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Chemical and morphological characterization of *Allium tuncelianum* (*Amaryllidaceae*) and its antioxidant and anticholinesterase potentials

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Abstract. Alzheimer's disease is the main reason for dementia, which increases with age. Cholinesterase inhibition and antioxidant potentials of extracts and essential oils from bulbs of *A. tuncelianum* (Kollmann) Özhatay & al., an endemic species to Tunceli (easthern Turkey), were evaluated. The fraction extracted of ethyl acetate had the highest phenolics level, 1,1-diphenyl-2-picrylhydrazyl, and thiobarbituric acid antioxidant capacity. Also, the ethyl acetate fraction presented the highest acetylcholinesterase (15.98 ± 2.76%), and butyrylcholinesterase inhibition (47.33 ± 3.27%). Diallyl disulfide (49.8%), diallyl trisulfide (27.9%) and allyl methyl trisulfide (6.9%) were found to be the major components of essential oil. This paper shows that the ethyl acetate fraction of *A. tuncelianum* could be a potent source of antioxidant and anticholinesterase components.

Keywords. *Allium tuncelianum*, anticholinesterase, endemic, essential oil, morphology.

Resumen. La enfermedad de Alzheimer es la causa principal de la demencia, cuya aparición aumenta según la edad. Se evaluaron la inhibición de la colinesterasa y el potencial antioxidante de los extractos y los aceites esenciales de los bulbos de *A. tuncelianum* (Kollmann) Özhatay & al., una especie endémica de Tunceli (este de Turquía). La fracción extraida de acetato de etilo presentó los niveles más altos de fenoles, 1,1-difenil-2-picrilhidrazilo y capacidad antioxidante, ácido tiobarbitúrico. Asimismo, la fracción de etil acetato presentó la mayor capacidad de inhibición de acetilcolinesterasa (15.98 ± 2.76%) y butirilcolinesterasa (47.33 ± 3.27%). El disulfuro de dialilo (49.8%), el trisulfuro de dialilo (27.9%) y el trisulfuro de metil alilo (6.9%) fueron los componentes principales del aceite esencial. Este artículo muestra que la fracción de etil acetato de *A. tuncelianum* podría ser una fuente potencial novedosa de componentes antioxidantes y anticolinesterasa.

Palabras clave. Aceite esencial, *Allium tuncelianum*, anticolinesterasa, endémico, morfología.

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease characterized by an accumulation of extracellular amyloid-beta peptide (Aβ) and intracellular neurofibrils resulting in a loss of memory. Aβ is the principal constituent of senile plaques that are thought to play a central role in the healing and progression of oligomer and fibril forms of AD. Besides, many studies have shown that oxidative stress and mitochondrial dysfunction might have a substantial role in AD and that they are suppressed or reduced by using antioxidant agents, suggesting a therapeutic intervention for AD patients. It is reported that numerous antioxidant components protect the brain from Aβ neurotoxicity (Sgarbossa & al. 2015). Pharmacological treatments available for AD occur by relaxation of the symptoms rather than targeting the etiological mechanisms (Ng & al. 2015).

Recent investigations have indicated that numerous medicinal herbs are a significant source of antioxidants. Plants include a large number of free radical scavenging molecules, such as alkaloids, tannins, steroids, rotenoids, carotenoids, dietary glutathione, anthocyanins, saponins, terpenoids, and flavonoids. Consequently, medical herbs become popular as a cheap source to discover novel antioxidants (Chand & al. 2018).

Volatile oils are found to have several pharmacological activities such as hepatoprotective, carminative, antispasmodic, antiviral, and anti-tumor, etc. Nowadays, many volatile oils have been characterized as natural antioxidants and proposed as potential sources for food preservation. Additionally, biologically active natural compounds are of interest to the pharmaceutics industry for the control of human ailments of microbial origin and for the containment of lipid peroxidative damage, which has been implicated in specific pathological diseases such as AD, ischemia-reperfusion injury, cancer, coronary, atherosclerosis, and aging (Mimica & al. 2004).

The representatives of family *Amaryllidaceae* J.St.-Hil. have been characterized by high phenolics content (Resetár & al. 2017) and some members of this family have been used in the treatment of AD. Galanthamine is an alkaloid of the *Amaryllidaceae* that is a competitive selective, long-acting, and reversible acetylcholinesterase inhibitor that maintains the beneficial effects even after treatment (López & al. 2002).

Garlic has been produced and eaten worldwide and has attracted attention due to its preservative potentials against various diseases. Previous investigations indicated that garlic has numerous biological and pharmacological effective compounds. It is used for medical aims since ancient times, and its usage for cancer treatment dates back to 3,500 years ago (Özkan & al. 2013).

The genus *Allium* L. (*Amaryllidaceae*; cf. APGIII 2009) consists of more than 900 species which naturally grow in the northern hemisphere (Duman & al. 2017; Ekşi & al. 2016). According to phylogenetic analyses, *A. tuncelianum* (Kollmann) Özhatay, B.Mathew & Şiraneci belongs to the *Allium* sect. *Allium*. Fritsch & Friesen (2002) have proposed that the wild progenitor of garlic species should grow in the district from South Central Asia to the Mediterranean, according to their taxonomic investigations. Mathew (1996) proposed that *A. tuncelianum* might be the wild progenitor of *A. sativum* L. and both share general characters such as the odor of leaves and bulbs.

Because of its similarity to the widespread garlic, it is locally named as 'Ovacik garlic' or 'Tunceli garlic' in the district. *Allium tuncelianum* generally consists of a single cloved white bulb, unlike garlic, which has bulbs with multiple cloves (Kiralan & al. 2013). Even though *A. tuncelianum* has been considered as a close relative of *A. sativum*, the precise phylogenetic or genetic relationships are not well known yet.

In this regard, the aim of this paper was to report the cholinesterase inhibitory and antioxidant activity of the methanol, hexane, dichloromethane, ethyl acetate, butanol, and aqueous extracts and essential oils of bulbs of *A. tuncelianum*. The total phenolic content of extracts and essential oil, as well as the essential oil composition and morphology of *A. tuncelianum*, were assessed.

MATERIAL AND METHODS

Plant specimen

Allium tuncelianum was gathered from Ovacık, Tunceli province (Eastern Turkey). The voucher specimens have been preserved at the Herbarium of Ataturk University, Faculty of Science under the code ATA-9877.

Extraction

Dried bulbs of *A. tuncelianum* (50 g) were crushed and macerated with methanol (3 times, 8 h) in a water-bath not exceeding 35°C (3 × 100 ml) by 200 rpm with using of a mechanical mixer. Combined bulbs extracts were filtered and concentrated by rotary evaporator to dryness, then dissolved in methanol: water (1 : 9) and fractionated three times with 150 ml of n-hexane, dichloromethane, ethyl acetate, and n-butanol, respectively.

On the other hand, 50 g of bulbs of *A. tuncelianum* were crushed and macerated with 200 ml of distilled water for 8 h/3 days at 30 to 35°C. The aqueous extract was filtered, frozen and lyophilized to attain aqueous extracts of bulbs. Amounts of the powdered parts of *A. tuncelianum* and acquired extracts/fractions are shown in Table 1.

Isolation of the essential oil, GC-FID and GC/MS analyses

The essential oil isolation, GC-FID, and GC/MS assays were done in accordance with Karakaya & al. (2016). The crushed part, the percentage of essential oil, and the color of the essential oil are displayed in Table 2.

Determination of total phenolic content

The total phenolic content of the samples was done in accordance with Karakaya & al. (2018). The procedure was repeated three times for each sample.

Antioxidant activity

The quantitative 1,1-diphenyl-2-picrylhydrazyl (DPPH) of the samples was done in Karakaya & al. (2018). The IC_{50} values of samples were established by linear regression analysis in triplicate.

Table 1. Amounts of the powdered parts of *A. tuncelianum* (Kollmann) Özhatay & al. and acquired extracts/fractions.

Extracts/Fractions (g)	Aerial part
MeOH	14.21
Hexane	2.08
CH_2Cl_2	5.11
EtOAc	1.03
BuOH	3.23
Methanolic residue	2.96
Lyophilised aqueous extract	15.44

Table 2. The crushed part, essential oil % yield *A. tuncelianum* (Kollmann) Özhatay & al. and color of essential oil (w/v, %).

Part	Crushed	Yield	Colour	Collection
bulbs	220 g	0.0046	White	2018

Table 3. Total phenolic contents of the extracts, fractions and essential oil from *A. tuncelianum* (Kollmann) Özhatay & al. [The data present the mean ± SD of three independent experiments ($p < 0.05$)].

Tested samples	Total phenolic contents (mg/g) ± SD
MeOH	377.25 ± 4.78
Hexane	67.78 ± 1.56
CH_2Cl_2	434.61 ± 4.12
EtOAc	666.45 ± 3.21
BuOH	124.05 ± 2.44
Methanolic residue	338.14 ± 5.34
Lyophilised aqueous extract	567.20 ± 3.07
Essential oil	209.01 ± 2.13

Anti-lipid peroxidation activity

The anti-lipid peroxidation activity of the samples was done according to Karakaya & al. (2018). The IC_{50} values were established through linear regression assay.

Evaluation of AChE and BuChE inhibition activities

The evaluation of AChE and BuChE inhibition followed Karakaya & al. (2018). The procedure was repeated three times for each plate. All data were denoted as mean ± SE of three independent tests.

Statistical analysis

Overall indications are denoted as mean ± SE and statistically analyzed through ANOVA one-way analysis followed by way of complementary analysis of Bonferroni ($P < 0.05$), planned to determine statistical significance.

RESULTS

General morphology

Allium tuncelianum (Kollmann) Özhatay, B.Mathew & Şiraneci, Kew Bull. 50 (4): 723 (Özhatay & Mathew 1995). *A. macrochetum* subsp. *tuncelianum* Kollmann, Notes Roy. Bot. Gard. Edinburgh 41: 262 (Kollmann & al. 1983), bason. Tipo: [Turkey] Tunceli, Munzur Da., Aksu Dere above Ovacik, 1800 m a.s.l., 21 Jul. 1957, *Davis 31498* leg. (holo-: E!; iso-: K!). Figs. 1 and 2.

Bulb 1.5–5.5 cm in diameter, ovoid; outer tunics thick, membranous, brownish to dirty yellowish-white; inner tunics thin, membranous, white; bulblets 1–2. Scape 50–150 cm. Leaves 4–8, 1–2.5 cm wide, flat, canaliculate, glabrous, shorter than scape. Spathe 10–20 cm, 1-valved, deciduous. Umbel 2–8 cm in diameter, spherical, dense (100–200 flowers), bracteolate. Perigone 2.5–3.5 mm, campanulate, pale pinkish to white, smooth. Stamens longer than perigone. Capsule 3–4 mm. Chromosome number (2*n*) 16.

Distribution and habitat.—East Anatolia; rocky areas, calcareous soils; 1000–2200 m a.s.l.

Phenology.—Flowering time from June to August.

Experimental section

The methanol extracts of bulbs of *A. tuncelianum* were fragmented using solvents with different polarities (C_6H_{14}, CH_2Cl_2, $C_4H_8O_2$, and $C_4H_{10}O$). Also, the lyophilised aqueous extract of bulbs was obtained. The extracts, fractions and essential oil were assessed for antioxidant and cholinesterase inhibitory activities. Also, the morphological study of *A. tuncelianum* was also evaluated.

The essential oils, extracts, and fractions of bulbs were estimated with regard to antioxidant capacity effect. The data of samples with regard to total phenolics content are displayed in Table 3. The fraction extracted of ethyl acetate (EtOAc) had the highest level of total phenolic (666.45 mg GAE g^{-1} DW) however the hexane fraction got the lowest phenolic content levels (67.78 mg GAE g^{-1} DW). DPPH analysis data were presented in Table 4 and the EtOAc fraction got the highest antioxidant activity (17.21 ± 4.33 µg/ml) and the hexane fraction had the lowest phenolic content (128.45 ± 3.56 µg/ml). The findings of the analysis of thiobarbituric acid (TBA) were exhibited in Table 5 as IC_{50} (µg/ml). The EtOAc fraction and lyophilized aqueous extract had the highest antioxidant potential (IC_{50} = 54.67 and 65.15 µg/ml, respectively) in TBA analysis.

Cholinesterase inhibitory activity of samples was revealed via colorimetric Ellman's method (Ellman & al. 1961), some changes were done following the mentioned method and donepezil was used as a standard (Yerdelen & Tosun 2015). In vitro cholinesterase inhibitory activity of samples at 100 µg/ml is displayed in Table 6. The EtOAc

Fig. 1. *Allium tuncelianum* (Kollmann) Özhatay, B.Mathew & Şiraneci: **a, b,** plant during the anthesis; **c,** plant during the pre-anthesis [AEF 24116; illustrated by Gülnur Ekşi].

Table 4. DPPH radical scavenging activity of the the extracts, fractions and essential oil from *A. tuncelianum* (Kollmann) Özhatay & al. (µg/ml) [The data present the mean ± SD of three independent experiments ($p < 0.05$)].

Tested samples	IC_{50} values (µg/ml) ± SD
MeOH	87.35 ± 3.42
Hexane	105.41 ± 3.34
CH_2Cl_2	31.43 ± 2.56
EtOAc	17.21 ± 4.33
BuOH	93.22 ± 1.66
Methanolic residue	128.45 ± 3.56
Lyophilised aqueous extract	52.57 ± 2.68
Essential oil	55.09 ± 3.22
Chlorogenic acid	2.41 ± 0.58
Propyl gallate	0.005 ± 0.21
Rutin	3.05 ± 0.89

Table 5. Antioxidant activities of the the the extracts, fractions and essential oil from *A. tuncelianum* (Kollmann) Özhatay & al. in TBA test [The data present the mean ± SD of four independent experiments ($p < 0.05$)].

Tested samples	IC_{50} values (µg/ml) ± SD
MeOH	451.61 ± 2.98
Hexane	>500
CH_2Cl_2	76.67 ± 3.77
EtOAc	54.67 ± 4.24
BuOH	166.29 ± 2.77
Methanolic residue	>500
Lyophilised aqueous extract	65.15 ± 1.79
Essential oil	89.25 ± 2.78
Chlorogenic acid	12.98 ± 4.89
Propyl gallate	3.44 ± 2.05
Rutin	9.65 ±3.09

and essential oil indicated remarkable inhibition against BuChE (47.33 ± 3.27 and 28.65 ± 2.58%, respectively) at 100 µg/ml. Also, EtOAc and CH_2Cl_2 fractions displayed inhibition against AChE (15.98 ± 2.76 and 14.12 ± 2.76%, respectively) at 100 µg/ml. On the other side, the methanolic residue fraction had no activity against both enzymes. Moreover, MeOH, lyophilized aqueous extracts and hexane, BuOH fractions had no activity against AChE. EtOAc fraction has been characterized by substantially higher total phenolic content than other samples.

The percentage yield of essential oil of *A. tuncelianum* and color of essential oil are presented in Table 2. The color of the essential oil was white. A total of five compounds making up 88.9% of the oil were defined in the bulbs of *A. tuncelianum*. Diallyl disulfide, diallyl trisulfide and allyl methyl trisulfide were the major components, amounting to 49.8%, 27.9 and 6.9%, respectively. Many of the defined

Table 6. In vitro AChE and BuChE inhibitory activities of samples from *A. tuncelianum* (Kollmann) Özhatay & al. at 100 µg/ml. [Superscript: a, standard error mean; b, no activity; c, not detected because of turbidity in the wells of microplates. The data present the mean ± SD of three independent experiments ($p < 0.05$)

Samples	Enyzmes	Percentile of inhibition ± S.E.M[a] against AChE and BuChE
MeOH	AChE	-[b]
	BuChE	9.68 ± 1.67
Hexane	AChE	ND[c]
	BuChE	2.56 ± 2.55
CH_2Cl_2	AChE	14.12 ± 2.76
	BuChE	23.56 ± 2.65
EtOAc	AChE	15.98 ± 2.76
	BuChE	47.33 ± 3.27
BuOH	AChE	-[b]
	BuChE	3.98 ± 2.56
Methanolic residue	AChE	-[b]
	BuChE	-[b]
Lyophilised aqueous extract	AChE	ND[c]
	BuChE	13.58 ± 1.93
Essential oils	AChE	7.59 ± 2.90
	BuChE	28.65 ± 2.58
Donepezil	AChE	82.45 ± 2.64
	BuChE	90.33 ± 4.16

Table 7. The essential oil composition of *A. tuncelianum* (Kollmann) Özhatay & al. RRI Relative retention indices calculated against n-alkanes. % calculated from FID data.

RRI	Compound	%
1292	Allyl methyl disulfide	1.9
1438	Allyl propenyl disulfide	2.4
1492	Diallyl disulfide	49.8
1607	Allyl methyl trisulfide	6.9
1811	Diallyl trisulfide	27.9
	Total	88.9

compounds were sulphur compounds. The compositions of essential oil are presented in Table 7.

DISCUSSION

Previously, it was observed that MeOH extract of the bulb of *A. tuncelianum* showed high antioxidant activities with the DPPH method (51.1 ± 5.5%) and greater content of total phenols (Yumrutaş & al. 2009). Moreover, another study about the antioxidant activity of *A. tuncelianum* showed that aqueous and EtOH extracts (Ağbaş & al. 2013) and MeOH extract (Şehitoğlu & al. 2018) of bulbs got a significant effect. Other research also found a substantial correlation between antioxidant capacity and total phenolic content (Sytar & al. 2015; Granato & al. 2018) as well.

Previous investigations indicated that the major constituents of essential oils of *A. tuncelianum* bulbs were diallyl tri- sulfides (30.90%) and diallyl disulfide (28.30%) (Takim & al. 2016); diallyl disulfide (in green and red garlic was 67.33% and 72.52%, respectively) (Kiralan & al. 2013).

Nowadays it is known that diallyl disulfide derivatives are chemical agents that modulate various facets of AD (Manral & al. 2015). Also, previous studies showed that some *Allium* species such as *A. sativum* and *A. tuberosum* Rottler ex Spreng. had significant effects on AD (Chauhan 2003; Kim & al. 2007; Ray & al. 2011).

AD is a neurodegenerative disease induced by oxidative stress with a further cholinergic lack in the brain. Particularly, AD is characterised by a reduction in the sum of acetylcholine delivered from cholinergic synapses. A therapy methodology has been stimulated to enhance or maintain the proportion of acetylcholine through inhibiting acetylcholinesterase (Dickson 1997). Essential oils are a miscellaneous family of low molecular weight organic compounds with circumstantial biological activity. Compounds that act as cholinesterase inhibitors still are the only pharmacological therapeutics for AD. Many in vitro examinations showed that some components, in essential oils, may have cholinesterase inhibitory activity (Karakaya & al. 2019).

This paper showed that the EtOAc fraction of A. tuncelianum has cholinesterase inhibitory and antioxidant effects. The uses of antioxidants may be beneficial for AD healing (Gibson & Huang 2005). To the literature surveys, this is the initial exploration of cholinesterase inhibitory activity of extracts, fractions and essential oil from *A. tuncelianum*.

Especially, EtOAc fraction of *A. tuncelianum* bulbs displayed a substantial cholinesterase inhibitory and antioxidant potentials. The studied essential oils, extracts and fractions exhibited radical scavenging capacity (RSC), which were detected to be in correlation to the content of phenolic compounds. The essential oil has been characterized by the presence of diallyl disulfide which was determined to have inhibition towards both cholinesterase inhibitory activities. This paper displays that the EtOAc fraction of *A. tuncelianum* could be a novel potency source of native antioxidant and anticholinesterase components.

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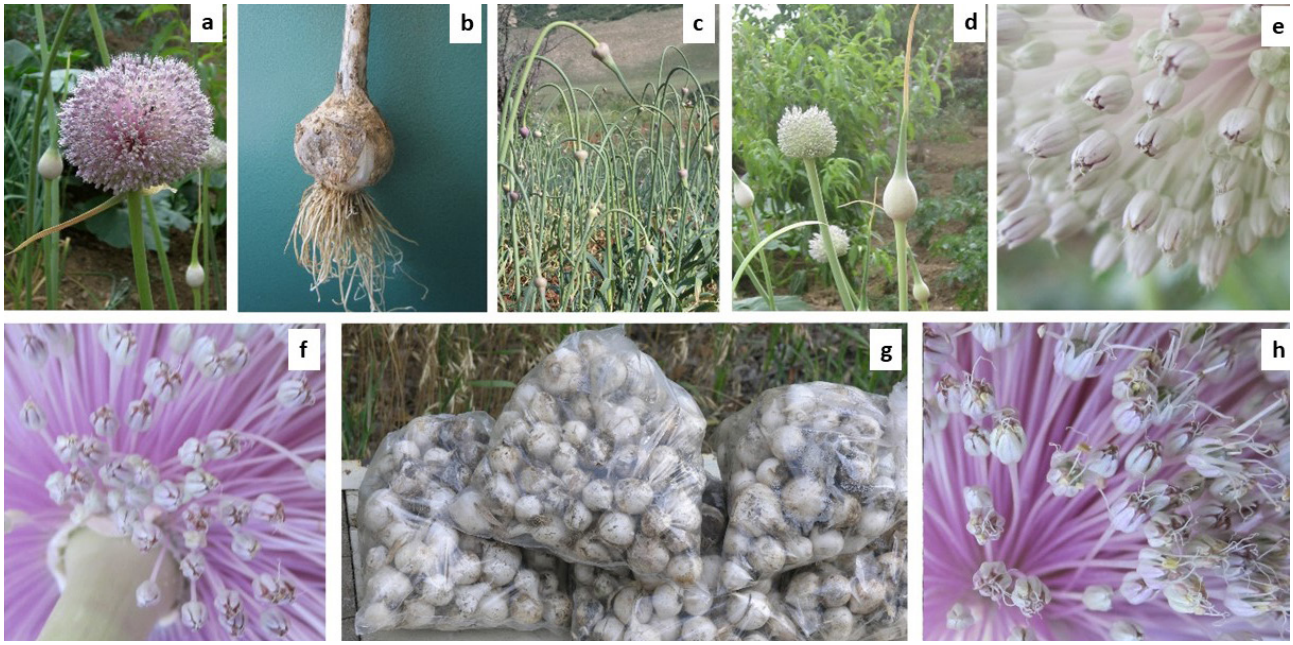

Fig. 2. *Allium tuncelianum* (Kollmann) Özhatay, B.Mathew & Şiraneci: **a, e, f, h,** anthesis; **b,** bulb; **c, d,** early stages of the anthesis; **g,** bulbs at a local market. [Photos: a, b, d-f, h, Gülnur Ekşi; c, g, Mehmet Koyuncu].

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