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Screening of non-alkaloid acetylcholinesterase inhibitors from extracts and essential oils of *Anthriscus nemorosa* (M.Bieb.) Spreng. (Apiaceae)

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ABSTRACT

This screening of biologically active compounds, cholinesterase inhibition and antioxidant potentials of extracts and essential oils from different plant parts such as fruits, aerial parts, roots and flowers of *Anthriscus nemorosa* was performed. GC analytical results of essential oil compositions have been detailed described. It was found that EtOAc fraction of root and root essential oil had the highest total phenolics content and antioxidant activity (DPPH test). The essential oil of roots showed the highest butyrylcholinesterase inhibition (88.51%). α-Pinene as the major component of root essential oil also indicated strong butyrylcholinesterase inhibitory activity (72.09%) and antioxidant effect. The GC-FID and GC–MS analysis assessed that major monoterpene of roots and aerial parts were α-pinene (25.5.%), myristicin (10.4%), *p*-cymene (8.2%), limonene (6.0%) and fatty alcohol 1-heptadecanol (7.5%). The root and aerial part canals revealed the smaller number of secretory canals which contains mainly monoterpene and oxygenated monoterpenes. The secretory canals of fruits and flowers were characterised by the largest shape and contain a high amount of sesquiterpene hydrocarbon bicyclogermacrene. The high content of sesquiterpene spathulenol (49.6%) was estimated in the extracts of the aerial part. These presented findings represented that the roots essential oil of *A. nemorosa* may be a novel alternative source of natural antioxidant and anticholinesterase.

1. Introduction

The primary effect of acetylcholinesterase (AChE) is the termination of nerve impulse conduction by rapid hydrolysis of acetylcholine (ACh) in cholinergic synapses. Inhibition of AChE acts as a strategy for the curation of Alzheimer's disease (AD), Parkinson's disease ataxia, myasthenia gravis, and senile dementia. There are several synthetical drugs, eg. donepezil, tacrine, and natural products rivastigmine based on for the curation of cognitive dysfunction and memory loss related to AD. These components have been reported to have side effects such as gastrointestinal disorders related to biocompatibility issues that interest in the presence of better AChE inhibitors from natural sources (Pulok et al., 2007).

AD is a fatal neurodegenerative ailment with gradual character and has become an important health problem specially in industrialised countries with high living standards. The pathogenesis of AD is not fully clarified, therefore, there is no treatment for the disease except for symptomatic curation against mild to moderate AD types. As AD is diagnosed with acetylcholine deficiency in the brain of patients with AD, acetylcholinesterase (AChE) inhibitors have become the most prescribed medication class in the curation of AD. At the same time, it is known that AD is related to metal accumulation in senile plaques formed in patients and oxidative stress. In this respect, it is substantial that any medication nominee that can be utilised for the curation of AD has both cholinesterase inhibitory as well as antioxidant effects (Orhan et al., 2011; Karakaya et al., 2019).

Essential oils are certificated to have varied pharmacological effects, like carminative, antipathogenic, spasmolytic, antiproliferative, and hepatoprotective effects, etc. (Ložienė et al., 2018). Numerous essential oils and their constituents have been researched for their effects on AChE and BuChE, and have indicated strong inhibitory activity. The Apiaceae and Lamiaceae families have been featured by high phenolics content and essential oils. They exhibited encouraging effectiveness on the central nervous system. For example, the essential oils from *Zosima absinthfolia* (Apiaceae) and *Rosmarinus officinalis* (Lamiaceae) showed significant anticholinesterase activity (Ložienė et al., 2018; Karakaya et al., 2019; Pulok et al., 2007).

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Some essential oils have been utilised as therapeutical agents since ancient times, and some of them have been scientifically certified to have medical properties, containing antitumour, anti-inflammatory, cytotoxic, antiviral, antifungal, and antibacterial effects. In plants, α-pinene has some biological activities such as anticholinesterase, antifungal, a natural insecticide and has been utilised for centuries to produce flavours and fragrances (Silva et al., 2012; Savelev et al., 2003).

Anthriscus belongs to the Apiaceae family (generally known as beaked parsley, rough chervil, beaked chervil,) is one of the aromatic plants, is utilised for therapeutic aims in conventional medicine in the world (Bagci et al., 2016). About 80 specific names have been identified in *Anthriscus*, however, about 14 species are known. In the flora of Turkey, *Anthriscus* genus is typified by eight species [*A. caucalis* M. Bieb., *A. cerefolium* (L.) Hoffm., *A. kotschyi* Fenzl ex Boiss., *A. lamprocarpa* Boiss., *A. nemorosa* (M.Bieb.) Spreng., *A. ruprechtii* Boiss., *A. sylvestris* (L.) Hoffm., *A. tenerrima* Boiss. & Spruner] (Menemen, 2012). *Anthriscus nemorosa* is known as 'gimigimi, peçek' in Turkey and the fruits of *A. nemorosa* have been utilised for inflammation gastrointestinal ailments, and rheumatism. It is one of the 25 plants which are utilised to make herby cheese (it is called 'otlu peynir') in the east and south-eastern areas of Turkey (specially in Van province). Ethnobotanical investigations recommend that essential oil of *A. nemorosa* can develop memory in AD. It has been investigated that essential oil of *A. nemorosa* prevents anxiety, depression and memory impairment (Bagci et al., 2016; Menemen, 2012).

This current paper reports the cholinesterase inhibition against acetylcholinesterase/butyrylcholinesterase enzymes and antioxidant activity of the MeOH extract, *n*-hexane, CH_2Cl_2, EtOAc, BUOH and aqueous fractions and essential oils of roots, aerial part, fruits and flowers of *A. nemorosa.* The content of total phenolics in the extracts and essential oils and also the composition of the essential oils were determined. Furthermore, structures of secretory canals were searched. As a result, the inhibitory activities of BuChE and AChE of the most active root essential oil major compound (α-pinene) were assessed through molecular docking studies.

2. Material and methods

2.1. Plant examples

Anthriscus nemorosa was picked up at fruity and flowering stages at Urban Forest, Palandöken Mountains in 2016 and 2017 from Erzurum, which was identified by Prof. Dr. Hayri Duman. The Herbarium of Ataturk University, Faculty of Pharmacy has stored the voucher specimens (AUEF 1265 and 1277).

2.2. Standards

α-Pinene was purchased from Merck-Schuchardt (Hohenbrunn, Germany).

2.3. Extraction and fractionation

Roots (50 g), aerial parts (50 g), flowers (50 g) and fruits (50 g) of *Anthriscus nemorosa* were milled and saturated with methanol (3 times × 8 h) in a water-bath not exceedance 45 °C (3 × 150 mL) utilising via use of mechanical mixer at 250 rpm. Received extracts were filtered. The concentration of extracts was done by rotating evaporator (Heidolph VV2000, Germany). After that, the extracts were dissolved in methanol:distillate water (1:9) with the next three instance fractionations with 100 mL *n*-hexane, CH_2Cl_2, EtOAc, BUOH, in turn. Sums of the attained extracts and comminuted parts of *A. nemorosa* are exhibited in Table 1.

Table 1
Sums of the crushed plants and gained extracts and fractions.

Species	Extracts/Fractions	Root	Aerial part	Fruit	Flower
Angelica purpurascens	MeOH (g)	11.81	12.98	11.86	13.88
	Hexane (g)	1.66	1.91	1.24	1.36
	CH_2Cl_2 (g)	4.30	4.73	4.45	4.80
	EtOAc (g)	1.51	1.22	0.87	1.77
	BuOH (g)	2.19	2.31	2.38	2.59
	Aqueous residue (g)	1.78	2.21	2.27	3.01

2.4. GC-FID and GC/MS methods for essential oils isolation

GC-FID and GC/MS methods phases of essential oils isolation were realised in proportion to Karakaya et al. (2016). The samples were subjected to hydrodistillation for 3 h utilising a Clevenger-type apparatus in accordance with the method suggested in the European Pharmacopoeia. Gained oils were dried over anhydrous sodium sulfate and stored in sealed vials at 4 °C in the dark until analysed and tested. The ground different parts such as roots, aerial parts, fruits, and flowers % yields of essential oils of *Anthriscus nemorosa* and essential oils colours were exhibited in Table 2.

2.5. Estimation of total phenolics

The total phenolic quantity of examples was realised in proportion to Karakaya et al. (2019). The absorbance for total phenolics 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. Antioxidant activity assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity assay of the examples was realised in proportion to Karakaya et al. (2019). The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\%\text{Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample.

2.7. Anti-lipid peroxidation activity

The anti-lipid peroxidation activity of the examples was realised in proportion to Karakaya et al. (2018). The linear regression analysis was used to establish IC_{50}values.

2.8. Determination of AChE and BuChE inhibition activities

The estimation of AChE and BuChE inhibition activities of the examples were realised in proportion to the methodology defined in work by Karakaya et al. (2018). This protocol for each plate was repeated three times and overall data were denoted as mean ± SE of three independent testings.

Table 2
Anthriscus nemorosa Essential oil yields (w/v, %).

Used parts	Crushed (g)	Yields	Colour	Collection time
Root	227	0.441	Yellow	2016
Aerial	244	0.041	Yellow	2016
Fruit	64	0.016	Brown	2017
Flower	30	0.333	Yellow	2016

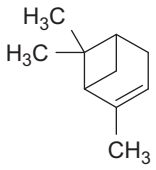

Fig. 1. Chemical structure of α-Pinene.

2.9. Microscopic analysis

The light microscope investigations of the examples were realised in proportion to Karakaya et al. (2019).

2.10. Protein preparation

The three-dimensional complex structures AChE(PDB ID: 1EVE) and BuChE (PDB ID: 1P0I) were gained from Protein Data Bank (Nicolet et al., 2003). The pdb file of the proteins was assessed and passed to AutodockTools (ADT ver.1.5.6) for pdbqt file preparation. Therefore, water molecules and non-standard residues were removed, only polar hydrogen was pursued, and Kollman charges were added for protein atoms by ADT.

2.11. Ligand preparation

The ligand of alpha-pinene was attained from PubChem. After molecular file in sdf format was converted to mol2 format using ChemDraw 3D. Gasteiger charges were calculated and saved as pdbqt file by using AutodockTools (ADT ver.1.5.6)

2.12. Docking procedure

Autodock4 (ver. 4.2.6) (Kryger et al., 1999; Nicolet et al., 2003) was utilised for docking simulations. Lamarckian genetic algorithm withal local search (GALS) was used as a research engine, with a sum of 30 runs. The area of interest, utilised through Autodock4 for docking runs and by Autogrid4 for affinity grid maps preparation, was described in such a method to contain the entire catalytic binding site utilising a grid of 40 × 40 × 40 points with a grid space of 0.375 Å, centers of the grid box. Cluster assay was carried out the docked data utilising an RMS tolerance of 2.0 Å. In the end; the more energetically favourable cluster poses were assessed through utilising Python Molecule Viewer (PMV ver.1.5.6) and PyMOL ver.1.1.7 (DeLano Scientific LLC).

2.13. Statistical analysis

The all experimental data indications are shown as mean ± SE and diversifications were statistically evaluated via one-way analysis ANOVA dogged via the method of complementary assay of Bonferroni ($P < .05$), conceived to display statistic significancy.

Table 3
Total phenolic contents of the extracts, fractions and essential oils from *Anthriscus nemorosa*.

Used part	Total phenolic contents (mg/g) ± SD[a]			
	Root	Aerial part	Fruit	Flower
MeOH	366.39 ± 2.61	199.55 ± 3.01	358.21 ± 1.59	135.46 ± 2.78
Hexane	71.63 ± 5.66	80.55 ± 2.61	34.41 ± 4.77	59.09 ± 1.69
CH_2Cl_2	677.31 ± 3.54	107.36 ± 4.09	168.39 ± 2.57	105.48 ± 2.82
EtOAc	409.34 ± 5.09	501.09 ± 2.78	298.57 ± 4.03	108.78 ± 5.33
BuOH	255.80 ± 2.71	477.80 ± 2.99	284.57 ± 2.93	482.61 ± 1.72
Aqueous residue	405.67 ± 1.83	444.32 ± 1.99	189.35 ± 5.60	388.51 ± 2.53
Essential oils	509.39 ± 2.51	183.54 ± 2.22	97.87 ± 1.70	77.52 ± 1.68

[a] Standard deviation. The data display the mean ± SD of three independent experiments ($p < .05$).

Table 4
DPPH radical scavenging activity of the extracts, fractions from *Anthriscus nemorosa* and α-pinene (μg/mL).

Used part	Total phenolic contents (mg/g) ± SD[a]			
	Root	Aerial part	Fruit	Flower
MeOH	66.48 ± 1.99	55.9 ± 4.77	48.29 ± 4.57	59.41 ± 2.74
Hexane	127.30 ± 2.57	130.44 ± 3.67	74.45 ± 2.76	109.07 ± 3.61
CH_2Cl_2	71.54 ± 2.58	87.21 ± 2.64	88.32 ± 3.50	55.45 ± 1.89
EtOAc	79.31 ± 2.45	49.05 ± 2.79	38.54 ± 1.05	26.67 ± 3.09
BuOH	68.69 ± 1.56	39.81 ± 1.96	71.54 ± 2.97	52.66 ± 1.73
Aqueous residue	113.59 ± 4.09	44.38 ± 3.04	46.99 ± 4.63	89.54 ± 3.59
Essential oils	19.90 ± 2.77	69.57 ± 3.27	87.22 ± 3.75	103.44 ± 1.81
α-Pinene	184.51 ± 1.29			
Chlorogenic acid	2.29 ± 1.09			
Propyl gallate	0.006 ± 0.62			
Rutin	3.07 ± 1.22			

The data display the mean ± SD of three independent experiments and the differences are * from control ($p < .05$).
[a] Standard deviation.

3. Results and discussion

The methanolic extracts of roots, aerial parts, fruits, and flowers of *Anthriscus nemorosa* were fragmented utilising solvers withal divergent polarities (*n*-hexane, CH_2Cl_2, EtOAc, BUOH, respectively), and attained extracts/fractions were estimated about antioxidant capacity and inhibitory activities opposed to AChE and BuChE enzymes. Besides, essential oils of different studied plants part *A. nemorosa* were assessed for their antioxidant activity and inhibitory activities against AChE and BuChE enzymes. Also, inhibitory activities of BuChE and AChE of the most active root essential oil major compound (α-pinene) were assessed through molecular docking studies. The chemical structures of α-pinene are given in Fig. 1.

The extracts, fractions and essential oils of different plant parts related to the antioxidant effect were analysed. The results of examples regarding total phenolics content are revealed in Table 3. The root essential oil and CH_2Cl_2 fraction had the utmost total phenolic level (509.39 and 677.31 mg GAE g^{-1} DW) but the fruit hexane fraction had the lowest phenolics content (34.41 mg GAE g^{-1} DW). The data of analysis antioxidant activity with DPPH exhibited that the root essential oil (19.90 μg/mL) and flower EtOAc fraction (26.67 μg/mL) had the highest antioxidant activity in proportion to the fruit extracts of *A. nemorosa* (Table 4). However, the findings of the antioxidant activity of examples from different *A. nemorosa* parts were substantial high contrasted to the standards (Table 4). Table 5 shows the TBA assay data presented as IC_{50} value (μg mL^{-1}). The root essential oil, root

Table 5
Antioxidant activities of the samples from *Anthriscus nemorosa* in the TBA test.

Tested samples	IC_{50} values (μg/mL) ± SD[a]			
	Root	Aerial part	Fruit	Flower
MeOH	157.51 ± 2.09	500>	171.65 ± 2.32	243.21 ± 1.88
Hexane	500>	266.15 ± 1.88	500>	458.92 ± 3.81
CH_2Cl_2	98.70 ± 2.57	215.12 ± 2.05	500>	380.22 ± 4.08
EtOAc	500>	118.66 ± 4.31	169.41 ± 1.45	109.61 ± 2.87
BuOH	375.61 ± 2.77	500>	500>	409.41 ± 1.66
Aqueous residue	500>	500>	500>	285.31 ± 3.54
Essential oils	68.54 ± 2.62	122.38 ± 2.71	281.16 ± 1.99	111.67 ± 3.08
α-Pinene	115.36 ± 1.08			
Chlorogenic acid	11.79 ± 3.77			
Propyl gallate	3.66 ± 1.99			
Rutin	8.98 ± 2.76			

The data display the mean ± SD of four independent experiments and the differences are * from control ($p < .001$).
[a] Standard deviation.

Table 6
In vitro AChE and BuChE inhibitory activities of samples from *Anthriscus nemorosa* at 20 μg/mL.

Samples	Enyzmes	Percentile of inhibition ± S.E.M[a] against AChE and BuChE			
		Root	Aerial part	Fruit	Flower
MeOH	AChE	8.66 ± 2.05	[c]	[b]	11.85 ± 2.86
	BuChE	67.49 ± 2.76	43.61 ± 2.09	46.34 ± 3.71	59.32 ± 1.03
Hexane	AChE	23.29 ± 2.42	[c]	7.29 ± 1.69	[b]
	BuChE	18.66 ± 1.68	50.22 ± 2.99	39.62 ± 1.76	[b]
CH_2Cl_2	AChE	18.69 ± 2.04	[c]	[c]	12.40 ± 2.54
	BuChE	75.62 ± 3.02	59.92 ± 1.66	69.23 ± 2.73	71.54 ± 1.93
EtOAc	AChE	5.04 ± 2.94	7.51 ± 1.65	40.06 ± 1.97	10.73 ± 1.69
	BuChE	83.09 ± 2.55	55.90 ± 2.67	46.21 ± 3.51	76.43 ± 3.03
BuOH	AChE	[b]	[b]	7.72 ± 3.04	[c]
	BuChE	[b]	[c]	[b]	21.24 ± 2.24
Aqueous residue	AChE	[c]	2.77 ± 3.07	[c]	[b]
	BuChE	[c]	[c]	[b]	26.01 ± 2.69
Essential oils	AChE	35.99 ± 1.85	17.61 ± 2.00	6.43 ± 1.76	19.13 ± 2.17
	BuChE	88.51 ± 2.08	72.91 ± 2.65	61.65 ± 3.51	67.54 ± 2.22
α-Pinene	AChE	19.41 ± 1.99			
	BuChE	72.09 ± 2.88			
Donepezil	AChE	81.99 ± 3.61			
	BuChE	91.09 ± 2.17			

The data display the mean ± SD of three independent experiments and the differences are * from control ($p < .005$).
[a] Standard error mean.
[b] No activity.
[c] Not detected because of turbidity in the wells of microplates.

Fig. 2. Schematic representation of the main interaction of α-pinene with AChE. α-Pinene imposed active side of 1P0I with −4.98 kcal/mol binding free energy.

CH_2Cl_2 and flower EtOAc fractions had the highest antioxidant potential (IC_{50} = 68.54, 98.70 and 109.61 μg/mL, in turn) in TBA assay. Moreover, among the major compounds of essential oils α-pinene had a strong antioxidant effect with 115.36 μg/mL IC_{50} value. Antioxidant capacity of many examples displayed on liposome beside the chlorogenic acid and rutin. We guess that it may be connected to subsistence some other metabolite that is accountable for the antioxidant capacity.

Anticholinesterase activity of examples was assessed by favour of colorimetric Ellman's method (Ellman et al., 1961), to a number of variegations through commercially existing donepezil as standard (Yerdelen and Tosun, 2015). The invitro acetylcholinesterase inhibition of examples at 20 μg/mL were exhibited in Table 6. Depending on findings of inhibition of enzymes MeOH extracts, CH_2Cl_2, and EtOAc fractions and essential oils of whole parts revealed substantial stoppage activities towards to butyrylcholinesterase. The root essential oil, root CH_2Cl_2 and flower CH_2Cl_2 fractions displayed strong inhibition against BuChE (88.51 ± 2.08, 83.09 ± 2.55 and 76.43 ± 3.03%, in turn) at 20 μg/mL. Also, the root essential oil and fruit EtOAc fraction exhibited remarkable inhibition against AChE (35.99 ± 1.85 and 40.06 ± 1.97%, in turn) at 20 μg/mL. Among the major compounds of essential oils, α-pinene revealed strong inhibition against AChE (19.41 ± 1.99%) and BuChE (72.09 ± 2.88%). All essential oils exhibited AChE and BuChE inhibition activities. Among the BuOH and aqueous residue fractions, only flower fraction had BuChE inhibition activity, also only aerial part aqueous residue and fruit BuOH fractions had AChE inhibition activity. Among the hexane fractions, only flower fraction flower had no butyrylcholinesterase inhibition activity. Most active compound against ChE (α-pinene) was docked at the binding sites of 1-P0I. No hydrogen bond formation in molecular docking results. The binding affinity was evaluated by binding free energies (ΔGb, kcal/mol). In the assay of the docked complexes, the binding free energies of α-pinene to acetylcholinesterase was found to be lower than the binding free energy to butyrylcholinesterase. In theoretically, this result suggests that α-pinene inhibits acetylcholine esterase better in in vitro experiments. The results have been presented in Figs. 2 and 3.

The colours of essential oils and % yields of *A. nemorosa* were exhibited in Table 2. The essential oils' colour from the root, aerial part and

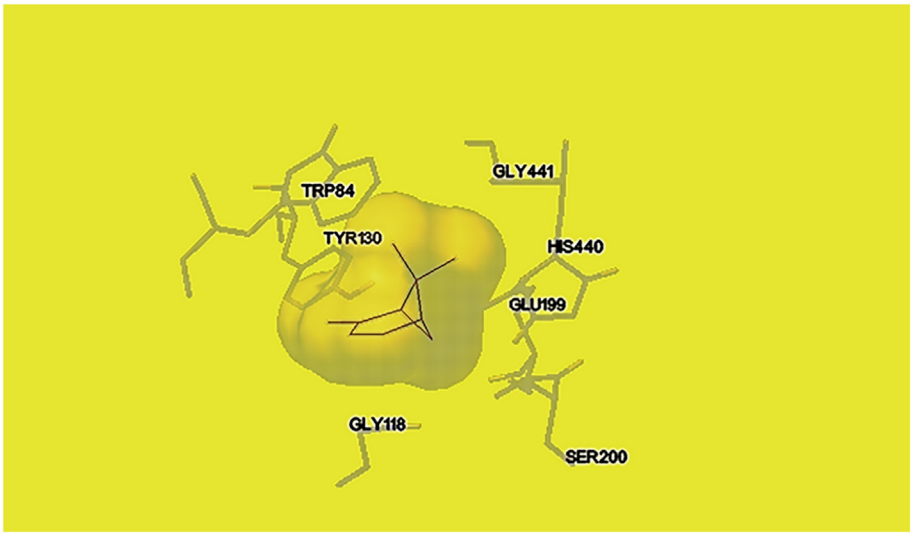

Fig. 3. Schematic representation of the main interaction of α-pinene with BuChE. α-Pinene imposed active side of 1EVE with −5.25 kcal/mol binding free energy.

flower were yellow, but fruit essential oil colour was brown. Generally, the yield of the fruit was low in comparison with the aerial part, roots, and flowers. The best results were attained in root and flower, respectively (0.441 and 0.333 *w*/*v*, %).

A total of 54 compounds finding 93.9% of the oil were defined in the roots of *A. nemorosa* essential oil. α-pinene (25.5.%), myristicin (10.4%) *p*-cymene (8.2%) and sabinene (8.0%) were the primal constituents of the root essential oil. The analysis of the aerial parts of *A. nemorosa* leads to the determining of 48 essential components finding 96.8% of the oil. In the essential oil found spathulenol with 49.6%, bicyclogermacrene with 8.9% and (*Z*)-β-farnesene with 6.1% as the main abounding compounds. Thirty compounds were identified in the fruits essential oil of *A. nemorosa* finding 97% of the oil. The primal constituents were detected to be bicyclogermacrene (35.8%), 1-heptadecanol (7.5%), and heptacosane (6.2%). The investigation on the fruits essential oil of *A. nemorosa* found 56 essential compounds finding 94.5% of the β-phellandrene at 47.6% was the main abounding compound, farther followed by isopropyl hexanoate (8.8%), α-phellandrene (8.5%), limonene (8.2%), α-pinene (7.1%). The essential oils compositions are displayed in Table 7. The detected components were categorised into two major classes owing to their different chemical structures: isoprenoids (oxygenated monoterpenes, terpene hydrocarbons) and nonisoprenoids variously functionalized (furans, aldehydes, lactones ketones, alkanes, esters, alcohols, and fatty acids). Monoterpene hydrocarbons, oxygenated sesquiterpenes, and sesquiterpene hydrocarbons, were in the essential oils one of the dominating group of compounds but, diterpenes were the lowest components of the essential oils (Table 7).

Previous studies on the *A. nemorosa* revealed that the main volatile compounds of essential oils, of roots, were n-nonane (12.1%), n-hexadecanol (6.9%), γ-cadinene (6.4%), and β-pinene (6.0%) (Pavlović et al., 2011). It was reported that the primal components of the aerial parts of *A. nemorosa* were (E)-nerolidol (41.7%), β-elemene (13.0%) and α-zingiberene (9.9%) (Nickavar et al., 2009). Caryophyllene (23.6%), caryophyllene oxide (12.3%),δ-cadinene (12.1%), and trans-pinocarveol (9.8%) were detected as major compounds of aerial part essential oil of *A. nemorosa* (Bagci et al., 2016). Caryophyllene (15.8%), caryophyllene oxide (14.5%), δ-cadinene (13.4%), germacrene D (8.9%) and trans-pinocarveol (6.2%) were found to be primal compounds of aerial parts oil of *A. nemorosa* (Kiliç, 2017). Hayta et al., 2015 found the main compounds of *A. nemorasa* essential oil were β-caryophyllene (23.6%), caryophyllene oxide (12.3%), δ-cadinene (12.1%), and trans pinocarveol (9.8%) as well. In all previous studies was done the analysis of essential oil of whole plant but in this presented work is done the primary comparable screening on essential oils components of different plant parts of *A. nemorosa*.

Nowadays is not presented information about the existence of α-pinene in the *Anthriscus* sp., which is characterised by antioxidant potential (Karthikeyan et al., 2018). The current GC analytical study revealed in the essential oil of roots of *A. nemorosa* α-pinene presence in a high amount. The root essential oil and flower fractions have been characterised by remarkable greater total phenolics content compared to the aerial part and fruit fractions. We have found no former searches about the antioxidant activity of *A. nemorosa* in the literature search. So, this is the first detailed research on antioxidant activity of *A. nemorosa.*

The structural diversification of the active anticholinesterase terpenoids inconveniences the estimation of potential structure–activity relationships. One feature related to AChE inhibition is a hydrophobic ligand. The hydrophobic active site of AChE is presented to be sensitive to hydrophobic interplays. Monoterpenes comprise a hydrocarbon skeleton, that might contribute to their cholinesterase inhibitory activity. Monoterpenes can be cyclic (e.g. α-pinene and 1,8-cineole) or acyclic (e.g. linalool and geraniol), a property that might also affect cholinesterase inhibitory activity (Pulok et al., 2007). Oxygenated derivatives of the monoterpene α-pinene are found in herb essential oils and utilised as fragrances and flavourings. In this investigation, α-pinene a monoterpene, was a major compound of the root essential oil from *A. nemorosa,* and displayed AChE and BChE inhibitory activities. As indicated in Fig. 1 α-pinene is structurally connected to camphor–camphor is a bicyclic-2,2,1 compound whereas α-pinene has the 3,1,1 structure. The carbonyl group in a camphor is absent from α-pinene and the C_{10} methyl group is on a dissimilar ring carbon in α-pinene. We presumed that the α-pinene inhibitory activity was principal resulting in the C_{10} methyl group.

The plants pertain to the Apiaceae family are characterised through the specific kind of essential oil secretory architecture which called secretory canals. The counts and forms of secretory canals may be diversified among the plants or within particular herbs. They get a big quantity of metabolites in the region between their secretory canals. Principally, they produce and reservoir essential oils in herbs (Karakaya et al., 2019). Our exploratory is the primary comparable research on the anatomy of different plant parts of *A. nemorosa.*

The composition of specific secondary metabolites in the roots, aerial parts, fruits, and flowers secretory canals were dissimilar. The fruits and flowers secretory canals are defined by sesquiterpene hydrocarbons. At the same time, the roots secretory canals contain monoterpene hydrocarbons while aerial parts secretory canals contain oxygenated

Table 7
The composition of the essential oils of *Anthriscus nemorosa*.

RRI_{lit}	$RRIe_{xp}$	Compound	R %	AP %	Fr %	F %	IM
1025	1032	α-Pinene	25.5	1.4	0.2	0.2	RRI, MS
1026	1035	α-Thujene	1.1	–	–	–	MS
1067	1065	2-Methyl decane	0.2	–	–	0.1	MS
1068	1076	Camphene	1.3	–	–	–	RRI, MS
1100	1100	Undecane	–	tr	–	0.3	RRI, MS
1110	1118	β-Pinene	0.8	tr	–	tr	RRI, MS
1122	1132	Sabinene	8.0	0.4	–	tr	MS
1146	1159	δ-3-Carene	0.9	–	–	tr	MS
1160	1174	Myrcene	3.8	0.4	0.6	0.7	RRI, MS
1167	1176	α-Phellandrene	0.6	0.1	0.3	–	MS
1177	1188	α-Terpinene	0.5	–	–	–	RRI, MS
1174	1194	Heptanal	0.3	–	–	–	RRI, MS
1201	1203	Limonene	6.0	0.6	–	0.1	RRI, MS
1209	1218	β-Phellandrene	1.7	–	–	0.1	MS
1245	1246	(*Z*)-β-Ocimene	0.4	tr	0.3	0.4	MS
1211–1251	1255	γ-Terpinene	4.8	0.3	0.3	0.4	RRI, MS
1232–1267	1266	(*E*)-β-Ocimene	0.5	0.9	1.8	2.9	MS
1246–1291	1280	*p*-Cymene	8.2	0.7	–	0.1	RRI, MS
1297	1290	Terpinolene	0.4	–	–	–	RRI, MS
1267–1312	1296	Octanal	0.8	0.3	–	–	RRI, MS
1300	1300	Tridecane	–	–	–	0.1	RRI, MS
1374–1415	1398	2-Nonanone	0.3	–	–	–	MS
1471–1495	1495	Bicycloelemene	–	tr	tr	0.3	MS
1491	1497	α-Copaene	–	1.3	0.9	1.1	MS
1477–1511	1499	α-Campholene aldehyde	0.3	–	–	–	MS
1500	1500	Pentadecane	–	0.1	–	tr	RRI, MS
1496–1546	1535	β-Bourbonene	–	1.0	–	0.1	MS
1509–1569	1548	(*E*)-2-Nonenal	0.3	–	–	–	MS
1507–1564	1553	Linalool	0.4	–	–	–	RRI, MS
1557–1625	1571	*trans*-*p*-Menth-2-en-1-ol	2.0	1.4	–	0.1	MS
	1595	Isothymol methyl ether	0.3	–	–	–	MS
1550–1603	1597	β-Copaene	–	–	–	0.1	MS
1565–1608	1600	β-Elemene	–	tr	0.3	0.4	RRI, MS
1563–1607	1604	Thymol methyl ether	0.8	–	–	–	RRI, MS
1564–1630	1611	Terpinen-4-ol	1.1	–	–	–	RRI, MS
1569–1632	1612	β-Caryophyllene	0.1	1.6	2.0	2.6	RRI, MS
1576–1614	1614	Carvacrol methyl ether	0.9	–	–	–	RRI, MS
1583–1668	1628	Aromadendrene	–	–	–	0.1	MS
1555–1645	1638	*cis*-*p*-Menth-2-en-1-ol	1.2	0.5	–	0.1	MS
1595–1662	1655	(*E*)-2-Decenal	0.4	–	–	–	MS
	1658	Sabinyl acetate	0.2	–	–	–	MS
1627–1668	1668	(*Z*)-β-Farnesene	tr	6.1	2.7	5.1	MS
1637–1689	1687	α-Humulene	tr	0.7	1.4	1.6	RRI, MS
1675–1761	1689	*trans*-Piperitol	0.8	0.6	–	–	MS
1644–1690	1690	Cryptone	0.2	–	–	–	MS
1655–1714	1704	γ-Muurolene	–	–	–	0.2	MS
1659–1724	1706	α-Terpineol	0.3	–	–	–	RRI, MS
	1708	Ledene	–	–	–	0.2	MS
1676–1726	1726	Germacrene D	–	1.5	4.5	7.1	MS
1713–1748	1737	(*Z*,*E*)-α-Farnesene	–	1.2	0.4	1.0	MS
1686–1753	1740	α-Muurolene	–	–	–	0.1	MS
1692–1757	1755	Bicyclogermacrene	0.4	8.9	35.8	51.3	MS
1714–1763	1758	(*E*,*E*)-α-Farnesene	–	1.4	0.4	–	MS
1668–1771	1758	*cis*-Piperitol	0.9	0.6	–	–	MS
1722–1774	1773	δ-Cadinene	0.3	3.1	3.8	5.2	MS
1735–1782	1776	γ-Cadinene	–	–	–	0.1	MS
	1786	Aromadendra-1(10),4(15)-diene	–	–	–	0.1	MS
	1799	Cadina-1,4-diene (= *Cubenene*)	–	–	–	0.1	MS
1734–1803	1807	α-Cadinene	–	–	–	tr	MS
1770–1834	1827	(*E*,*E*)-2,4-Decadienal	0.2	–	–	–	MS
1805–1850	1845	*trans*-Carveol	0.3	–	–	–	RRI, MS
1778–1854	1854	Germacrene-B	–	–	–	0.2	MS
1795–1865	1857	Geraniol	0.3	–	–	–	RRI, MS
1826–1878	1878	2,5-Dimethoxy-p-cymene	0.3	0.4	–	–	MS
1854–1928	1900	*epi*-Cubebol	tr	0.3	–	0.1	MS
1901–1944	1933	Tetradecanal	–	0.5	–	0.1	RRI, MS
1893–1941	1941	α-Calacorene	–	0.7	–	0.1	MS
1884–1964	1957	Cubebol	0.1	0.3	–	0.2	MS
1892–1958	1958	(*E*)-β-Ionone	–	0.3	–	–	MS
1924–1980	1973	1-Dodecanol	0.1	–	–	0.1	MS
2000	2000	Eicosane	–	–	0.5	–	RRI, MS
1936–2023	2008	Caryophyllene oxide	0.1	1.6	–	0.1	RRI, MS
1995–2055	2050	(*E*)-Nerolidol	–	0.7	–	0.1	MS
2000–2070	2069	Germacrene D-4β-ol	–	0.4	1.6	1.9	MS
2024–2071	2071	Humulene epoxide-II	–	0.9	–	–	MS
	2077	1-Tridecanol	0.2	–	–	–	MS
2011–2089	2084	Octanoic acid	0.3	–	–	–	RRI, MS
2082	2098	Globulol	–	–	0.4	0.4	MS
2100	2100	Heneicosane	–	–	–	0.4	RRI, MS
2089	2104	Viridiflorol	–	–	–	0.3	MS
2096–2131	2131	Hexahydrofarnesyl acetone	–	0.5	3.0	0.2	MS
2074–2150	2144	Spathulenol	1.7	49.6	3.0	3.4	MS
2128–2155	2145	Valeranone	–	–	–	0.5	MS
2123–2174	2179	1-Tetradecanol	0.4	–	–	0.2	MS
2165	2187	T-Cadinol	–	–	–	0.2	MS
2200	2200	Docosane	–	–	–	0.1	RRI, MS
2145–2178	2209	T-Muurolol	–	–	0.6	0.2	MS
	2219	δ-Cadinol (= *Torreyol*)	–	–	–	0.3	MS
2214–2260	2245	Elemicin	2.5	–	–	–	MS
	2247	*trans*-α-Bergamotol	–	1.5	–	0.3	MS
2180–2255	2255	α-Cadinol	–		–	0.9	MS
2225–2296	2296	Myristicin	10.4	0.6	–	–	MS
2300	2300	Tricosane	–	1.2	4.8	3.7	RRI, MS
	2384	1-Hexadecanol	–	–	1.9	0.1	MS
2400	2400	Tetracosane	–	–	–	0.4	RRI, MS
	2475	1-Heptadecanol	–	–	7.5	–	MS
2500	2500	Pentacosane	–	0.3	6.0	1.2	RRI, MS
2600	2600	Hexacosane	–	–	–	tr	RRI, MS
2510–2633	2622	Phytol	–	1.7	–	0.4	MS
2700	2700	Heptacosane	–	–	6.2	0.5	RRI, MS
2900	2900	Nonacosane	–	0.1	3.9	tr	RRI, MS
2862–2945	2931	Hexadecanoic acid	–	0.1	1.9	0.2	RRI, MS
		Monoterpene hydrocarbons	64.5	4.8	3.5	4.9	
		Oxygenated monoterpenes	9.8	3.1	–	0.2	
		Sesquiterpene hydrocarbons	1.0	27.5	52.2	77.1	
		Oxygenated	1.9	55.3	5.6	8.9	

Table 7 (*continued*)

RRI_{lit}	$RRIe_{xp}$	Compound	R %	AP %	Fr %	F %	IM
		sesquiterpenes					
		Fatty acid + esters	0.3	0.1	1.9	0.2	
		Diterpenes	–	1.7	–	0.4	
		Others	16.4	4.3	33.8	7.5	
		Total	93.9	96.8	97	99.2	

RRI_{lit}: Relative retention indices for the volatile compounds from literature (Babushok et al., 2011; Acree and Arn, 2019).
RRI_{exp}: Relative retention indices calculated against *n*-alkanes.
%: calculated from FID data.
tr: Trace (< 0.1%).
IM: Identification method based on the relative retention indices (RRI) of authentic compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data; R: Root; AP: Aerial part; Fr: Fruit; F: Flower.

monoterpenes. Moreover, except fruit canals, all parts canals contain oxygenated monoterpenes. Only, aerial part and fruit canals contain terpenes. The distribution of different classes of compounds of the examples are displayed in Table 7. The flower canals contain the most sesquiterpene hydrocarbons (77.1%).

The stems, peduncles, rays, pedicels, and fruits of *Anthriscus nemorosa* got micrographs which were acquired from ethanol sample via utilising light (Figs. 4–8). The secretory canals were only found in the cortex at stems, peduncles, rays, and pedicels. The number of canals was higher at ray while they were found the least at the peduncle. However, the shape of secretory canals was larger than stems, rays, and pedicels. The secretory canals of the fruit were wide and very massive.

The inhibition of cholinesterase is a prominent aim in the fight against AD. Since several adverse effects have been reported with the latterly existing anticholinesterase agents, by which only symptomatic therapy could be reached, it is necessary to discover novel medication candidates for AD. Many aromatic herbs including a spectacular amount of essential oil have been reported to have inhibitory effects against AChE and BChE enzymes in varied investigations (Orhan et al., 2011).

Anthriscus nemorosa is among the significant medicinal herbs including essential oil and has been reported to be utilised traditionally in folk medicine and as a spicery. The researchers noted that the root essential oil and root CH_2Cl_2 fraction of *A. nemorosa* get inhibition against BuChE and AChE enzymes and strong antioxidative effects. Inhibition of BuChE is rather important since this enzyme lingers as the primary cholinesterase in the brains of AD patients being in the late phases (Orhan et al., 2011).

The antioxidants usage may be rewarding at AD curation (Galasko et al., 2012). To support such point of our inquisitiveness, the paper is the new exploration of anticholinesterase activity of extracts, fractions and essential oils from *A. nemorosa*.

Otherwise, synergistic interactions of α-pinene, 1,8-cineol, and camphor; the monoterpenes found in the essential oil of *Salvia officinalis*, were presented to create strong AChE inhibitory activity (Perry et al., 2000). α-Pinene was the dominant component of root essential oil from *A. nemorosa* (25.5%). Therefore, all these former findings propose that the effective monoterpenes (α-pinene) detected in essential oils against AChE, which could be also existing in the CH_2Cl_2 extracts, are either not existing or not enough spontaneously and this may support to clarify the occurrence of low inhibitory activity of the aforenamed *A. nemorosa* extracts against cholinesterases. An evaluation of the enzyme inhibitory activity of a combination of chemicals with regard to zero interplay, synergy or antagonism consists of a definition of what the anticipated answer of a mix should be. The interplays of a described combination of components could be usually defined as having a zero interaction in which the response of the combination is that supposed from the individual dose–response curves; synergy in which the response is greater than supposed; and antagonism in which it is less (Savelev et al., 2003). Essential oils contain many components and it is difficult to analyse chemical interactions to mimic the combinations of these components regardless of the number.

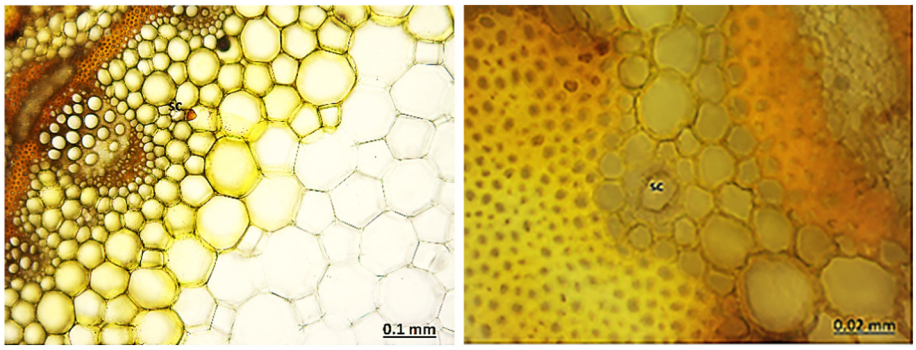

Fig. 4. Secretory canals at the stem of *Anthriscus nemorosa* by light microscopy.

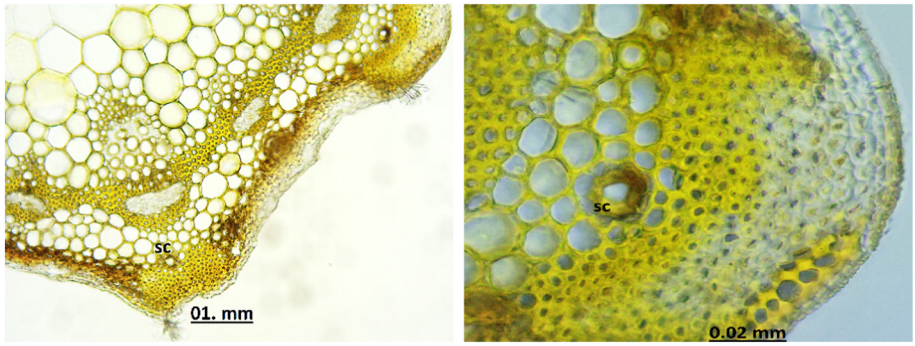

Fig. 5. Secretory canals at the peduncle of *Anthriscus nemorosa* by light microscopy.

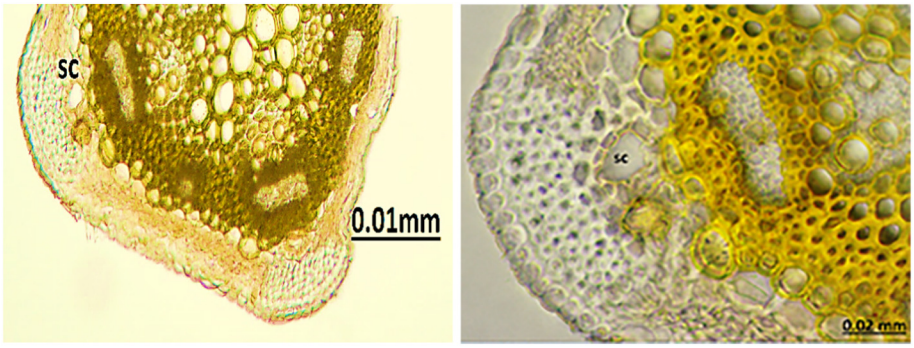

Fig. 6. Secretory canals at the ray of *Anthriscus nemorosa* by light microscopy.

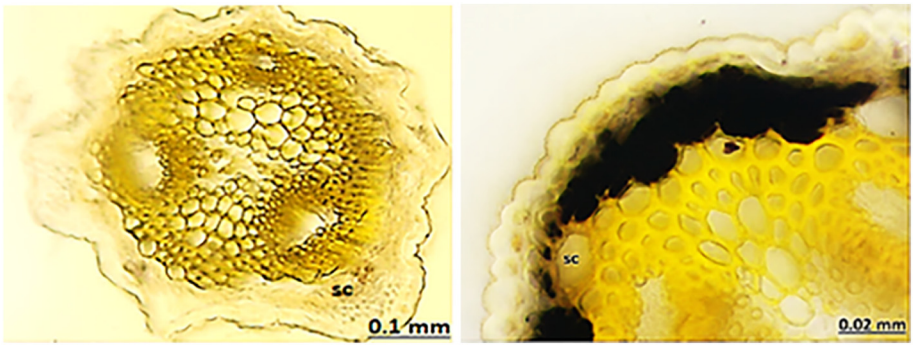

Fig. 7. Secretory canals at the pedicel of *Anthriscus nemorosa* by light microscopy.

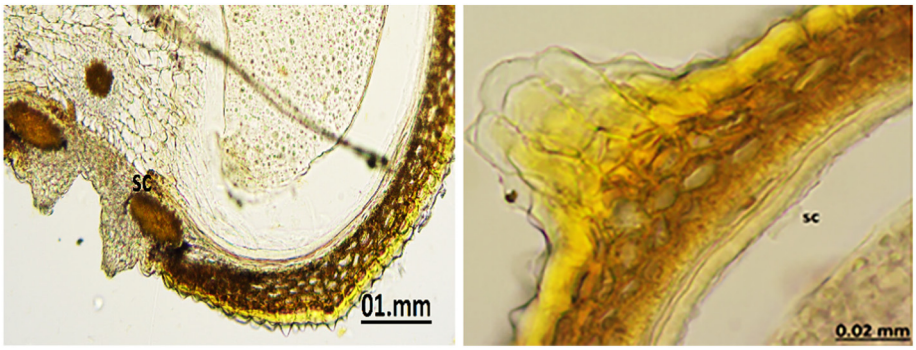

Fig. 8. Secretory canals at the fruit of *Anthriscus nemorosa* by light microscopy.

4. Conclusion

It is expected that 114 million human beings will be suffered from AD in 2050. It is a substantial public health problem because it raises pressure on caregivers and financial resources owing to the growing number of patients in countries. This current study emphasises the novel cholinesterase inhibitors which have fewer side-effects compared to the other cholinesterase inhibitors like alkaloids. Specifically, root essential oil and root CH_2Cl_2 fraction of *Anthriscus nemorosa* and major compound of root essential oil α-pinene got a worthy anticholinesterase and antioxidant capacities. The investigated extracts and essential oils revealed strong RSC, which were shown to be in correl to the phenolic compounds content. As a consequence, we can conclude that *A. nemorosa* may be utilised in AD and can utilise as an herbal substitute to synthetical medications.

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Declaration of Competing Interest

The authors report no declarations of interest.

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