SEM) was done (Karakaya et al., 2019). The electron microscopy (Jeol JSM 6490LV scanning electron microscope) is presented at the Research Center Laboratory of Turkish Petroleum International Company (TPAO), Ankara.

2.14. Statistical analysis

The results are displayed as mean ± SE, and variations analysis were statistically carried out via ANOVA one-way analysis determined through way of Bonferroni complementary analysis ($p < 0.05$), which was conceived to demonstrate statistic significance. All results were repeated at least three times.

3. Results and discussion

3.1. Extraction, isolation, and identification of the compounds

The anti-lipid peroxidation, antioxidant, anticholinesterase, and inhibition of isoenzymes I and II for carbonic anhydrase of the methanolic extracts of different plant parts of *Angelica purpurascens*, fractions of different polarities, essential oils (roots part, aerial part, flowers and fruits), and isolated compounds including stigmasterol (**1**), β-sitosterol (**2**), bergapten (**3**) and oxypeucedanin (**4**) from fruits were estimated. Also, AChE and BChE inhibitory potential of the most active component oxypeucedanin were determined through molecular docking.

Stigmasterol **(1)**. White powder, $C_{29}H_{48}O$. ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.27 (C-1), 28.91 (C-2), 71.84 (C-3), 42.30 (C-4), 140.76 (C-5), 121.73 (C-6), 31.66 (C-7), 31.91 (C-8), 50.16 (C-9), 36.52 (C-10), 24.38 (C-11), 39.70 (C-12), 42.34 (C-13), 56.80 (C-14), 25.42 (C-15), 29.71 (C-16), 55.99 (C-17), 12.32 (C-18), 19.41 (C-19), 40.51 (C-20), 21.10 (C-21), 138.34 (C-22), 129.31 (C-23), 51.26 (C-24), 31.89 (C-25), 19.01 (C-26), 19.07 (C-27), 29.71 (C-28), 11.91 (C-29). ^1H NMR (400 MHz, $CDCl_3$) δ 3.57 (1H, m, H-3), 5.38 (1H, bd, J = 5.16 Hz, H-6), 0.71 (3H, s, H-18), 1.05 (3H, s, H-19), 5.19 (1H, dd, J = 15.1/8.6 Hz, H-22), 5.06 (1H, dd, J = 15.1/8.6 Hz, H-23), 0.84 (3H, d, J = 7.1 Hz, H-26), 0.83 (3H, d, J = 7.0 Hz, H-27).

β-Sitosterol **(2)**. White powder, $C_{29}H_{50}O$. ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.28 (C-1), 31.67 (C-2), 71.81 (C-3), 42.31 (C-4), 140.77 (C-5), 121.74 (C-6), 31.68 (C-7), 31.92 (C-8), 50.16 (C-9), 36.53 (C-10), 21.24 (C-11), 39.80 (C-12), 42.24 (C-13), 56.89 (C-14), 25.32 (C-15), 28.26 (C-16), 56.09 (C-17), 12.02 (C-18), 19.42 (C-19), 36.16 (C-20), 18.93 (C-21), 33.98 (C-22), 26.12 (C-23), 45.87 (C-24), 29.18 (C-25), 19.82 (C-26), 19.43 (C-27), 23.09 (C-28), 12.12 (C-29). ^1H NMR (400 MHz, $CDCl_3$) δ 3.57 (1H, m, H-3), 5.38 (1H, bd, J = 5.1 Hz, H-6), 0.71 (3H, s, H-18), 1.05 (3H, s, H-19), 0.96 (3H, d, J = 6.6 Hz, H-21), 0.85 (3H, d, J = 7.1 Hz, H-26), 0.82 (3H, d, J = 7.0 Hz, H-27).

Bergapten **(3)**. White powder, $C_{12}H_8O_4$. ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.23 (C-2), 112.54 (C-3), 139.25 (C-4), 149.50(C-5), 112.67 (C-6), 158.41 (C-7), 93.90 (C-8), 152.78 (C-9), 106.47 (C-10), 144.78 (C-2′), 105.08 (C-3′), 60.06 (OMe). ^1H NMR (400 MHz, $CDCl_3$) 4.29 (3H, s, OMe), 6.27 (1H, d, J = 9.81 Hz, H-3), 8.19 (1H, d, J = 9.79 Hz, H-4), 7.18 (1H, s, H-8), 7.63 (1H, d, J = 2.11 Hz, H-2′), 7.06 (1H, bs, H-3′). ESIMS m/z 217.20 $[M + H]^+$.

Oxypeucedanin (**4**). White powder, $C_{16}H_{14}O_5$. ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.03 (C-2), 113.17 (C-3), 139.03 (C-4), 148.74 (C-5), 114.35 (C-6), 158.27 (C-7), 94.86 (C-8), 152.74 (C-9), 107.51 (C-10), 145.80 (C-2), 104.81 (C-3), 71.73 (C-1a), 73.77 (C-2a), 74.73 (C-3a), 26.65 (C-4a), 25.38 (C-5a). ^1H NMR (400 MHz, $CDCl_3$) δ 6.28 (3C-H), 8.20 (4C-H), 7.14 (8C-H), 7.58 (2C-H), 6.91 (3C-H), 3.94 (—CH═), 1.35 (CH3-H), 4.60 (CH2-H). ESIMS m/z 286.28 $[M + H]^+$. Chemical structures of compounds **1**–**4** were represented in Fig. 1.

3.2. Antioxidant activity assay

Analysis of materials of various studied plant parts regarding antioxidant effect was done. It is known that antioxidant impact

can be connected with the phenolic compounds presence (Sytar et al., 2018) therefore we were interested to study total phenolic content of methanolic extracts of *Angelica purpurascens* (Fig. 2). In the root and fruit extracts was observed the utmost total phenolic level. The lower phenolics content was observed in extracts from the aerial part (42.42 mg GAE g^{-1} DW). The results of DPPH antioxidant activity measurements with displayed that the roots and fruits extracts had the best effect in comparison to the aerial part of *A. purpurascens* extracts (Fig. 3).

3.3. Anti-lipid peroxidation activity

TBA analysis results were seen as presented IC_{50} values (μg mL^{-1}) in Table 2. The fruit fractions of hexane, CH_2Cl_2 and root essential oil had the best anti-lipid peroxidation potential (IC_{50} = 51.93, 87.24 and 89.37 μg/mL, in order of) in TBA analysis. Besides, amongst the isolated bergapten and oxypeucedanin had a strong anti-lipid peroxidation effect with 56.99 and 91.27 μg/mL IC_{50} values. Though, the results of the anti-lipid peroxidation effect of sample extracts from various parts of *A. purpurascens* were considered high compared to the standards (Table 3). Anti-lipid peroxidation capacity of many examples exhibited on liposome in proportion to the chlorogenic acid and rutin. The correlation coefficient between total phenolics content and anti-lipid peroxidation activity is considerable high (0.95). We presume that it may also depend on the existence of another metabolite responsible for antioxidant capacity.

3.4. Analysis of AChE and BChE inhibition activities

The virtue of colorimetric Ellman's method was utilized to estimate the anticholinesterase activity of specimens (Ellman et al., 1961), with several varieties whereby donepezil commercially present as standard (Yerdelen and Tosun, 2015). The MeOH, hexane, CH_2Cl_2, and EtOAc extracts and whole essential oils of presented significant inhibition towards BChE. The fruit hexane and CH_2Cl_2 fractions and root essential oil have been exhibited strong inhibition towards BChE (84.02 ± 1.28, 76.43 ± 2.98 and 74.98 ± 2.24%, in order of) at 20 mg/mL. Also, hexane and CH_2Cl_2 fractions of fruit indicated considerable inhibition against AChE (39.86 ± 2.63 and 29.49 ± 3.08%, in order of) at 20 μg/mL. The oxypeucedanin displayed significant inhibition towards AChE (19.36 ± 1.87%) and BChE (36.89 ± 1.23%) among the purified compounds. Whole essential oils showed AChE and BChE inhibition activities. At the same time the only root fraction among the aqueous residue fractions shown AChE and BChE inhibition activities, also only root

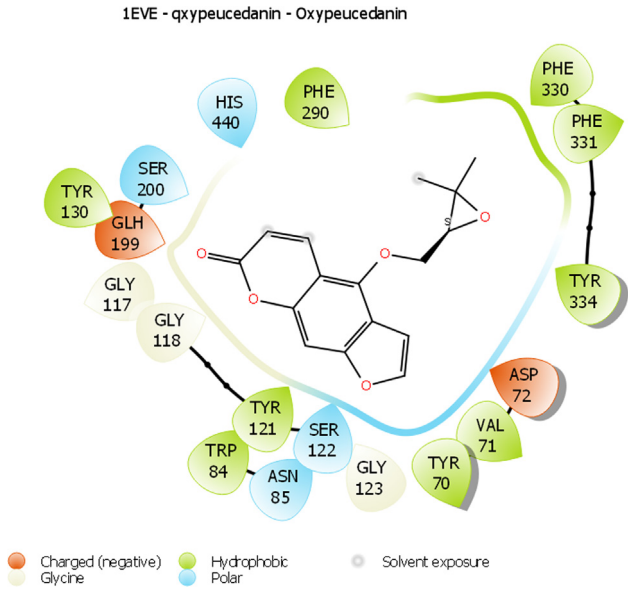

Fig. 3. Schematic description of the principal interaction of oxypeucedanin with AChE (1-EVE). Hydrophobic interactions are shown by green color, polar interactions are shown by light blue color and negatively charged residues by red color.

lyophilized aqueous fraction showed activity against BChE. On the other side, among the BuOH fractions, the only flower represented no BChE inhibition potential. The stigmasterol and β-sitosterol amongst the isolated compounds had no activity against AChE and did not show considerable inhibition towards BChE. The results were displayed in Table 3.

3.5. Molecular docking studies

For molecular docking studies was chosen oxypeucedanin as a sample compound that shown the highest AChE and BChE inhibition. For the present medications for the AD treatment, though they vary to each other according to the structure of their active sites, both AChE and BChE are the potency goals to be fixated. The AChE active site contains two principal binding sites, named the peripheral anionic site (PAS) and the catalytic active site (CAS). CAS is situated at the base of the enzyme active-site gorge and shows the enzyme catalytic effect. Otherwise, PAS is situated at the entry of the active site-gorge and augments the enzyme catalytic effect by heading acetylcholine toward the effective spot

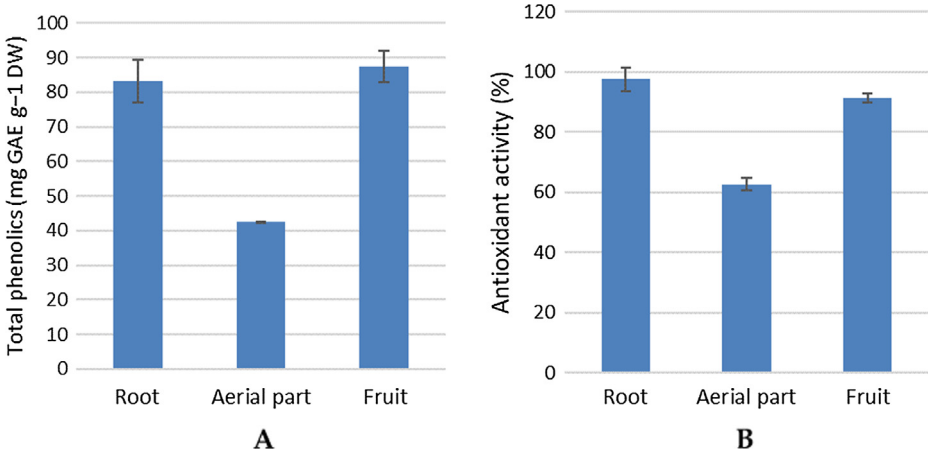

Fig. 2. The total phenolic contents (A) and antiradical scavenging activity (DPPH test) (B) of methanolic extracts of *Angelica purpurascens*.

Table 3
In vitro AChE and BChE inhibitory effects of *Angelica purpurascens* at the same concentration (20 μg/mL).

Samples	Percentile of inhibition ± S.E.M[a] against AChE and BChE									
	Aerial part		Root		Flower		Fruit		Compounds	
	AChE	BChE	AChE	BChE	AChE	BChE	AChE	BChE	AChE	BChE
MeOH	[c]	32.42 ± 2.23	7.46 ± 2.98	26.67 ± 3.87	[b]	31.39 ± 2.77	16.87 ± 3.91	69.35 ± 1.79		
Hexane	4.21 ± 3.48	21.39 ± 2.36	2.26 ± 4.09	35.21 ± 3.03	8.27 ± 2.66	21.67 ± 2.78	39.86 ± 2.63	84.02 ± 1.28		
CH_2Cl_2	[c]	58.61 ± 2.87	[b]	68.77 ± 2.34	26.55 ± 2.67	55.20 ± 3.76	29.49 ± 3.08	76.43 ± 2.98		
EtOAc	4.91 ± 1.99	16.56 ± 3.08	[b]	31.03 ± 1.87	[c]	38.22 ± 2.01	11.76 ± 2.67	44.41 ± 2.77		
BuOH	[b]	12.33 ± 1.66	[c]	23.50 ± 3.80	5.78 ± 2.68	[b]	[b]	19.28 ± 1.23		
Aqueous residue	[b]	[c]	3.88 ± 2.91	16.48 ± 1.66	[b]	[b]	[c]	[b]		
Lyophilized aqueous	[b]	[c]	[b]	7.90 ± 2.38	[b]	[c]	[b]	[b]		
Essential oils	6.48 ± 2.87	28.53 ± 3.41	19.60 ± 3.10	74.98 ± 2.24	5.49 ± 1.88	59.61 ± 1.57	9.18 ± 3.12	68.48 ± 3.06		
Stigmasterol									[c]	13.34 ± 2.99
β-sitosterol									[c]	9.93 ± 1.78
Bergapten									18.98 ± 2.98	31.00 ± 3.02
Oxypeucedanin									19.3 ± 1.87	36.89 ± 1.23
Donepezil									82.45 ± 2.64	90.33 ± 4.16

[a] Standard error mean.
[b] No activity.
[c] Not detected owing to turbidity in the wells of microplate.

(Kilic et al., 2018). Oxypeucedanin exhibits high dock score against acetylcholinesterase (1EVE) with −7.523 kcal/mol and for 1P0I with −4.232 kcal/mol) (Fig. 4). Hydrophobic interaction has occurred with residues on the epoxide ring of PHE330 with the groups of methyl, PHE331 with the groups of methyl on the epoxide ring, TYR334, PHE290, TYR130, TYR121, TRP84, TYR70, and VAL71. The polar interactions were realized by HIS440 with the coumarin ring, SER200 with the coumarin ring, SER122 with the furan ring, and ASN85 with the furan ring. As a supplement, negative load interactions were observed between GLH199, ASP72 residues and oxypeucedanin. On the other hand, in complex BChE and oxypeucedanin, hydrophobic interaction has developed with residues of TYR332, PHE329, PHE398, TRP231, VAL288, LEU286, PRO285, ALA277 (Fig. 4). Besides, stacking π-π interaction was formed between phenyl ring of PHE329 (5.35 Å) and pyran ring of the oxypeucedanin. Another stacking π-π interaction was seen among the phenyl ring of TYR332 and the furan group of the molecule (5.22 Å). Also, the polar interactions were completed by ASN289, SER287, GLN119, THR120, and ASN68.

3.6. Carbonic anhydrase isoenzymes I and II inhibition potential

The CA isoforms (hCA I and II) were utilized in this investigation. The dichloromethane fractions of the plant showed high anticholinesterase activity, so we evaluated the activity of enzymes hCA I, and II of the dichloromethane fractions of aerial part, root, flower, and fruit the plant. Also, the coumarins (bergapten and oxypeucedanin) which isolated from the most active fraction of fruit were evaluated against hCA I, and II enzymes.

The findings of hCA I, and II inhibition values were presented in Table 4. When experimented entire other kinds of CAIs, like inorganic complexed anions, thiophenols, sulfonamides or phenols preparation time of 10–15 min is let for the enzyme-inhibitor adduct formation. Under the similar conditions studying shown

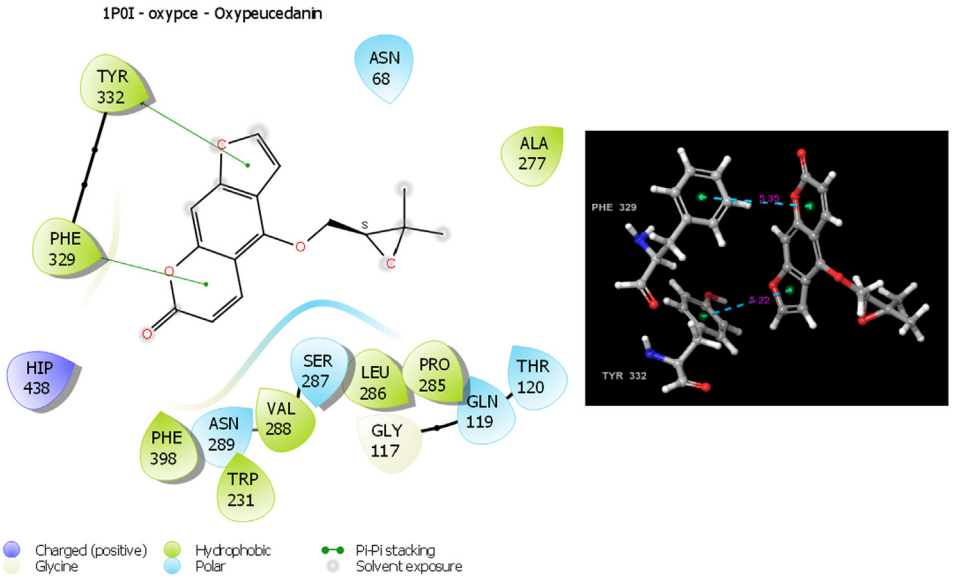

Fig. 4. Schematic description of the principal interaction of oxypeucedanin with BChE (1-P0I). Hydrophobic interactions are shown by green color, polar interactions are shown by light blue color and negatively charged residues by red color.

Table 4
Human carbonic anhydrase isoenzymes (hCA I and II) inhibition values (μM).

Compounds	IC_{50} (μM)			K_I (μM)		
	hCA I	r^2	hCA II	r^2	hCA I	hCA II
Bergapten	7.294	0.9825	8.25	0.9870	4.791 ± 0.57	12.823 ± 1.92
Oxypeucedanin	6.728	0.9833	5.290	0.9843	5.325 ± 1.08	8.848 ± 1.05
DAAp	2.145	0.9825	2.750	0.9755		
DAR	2.466	0.9861	2.576	0.9723		
DAFl	1.650	0.9870	2.520	0.9741		
DAFr	2.325	0.9895	2.020	0.9868		
AZA	1.008	0.9935	0.222	0.9943	0.734 ± 0.12	0.159 ± 0.04

DAp: Dichloromethane fraction of aerial part of *Angelica purpurascens,* ADR: Dichloromethane fraction of root of *A. purpurascens,* DAFl: Dichloromethane fraction of flower of *A. purpurascens,* LFr: Dichloromethane fraction of *fructus of A. purpurascens.*

with coumarins and extracts only a weak micromolar inhibition. The coumarins and extracts were analyzed for their inhibition capacities towards isoenzymes hCA I, and II, demonstrating, in general, an efficacious inhibition. It has been reported that establishing of isoenzyme-specific CAIs should be greatly helpful in acquiring new categories of medicaments deprived of varied undesirable side-effects (Gul et al., 2016). This is the initial research on the inhibitory effects of bergapten and oxypeucedanin and *Angelica purpurascens* dichloromethane fractions opposing hCA I and II utilizing esterase activity. The high doses of hCA I may be found in the gastrointestinal tract and blood. The low level of cytosolic isoenzyme hCA I found everywhere in the body. Although hCA I, in particular, is present in many tissues, it has been shown that current isozyme is associated with brain and retinal edema, and its prevention can be a significant tool in combating these conditions. Furthermore, if the *K*i value of a compound being analyzed is lower than 50, low inhibition is considered and this inhibitor has been confirmed to be ineffective against hCA I.[31] The data acquired from this research clearly displays that isolated coumarins possess efficient profile of inhibition towards slow cytosolic hCA I isoform, and cytosolic dominant quick hCA II isozymes with low micromolar range. Bergapten and oxypeucedanin in the micromolar scope connect to hCA I. K_i values are in the diapason of 4.791 ± 0.57 and 5.325 ± 1.08 μM for hCA I isoenzyme. Otherwise, AZA being a wide range specificity CA inhibitor because of its extensive CAs inhibition, exhibited K_i value of 0.734 ± 0.12 μM against hCA I. Bergapten, possessing 5-methoxy was observed to have the highly potential hCA I inhibitor (K_i: 4.791 ± 0.57 μM). Among the extracts, the CH_2-Cl_2 fraction of the flower showed the highest inhibition towards hCA I with 1.650 and the dichloromethane fraction of fruit showed the highest inhibition towards hCA II with 2.020 μM IC_{50} values.

CA II plays a prominent role in certain ailments containing epilepsy, glaucoma, oedema and presumably altitude ailments (Yiğit et al., 2019; Zengin et al., 2018). The compound oxypeucedanin was estimated to have the highly potential hCA II inhibitor (K_i: 8.848 ± 1.05 μM). Though the medium value of K_i of isolated coumarins was observed to be 5.058 μM for hCA I. On the other hand, the average K_i value of the coumarins for hCA II was found to be 10.8355 μM. Such findings displayed that the isolated coumarins possess greater inhibition affinity against hCA I compared to the hCA II isoenzyme.

Coumarin derivatives were in these days exhibited to form an accurately novel group of the zinc metalloenzyme carbonic anhydrase inhibitors, which are hydrolyzed to 2-hydroxycinnamic acids within the CA active site (Maresca et al., 2009).

3.7. GC-FID and GC/MS methods for essential oil isolation

The next step of the presented complex study was based on the screening of biochemical composition and capacities of different parts essential oils from *Angelica purpurascens*. The colors of essential oils and % yields of the species and shown in Table 4. The color of essential oils from different parts of *A. purpurascens* was different. The roots and fruits essential oils of *A. purpurascens* shown white color however flower and aerial part essential oils got light brown and yellow, in turn. The fruit % yield was lesser level compared to the aerial part, flowers and roots. The best findings were observed in the root (Table 5).

A total of 80 identified compounds composing 95.6% of the oil of the aerial parts of *A. purpurascens* were defined. α-Pinene and β-phellandrene were the primal constituents, amounting to 33.7%, and 4.3%, in turn. A total of eighty-four compounds composing 93.2% of the oil of the roots of *A. purpurascens* were described. In the essential oil was identified α-pinene with 32.8% and α-bisabolol with 29.0% as the principal abundant compounds. In the essential oil of flowers of *A. purpurascens* found 60 compounds with 82.5% of the oil. The primary constituents were identified to be β-caryophyllene (12.1%), octyl acetate (11.9%), (*Z*)-β-ocimene (7.6%), γ-terpinene (4.7%), germacrene D (4.5%). The study on the essential oil of fruits of *A. purpurascens* has been established 52 essential compounds with 96.5% of the hexyl octanoate. The hexyl octanoate at 55.1% found as major abundant compound, farther followed by octyl octanoate (14.8%), hexyl hexanoate (10.8%). The essential oil compositions are exhibited in Table 5. Monoterpene hydrocarbons (it was found mostly in the fruit (75.1%)), sesquiterpene hydrocarbons (34.5% most of the root), and oxygenated sesquiterpenes (19.5% most of the flower) in the studied essential oils found to be the main group of compounds (Table 6).

3.8. Microscopic analysis

The GC screening of the essential oils biochemical composition we would like to connect with the presence of some active biological compounds in secretory channels of a specific parts of the plant. In this case is important to use microscopic analysis for detailed characterization of secretory channels. The pedicels, peduncles, rays, fruits, and leaf of *A. purpurascens* had micrographs which were gained from 70% ethanol specimen via utilizing light (Figs. 5–9) and from the dried examples via Scanning Electron Microscopy Jeol JSM 6490LV (Fig. 10a–f). In the cortex at peduncle found that the quantity of secretory canals in the center is almost the same. The secretory canals are observed in the cortex of the ray and pedicel. The canals number was greater at ray however they are larger at pedicel. The fruit secretory canals are wide and very massive. The secretory canals are found at the vascular bundle

Table 5
Essential oil yields of *Angelica purpurascens* (w/v, %).

Plant Parts	Crushed (g)	Yields	Color	Collection Time
Aerial	234	0.427	Light yellow	2017
Root	238	1.177	White	2017
Flower	64	0.016	Brown	2018
Fruit	72	0.014	White	2018

Table 6
Percentage composition (%) of the essential oils from *Angelica purpurascens*.

RRI	Compound	Aerial part (%)	Root (%)	Flower (%)	Fruit (%)
1032	α-Pinene	tr	20.5	1.7	–
1048	2-Methyl-3-buten-2-ol	–	0.1	–	–
1076	Camphene	–	0.1	0.1	–
1093	Hexanal	–	0.1	–	–
1118	β-Pinene	0.2	63.3	0.2	–
1132	Sabinene	–	0.6	tr	–
1159	δ-3-Carene	–	6.4	0.1	–
1174	Myrcene	–	2.2	0.9	–
1176	α-Phellandrene	–	0.4	–	–
1203	Limonene	0.3	–	1.1	–
1218	β-Phellandrene	–	–	0.5	–
1244	2-Pentyl furan	–	tr	–	–
1246	(*Z*)-β-Ocimene	–	–	0.5	–
1255	γ-Terpinene	–	0.1	tr	–
1266	(*E*)-β-Ocimene			0.5	
1280	*p*-Cymene	0.7	0.4	0.1	–
1286	Isoterpinolene	–	0.1	–	–
1290	Terpinolene	–	1.0	0.1	–
1360	Hexanol	0.4	–	tr	0.7
1400	Nonanal	0.2	–	–	–
1424	Hexyl butyrate	0.2	–	1.6	0.9
1444	Ethyl octanoate	–	–	0.1	0.1
1452	α,*p*-Dimethylstyrene	–	0.1	–	–
1477	4,8-Epoxyterpinolene	–	tr	–	–
1483	Octyl acetate	–	–	14.7	0.9
1497	α-Copaene	0.1	–	tr	–
1535	β-Bourbonene	0.2	–	tr	0.1
1553	Linalool	–	–	0.3	–
1562	Octanol	0.2	–	3.2	0.3
1571	*Trans*-*p*-Menth-2-en-1-ol	–	–	1.1	–
1582	*Cis*-Chrysanthenyl acetate	–	–	1.0	–
1586	Pinocarvone	–	0.3	–	–
1589	β-Ylangene	tr	–	–	–
1591	Bornyl acetate			1.0	
1600	β-Elemene	0.3	–	tr	0.1
1611	Terpinen-4-ol	–	0.1	–	–
1612	β-Caryophyllene	–	–	1.8	0.1
1617	Lavandulyl acetate	–	–	0.3	–
1617	Hexyl hexanoate	0.4	–	7.6	10.8
1623	Octyl butyrate	–	–	0.8	0.3
1634	Octyl 2-methyl butyrate	–	–	0.4	tr
1638	*Cis*-*p*-Menth-2-en-1-ol	–	–	0.4	–
1647	Ethyl decanoate	–	–	–	tr
1648	Myrtenal	–	0.2	–	–
1668	Citronellyl acetate	–	–	0.4	–
1670	*Trans*-Pinocarveol	0.2	0.3	–	–
1687	Decyl acetate	–	–	0.3	–
1687	Methyl chavicol	–	0.1	–	–
1687	α-Humulene	–	–	0.1	–
1690	Cryptone	0.1	–	–	–
1704	γ-Curcumene	–	–	0.7	–
1704	γ-Muurolene	0.2	–	–	0.1
1706	α-Terpineol	0.1	0.2	0.3	–
1709	α-Terpinyl acetate	–	–	0.1	–
1718	Hexyl heptanoate	–	–	–	tr
1722	Dodecanal	0.1	–	–	–
1726	Germacrene D	–	–	3.7	0.2
1740	α-Muurolene	0.1	–	–	–
1755	Bicyclogermacrene	–	–	2.0	–
1758	*Cis*-Piperitol	–	–	0.2	–
1765	Geranyl acetate	–	–	0.1	–
1772	Citronellol	–	–	0.2	–
1773	δ-Cadinene	0.3	–	–	0.3
1776	γ-Cadinene	0.1	–	–	0.1
1786	*Ar*-Curcumene	–	–	0.1	0.1
1804	Myrtenol	–	0.2	–	–
1829	Hexyl octanoate	2.8	–	1.4	55.1
1829	Octyl hexanoate	–	–	0.4	–
1849	Cuparene	0.4	–	–	–
1856	(*Z*)-4-octenyl hexanoate	–	–	0.5	–
1856	*m*-Cymen-8-ol	–	0.1	–	–
1864	*p*-Cymen-8-ol	0.3	0.2	–	–
1871	1-Undecanol	0.9	–	–	–
1878	2,5-Dimethoxy-p-cymene	–	tr	–	tr
1893	Geranyl isovalerate	–	–	0.3	–
1893	Dodecyl acetate	–	–	5.5	–
1904	Geranyl 2-methyl butyrate	0.1	–	–	–
1933	Neryl valerate	0.1	–	–	0.1
1933	Tetradecanal	0.3	–	–	–
1945	1,5-Epoxy-salvial(4)14-ene	0.2	–	–	0.1
1958	(*E*)-β-Ionone	–	–	0.1	0.1
1973	1-Dodecanol	7.8	–	3.3	0.1
2000	Citronellyl hexanoate	–		0.2	–
2008	Caryophyllene oxide	0.8	–	0.3	0.3
2020	Octyl octanoate	0.7	–	0.2	14.8
2050	(*E*)-Nerolidol	0.2	–	–	–
2069	Germacrene D-4β-ol	–		0.4	–
2084	Octanoic acid	0.2	–	–	0.4
2071	Humulene epoxide-II	0.3	–	–	–
2080	1,10-*diepi*-Cubenol	0.3	–	–	0.1
2088	1-*epi*-Cubenol	0.2	–	–	–
2096	Elemol	4.1	–	–	1.5
2130	Salviadienol	0.3	–	–	–
2131	Hexahydrofarnesyl acetone	3.0	–	0.1	0.6
2144	Spathulenol	0.9	–	0.8	0.4
2179	Tetradecanol	4.2	–	0.1	–
2183	γ-Decalactone	–	–	–	0.1
2185	γ-Eudesmol	–	–	–	1.0
2192	Nonanoic acid	0.5	–	–	0.2
2209	T-Muurolol	–	–	0.6	0.3
2219	δ-Cadinol	0.3	–	–	–
2232	α-Bisabolol	0.8	–	–	0.1
2250	α-Eudesmol	1.1	–	–	0.3
2255	α-Cadinol	3.8	–	–	0.8
2262	Ethyl hexadecanate	0.3	–	–	0.1
2271	(2*E*,6*E*)-Farnesyl acetate	–	–	1.8	0.2
2278	Torilenol	0.4	–	–	–
2289	Oxo-α-Ylangene	0.1	–	–	–
2296	Myristicine	4.7	0.8	–	0.9
2298	Decanoic acid	0.7	–	–	0.4
2300	Tricosane	–	–	1.8	–
2320	*Unknown I (bp98, M⁺ 270)*	0.8	–	12.6	0.5
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol II*)	0.3	–	–	–
2369	(2*E*,6*E*)-Farnesol	–	–	1.1	–
2369	Eudesma-4(15),7-dien-4β-ol	1.5	–	–	–
2372	*Unknown II (bp81, M⁺ 208)*	tr	–	4.1	tr
2384	1-Hexadecanol	1.5	–	–	0.4
2384	Dill apiole	4.1	0.2	–	0.7
2400	Undecanoic acid	0.4	–	–	0.1
2450	*Unknown III (bp98, M⁺ 228)*	0.5	–	7.2	0.2
2475	1-Heptadecanol	2.1	–	–	–
2500	Pentacosane	0.3	–	1.3	–
2503	Dodecanoic acid	4.4	–	–	0.7
2607	1-Octadecanol	–	–	–	0.4
2622	Phytol	0.2	–	0.5	–
2670	Tetradecanoic acid	3.9	–	–	0.5
2700	Heptacosane	0.3	–	–	–
2822	Pentadecanoic acid	1.3	–	–	–
2857	Palmito-γ-lactone	0.4	–	1.0	–
2931	Hexadecanoic acid	11.1	–	0.1	0.6
	Identified Total %	77.0	98.1	70.1	96.5

RRI Relative retention indices determined against n-alkanes; % determined from FID data; tr Trace (<0.1%).
Ap: Aerial part; Fr: Fruit; Fl: Flower; R: Root.

and they are the two at the leaf. The flower, leaf, fruit, and stem, examples of *A. purpurascens* were investigated in detail secretory structures with scanning electron microscopes and utilizing light. However, we have only seen secretory trichomes on fruit.

Monoterpenes and sesquiterpenes consisting of carbohydrates, aldehydes, phenols (especially coumarins), ethers, ketones, and alcohols are important for the biological activity of medicinal and aromatic plants. Many of them are plant volatiles class of

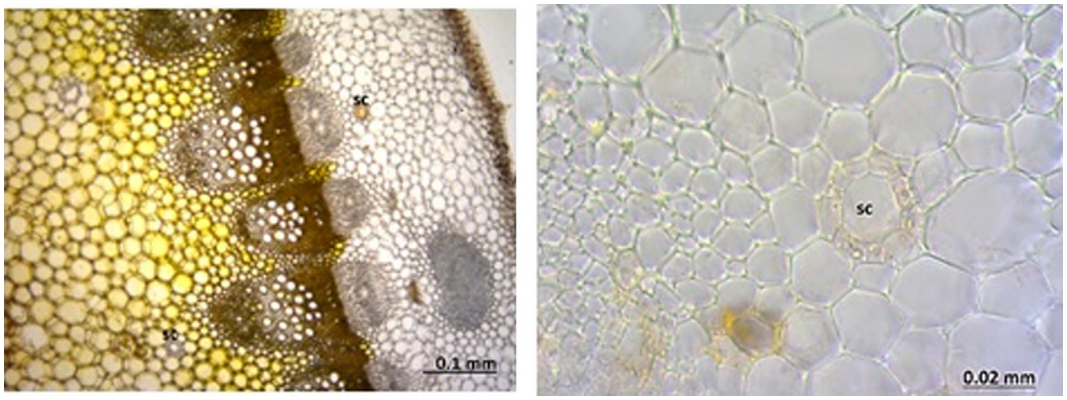

Fig. 5. Secretory canals at the peduncle of *Angelica purpurascens* by light microscopy.

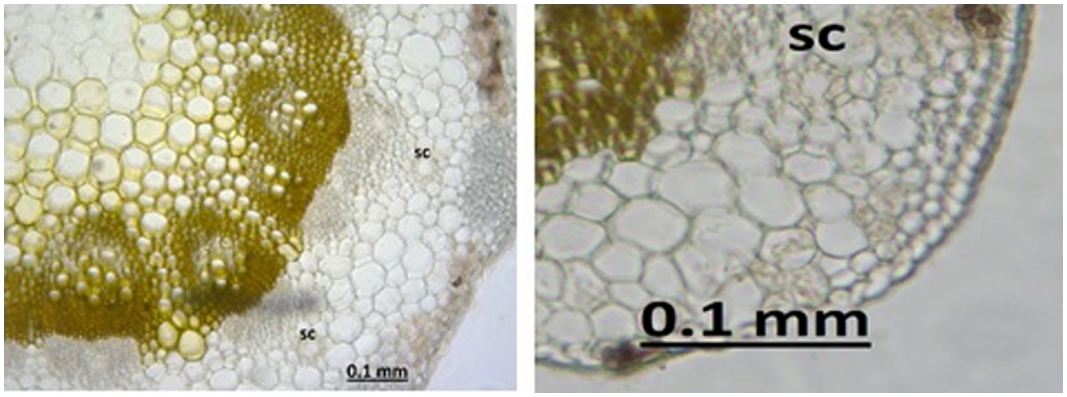

Fig. 6. Secretory canals at the ray of *Angelica purpurascens* by light microscopy.

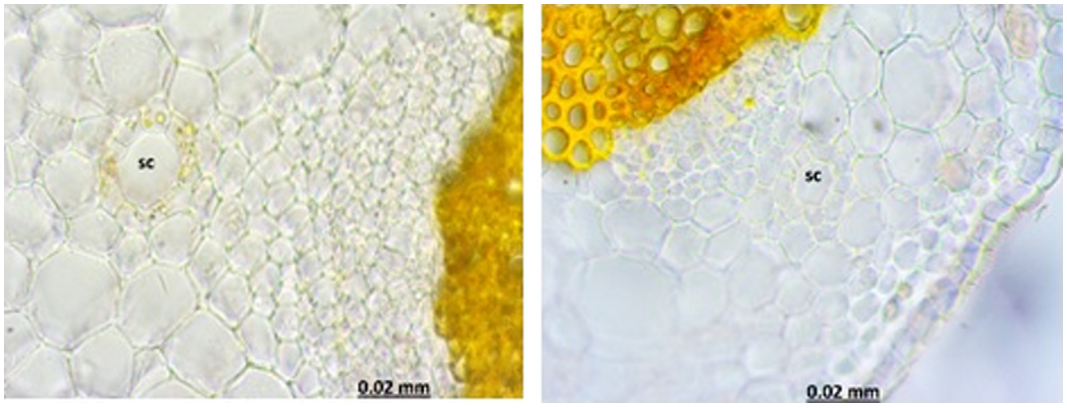

Fig. 7. Secretory canals at the pedicel of *Angelica purpurascens* by light microscopy.

compounds, which can be present in the extracts and also in plants essential oils. Plant volatiles compounds are greater products of lipophilic nature with molecular masses under 300. Many of them can be referred in order of decreasing size to the next classes: terpenoids, fatty acid derivatives consisting of C_5-branched compounds, benzenoids, lipoxygenase pathway products, phenylpropanoids, different sulfur and nitrogen-containing compounds (Dudareva et al., 2004). All these secondary metabolites have variegated pharmacological effects like antitubercular, anticoagulant, antibacterial, anti-inflammatory, hypoglycemic, neuroprotective, anticonvulsant, antioxidant, antiadipogenic, anticancer, antifungal, hypotensive, antiviral, and antidiabetic (Karakaya et al., 2018; El-Zaeddi et al., 2016). Compounds that react as inhibitors of cholinesterase still symbolize just pharmacological AD curation. The some *in vitro* searches exhibited the role of several components, got in essential oils, get a hopeful anticholinesterase effect, such as α- and β-asarone, α-pinene, thymohydroquinone, carvacrol, anethole, 1,8-cineole, δ-3-carene, etc (Burcul et al., 2018).

In the presented study was found that root and fruit fractions have been characterized by substantially higher total phenolic content by comparison with the aerial part fractions. We have not found any previous studies about the antioxidant and anti-lipid peroxidation activities of *A. purpurascens* or its synonym *X. purpurascens* in the literature search. In this investigation, oxypeuce-

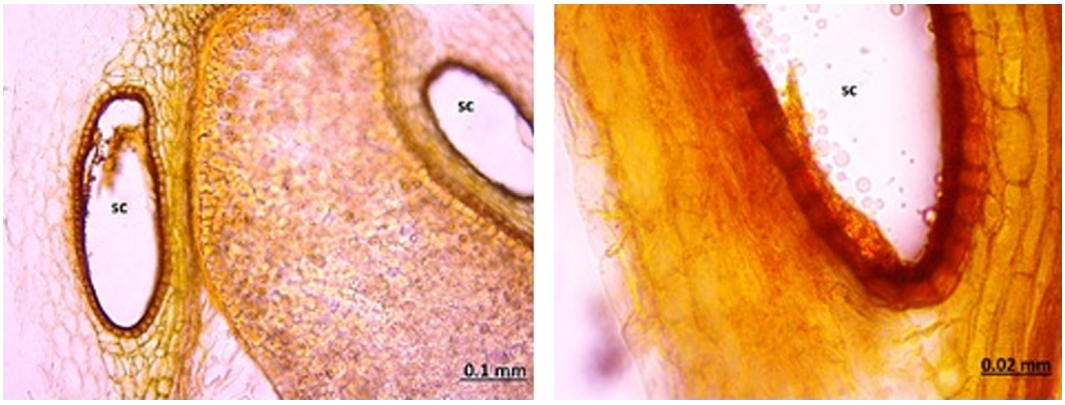

Fig. 8. Secretory canals at the fruit of *Angelica purpurascens* by light microscopy.

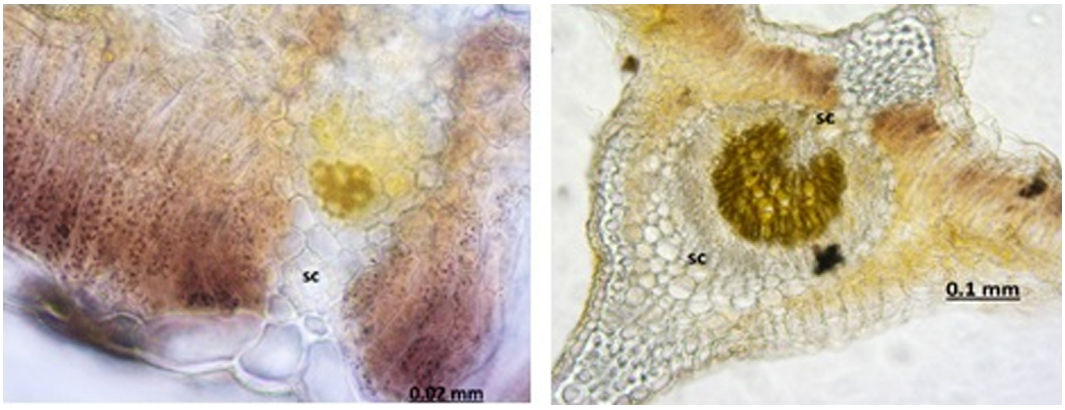

Fig. 9. Secretory canals at the leaf of *Angelica purpurascens* by light microscopy.

danin α-benzopyrone-type coumarin was purified from *A. purpurascens*, and presented the highest anticholinesterase activity compared to the other isolated compounds. Oxypeucedanin hydrate monoacetate purified from another species of *A. dahurica* showed inhibition of cancer cell migration and significant down-regulation of the expression of pPI3K and pAkt in cells Caco-2 (Liu et al., 2016). At the same time, another purified furanocoumarin bergapten exhibited also the high AChE and BChE inhibitory activities at 20 μg/mL, while sterols demonstrated a low level of inhibition. We supposed study that the oxypeucedanin inhibitory activity was primary resulting in the C-5 position from the Epoxy group. Also, we supposed that the inhibitory activity of bergapten was derived from owing at the C-5 position in the methoxy groups.

Previous researches performed on the primary compounds of essential oils of *A. purpurascens* showed that, especially of the fruit compounds were kessane (6.6%), spathulenol (6.9%), β-phellandrene (7.1%), and bicyclogermacrene (12.0%) (Baser et al., 2001). It was discovered that the primal compounds of the fruit oil of *A. purpurascens* were bicyclogermacrene (3%), α-pinene (3.2%), p-cymene (3.7%), limonene (5.3%), isopropyl hexanoate (6%), β-phellandrene (23%), α-phellandrene (32%) (Ozek et al., 2006). β-caryophyllene (11.3%), and β-phellandrene (20.1%) were established as major compounds of the flower and aerial part essential oils of *A. purpurascens* (Assadian et al., 2005). 1,8-cineole (17.6%) was established as main compounds of the leaf oil of *A. purpurascens* (Masoudi et al., 2012). In presented work was also found the same major components such as β-caryophyllene, β-phellandrene, and limonene. First time via GC/MS and GC-FID measurements were identified in the *A. purpurascens* extracts and essential oils α–pinene and octyl acetate. α–pinene and octyl acetate are characterized by high antioxidant potential (Karthikeyan et al., 2018; Villa et al., 2018). The high capacity of octyl acetate and probable anticancer and antioxidative capacities were forecasted in leaves essential oils of genus *Pittosporum* members (Pittosporaceae) (Weston, 2004), also in *Commiphora pyracanthoides* and *Boswellia carterii* plants from Ethiopia (Chen et al., 2013).

The results of original comparable research on compositions of essential oils of different parts of *A. purpurascens* and secretory canals in the roots, aerial parts, and fruits found to be dissimilar. Previously, some representatives of the family Apiaceae are defined via the particular type of architecture of essential oil secretory canal. The numbers and shapes of them can be different within particular herbs or among species. There is a large number of secondary metabolites among the secretory canals in the region. Specially, the secretory channels produce and reservoir volatile oils in plants (Cheniclet and Carde, 1985; Figueiredo et al., 2008). Anatomy of the fruits of *Angelica* species was as described in advance (Huizhen et al., 1995). In the current study for *A. purpurascens* found that a greater number of secretory canals were at peduncle while the shape of secretory canals was the largest at the fruit. The higher number of secretory canals was at the leaf. Furthermore, the aerial parts, roots, and fruits secretory canals are defined by a greater presence of monoterpene hydrocarbons. The fruits canals contain the most monoterpene hydrocarbons (75.1%). Moreover, the flower secretory canals showed a higher presence of sesquiterpenes β-caryophyllene, germacrene D and ether octyl acetate. The

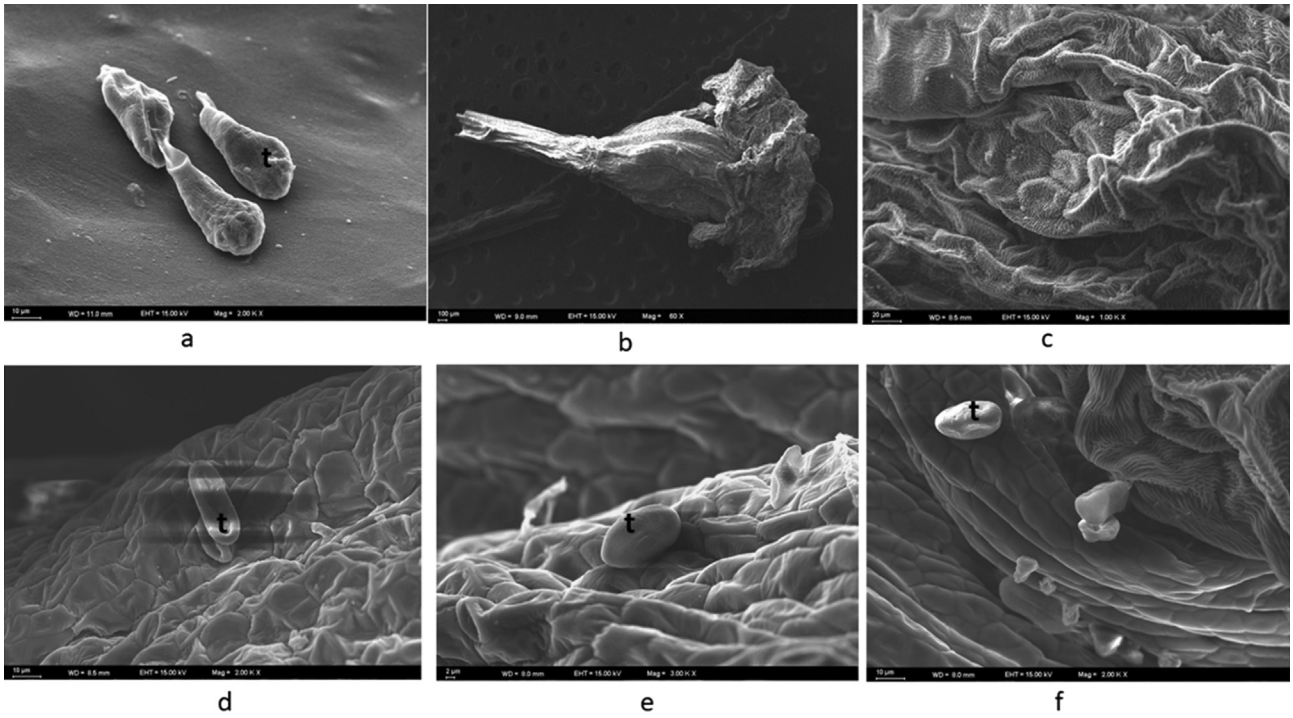

Fig. 10. Trichomes on the fruit (a), aerial part, root, and flower (b-k) by SEM.

canals of flowers don't include oxygenated monoterpenes. Moreover, canals of aerial parts and fruits contain diterpenes. The dispersion of various classes of specimens compounds is shown in Table 5.

Nowadays a remedy technique, which is beneficial at AD curation, has been used to enhance or defend acetylcholine levels through suppressing AChE (Dickson, 1997). The researchers noted that the hexane and CH_2Cl_2 fractions of fruit from *A. purpurascens* exhibited inhibition towards BChE and AChE enzymes and remarkable antioxidant effect. In current work was observed that fruit dichloromethane fraction demonstrated the highest acetylcholinesterase inhibition, while the fruit hexane fraction displayed the best inhibition towards butyrylcholinesterase. The data regarding biochemical composition in *A. purpurascens* different parts extracts with the use of different solvents withal variegated polarities can be a background to develop new techniques of use *Angelica* sp. extracts and essential oils. To promote such a point of our interestingness, the study of anticholinesterase activity is the novel investigation on *A. purpurascens* extracts.

4. Conclusion

The studied essential oils and extracts exhibited remarkable antioxidant potential, that were exhibited to be in correlation to the content of phenolics. The results of original comparable research on essential oils components of *A. purpurascens* different parts (roots, aerial part, flowers, fruits) and secretory canals of these plant parts found to be dissimilar with high presence of monoterpene hydrocarbons in the roots, aerial parts and fruits secretory canals and sesquiterpenes in the flowers secretory canals. Most particularly, hexane and CH_2Cl_2 fractions of fruit from *Angelica purpurascens* and isolated compounds oxypeucedanin and bergapten got a notableness anticholinesterase and antioxidant capacities. Therefore, we can conclude that *A. purpurascens* may be utilized as an herbal substitute to synthetically medications in the AD prophylaxis.

Disclosure statement

No potency conflict of interest was reported by the authors.

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