DOI:10.34108/eujhs.1384325

SAĞLIK BİLİMLERİ DERGİSİ
JOURNAL OF HEALTH SCIENCES
Erciyes Üniversitesi Sağlık Bilimleri Enstitüsü Yayın Organıdır

Araştırma 2024; 33(2): 190-198

ANALYSIS OF VOLATILE COMPOUNDS OF *ALCEA CALVERTİİ* BOİSS. AND ITS ANTIMICROBIAL, ANTICHOLINESTERASE AND ANTITYROSINASE POTENCY

ALCEA CALVERTİİ BOİSS.'İN UÇUCU BİLEŞENLERİNİN ANALİZİ AND ANTİMİKROBİYAL, ANTİKOLİNESTERAZ VE ANTİTİROZİNAZ ETKİSİ

Sıla Özlem ŞENER[1], Şeyda KANBOLAT[2], Nevin ULAŞ ÇOLAK[3], Merve BADEM[2], Rezzan ALIYAZICIOĞLU[2], Ufuk ÖZGEN[4], Şengül ALPAY KARAOĞLU[5], Ali KANDEMİR[6]

[1]*University of Health Sciences, Gülhane Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Türkiye*
[2]*Karadeniz Technical University, Faculty of Pharmacy, Department of Biochemistry, Trabzon, Türkiye*
[3]*Karadeniz Technical University, Faculty of Pharmacy, Department of Analytical Chemistry, Trabzon, Türkiye*
[4]*Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmacognosy, Trabzon, Türkiye*
[5]*Recep Tayyip Erdogan University, Faculty of Art and Science, Department of Biology, Rize, Türkiye*
[6]*Erzincan Binali Yildirim University, Faculty of Art and Science, Department of Biology, Erzincan, Türkiye*

ABSTRACT

The research aimed to analyze the volatile compounds by using SPME-GC-MS and to detect anticholinesterase, antityrosinase, and antimicrobial potential of metanol extract of *Alcea calvertii* Boiss., an endemic species for Türkiye. The inhibitory effects of tyrosinase, acetyl cholinesterase, and butyrylcholinesterase of the plant were determined by spectroscopic technique and the plant's antimicrobial activity was assessed using the agar diffusion method. A total of 18 volatile compounds were specified belonging to terpenes classes. *o*-cymene (10.60%) and sesquicineole (15.55%) were detected as major volatile components of the species by the SPME-GC-MS technique. Meaningful antimicrobial activity was observed on *Candida tropicalis, Enterococcus faecalis, Escherichia coli, Mycobacterium smegmatis, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. The half maximal inhibitory concentration (IC_{50}) value of the plant was determined 60.12 ± 1.75 µg/mL as a result of tyrosinase assay. IC_{50} value was found 111.54 ± 1.75 µg/mL, according to butyrylcholinesterase inhibition studies. Considering all the findings, it has been determined that the plant includes diverse volatile compounds and showed promising antimicrobial, tyrosinase inhibitory, and moderate butyrylcholinesterase inhibitory effects so *Alcea calvertii* may be the up-and-coming source of natural medicine for microbial and dermatological diseases although limited to its effects for Alzheimer's disease.

Keywords: *Alcea calvertii* Boiss., ant cholinesterase, antimicrobial, antityrosinase, GC-MS.

ÖZ

Bu araştırmanın amacı Türkiye için endemic bir tür olan *Alcea-calvertii* Boiss'in uçucu bileşenlerin SPME-GC-MS kullanılarak analiz edilmesi ve bitkinin methanol ekstresinin antikolinesteraz, antitirozinaz ve antimikrobiyal potansiyelinin belirlenmesidir. Türün tirozinaz, asetilkolinesteraz ve bütirilkolinesterazin hibitör etkileri spektroskopik teknikle belirlenmiş olup, antimikrobiyal aktivitesi için agar difüzyon yöntemi kullanılmıştır. Çalışma sonucunda terpenler sınıfı ait toplam 18 uçucu bileşen belirlenmiştir. SPME-GC-MS tekniği ile belirlenen uçucu bileşenelerinden *o*-simen (%10.60) ve seskisinol (% 15.55) türün ana uçucu bileşenleri olarak tespit edilmiştir. *Candida tropikalis, Escherichia coli, Enterococcus faecalis, Mycobacterium smegmatis, Pseudomonas aeruginosa* ve *Staphylococcus aureus* üzerinde ekstrenin anlamlı antimikrobiyal aktivite gösterdiği gözlemlenmiştir. Antitirozinaz aktivite çalışmaları sonucunda ekstrenin yarı maksimum inhibisyon konsantrasyonu (IC_{50}) değeri 60.12 ± 1.75 µg/mL olarak belirlenmiştir. Bütirilkolinesteraz inhibisyon çalışmalarına göre IC_{50} değeri 111.54 ± 1.75 µg/mL olarak tespit edilmiştir. Tüm bulgular gözönüne alındığında, türün zengin uçucu bileşen içeriğine sahip olduğu ve ümit verici antimikrobiyal, tirozinazinhibitör ve orta derecede bütirilkolinesteraz inhibitor etkiler gösterdiği, dolayısıyla *Alcea calvertii*'nin Alzheimer hastalığı için tedavi edici değeri sınırlı olmakla birlikte mikrobiyal, dermatolojik ve gelecek vaat eden doğal ilaç kaynağı olabileceği değerlendirilmiştir.

Anahtar kelimeler: *Alcea calvertii* Boiss., antikolinesteraz, antimikrobiyal, antitirozinaz, GC-MS.

Corresponding Author: Asst. Prof. Dr., Sıla Özlem ŞENER, silaozlem.sener@sbu.edu.tr, 0000-0001-7679-7165, Department of Pharmacognosy, Gülhane Faculty of Pharmacy, University of Health Sciences, 06018 Ankara, Türkiye

Authors: Asst. Prof. Dr., Şeyda KANBOLAT seydaakkaya@ktu.edu.tr, 0000-0001-7261-7067
PhD., Nevin ULAŞ ÇOLAK, nevinulas@ktu.edu.tr, 0000-0003-3200-6688
Asst. Prof. Dr., Merve BADEM, mervecolakoglu@ktu.edu.tr, 0000-0002-1265-5616
Prof. Dr., Rezzan ALIYAZICIOĞLU rezzan@ktu.edu.tr, 0000-0003-0143-8795
Prof. Dr., Ufuk ÖZGEN, uozgen@ktu.edu.tr, 0000-0001-9839-6717

Makale Geliş Tarihi : 01.11.2023
Makale Kabul Tarihi: 21.05.2024

INTRODUCTION

Plants possess comprehensive utilizations for different purposes for a long time.[1] Medicinal and aromatic plants have been utilized in the protection of health, prevention, and treatment of diseases for many years. Also, they are utilized in the field of food as dietary supplements, herbal tea, or flavor. In addition, they are used in perfumery and cosmetic industries as body care products.[2-4] So, phytochemical and biological activity studies on natural sources present new opportunities for scientific and industrial areas.

Alcea L. genus (Malvaceae) is represented by about 70 species in the world. The genus is found in Asia, especially in Iran and Türkiye. *Alcea calvertii* Boiss., one of the endemic species for Türkiye, is growing in Tunceli province. *Alcea* species have been used for anti-inflammatory effects, cough, intestinal disorders, kidney stones, pulmonary disorders, skin disorders, stomach ailments, urinary system disorders, etc., as a folk medicine for a long time. According to previous biological activity studies, *Alcea* species have diverse medicinal activities such as antioxidant, antimicrobial, antiviral, and hepatoprotective effects. *A. calvertii* has been used for kidney stones, lung disorders, pulmonary disorders, skin disorders, stomach disorders, and urinary system disorders, traditionally, too.[2,5,6]

The cholinergic system plays a vital role in brain functions. Acetylcholine (ACh) is accepted as a significant neurotransmitter for synaptic transmission of cholinergic system. Acetyl cholinesterase (AChE) and butyrylcholinesterase (BuChE) are enzymes in charge of hydrolysis of acetylcholine in synapse. Cholinesterase inhibitors inhibit the degradation of acetylcholine, thereby enhancing central and peripheral cholinergic function. ACh is hydrolysed by the AChE to allow the further transmission of impulses after the mediation of the impulse transmission. Another type of ChE, known as BuChE, is distributed all over the body and has a range of physiological roles. BuChE is discovered to play a role in both normal cholinergic function, and also the formation and progression of Alzheimer's Disease. AChE is the primary enzyme in the normal brain, while BuChE acts as a supporter when ACh concentrations are high. It has been suggested that in order to preserve optimal brain function, BuChE may assist the hydrolysis of excess ACh in the cholinergic system and balance to substrate-inhibited AChE. Cholinesterase inhibitors cause the rise of low choline levels and are clinically used in Alzheimer's Disease.[7]

Tyrosinase (TYR) enzyme is important in hyperpigmentation problems such as skin spots caused by excessive melanin synthesis in the body. Therefore, agents that inhibit TYR enzymes can be used to treat hyperpigmentation problems.[8-10]

Essential oils are oily mixtures obtained by water or steam distillation, which can be found in liquid or frozen form at room temperature. Essential oils obtained from plants are an indispensable part of the pharmaceutical, food, and cosmetic industries. The vast majority of essential oils (about 90%) are composed of terpenic substances. In chemical terms, terpenes are defined as a group of molecules that have a diverse but specific number of isoprene units. In recent years, natural antimicrobial agents have compelled the attention of both food and medicine industries due to their potential applications as food and medicine preservatives. Some secondary metabolites and essential oils found in plants can be used as food and medicine preservatives because of their antimicrobial effects for this purpose.[11,12]

Plant essential oils have been used frequently in cosmetic, food, and medicine areas for many years. So, the attention on obtaining, detecting, and quantifying natural volatile compounds has been scaling up for the food and medicine industries daily. Solid phase microextraction (SPME) is one of the detection and quantification techniques of natural volatile organic compounds which possess lots of advantages like running without solvent, portability, the probability of automation, rising sensitivity, passive sampling, etc.[13,14]

In light of all this information, the targets of this study were to carry out SPME-GC-MS analysison the species and to evaluate the antimicrobial and enzyme inhibition properties of *A. calvertii.*

MATERIAL AND METHODS

Chemicals and Instrumentation

Mushroom tyrosinase (EC 1.14.1.8.1, 30 U), levodopa (L -DOPA), disodium phosphate, sodium dihydrogen phosphate, 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB), acetylcholinesterase from *Electrophorus electricus* (electric eel) (AChE), acetylthiocholine iodide, butyrylcholinesterase human (BChE), butyrylcholine iodide, and galanthaminehydrobromide from *Lycoris* sp., were provided from Sigma-Aldrich. Methanol was purchased from Merck (Darmstadt, Germany). All microorganisms used in antimicrobial studies were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Türkiye). Ampicillin and fluconazole were purchased from Mustafa Nevzat and Pfizer, respectively. Mueller Hinton agar and broth, potato dextrose agar, brain heart ı-infusion agar (BHI), and broth were purchased from Merck (USA). All absorbance values were determined using a BMG LABTECH SPECTROstar® Nano spectrophotometer. Evaporation procedures were employed using the Heidolph rotary evaporator system (Schwabach, Germany). A manual fıber SPME device was obtained from Supelco (USA). GC analysis was carried out using a Shimadzu 2010 Plus (USA) device attached to a Shimadzu QP2010 (USA) Ultra mass selective detector and flame ionization detector concurrently.

Plant Material and Preparation of Samples

A. calvertii specimens were collected in 2017 from Erzincan, Türkiye and were identified by Prof. Dr. Ali KANDEMİR. The species was deposited in the Erzincan Binali Yildirim University Science Faculty herbarium (Herbarium Number: 10955). Primarily, the aerial parts of the plant were dried in the shade at room temperature and properly powdered. Forty grams of dried plant material was extracted with 400 mL methanol overnight at room temperature three times. The methanolic extract was filtered, and afterward, the filtrate was evaporated using a rotary evaporator to dry. The obtained extract was stored at 4 °C for use in biological studies.

SPME-GC-MS Analysis

Solid-phase microextraction

Polydimethylsiloxane/divinyl-benzene (PDMS/DVB, 65

µm-blue hub plain) fiber was identified in a manual fiber SPME device incorporated for exposing volatile components; the fiber was preconditioned for 30 minutes at 250 °C in the GC injection port. Following the sample process, the SPME device was inserted into the GC and GC-MS injectors for the duration of the 62-minute GC analysis on an RTX-5M column. The SPME fibers were prepared in the GC injector at 250 °C for 30 minutes. At 50 °C, extractions were achieved after 30 minutes of incubation and 10 minutes of extraction.

Gas chromatography-mass spectrometry/flame ıonization detector (SPME-GC-MS-FID)

Approximately 1.00 g of plant material was added to a 10-mL vial as part of the SPME method. Each extraction process used magnetic stirring. Next, in split mode, fibers containing extracted aroma components were introduced into the GC injector with a split ratio of 1:10. For four minutes, thermal desorption was conducted at 250 °C. To help with the separation processes, a Restek Rxi-5MS capillary column was used. After two minutes at 60 °C, the oven's temperature was raised to 240 °C at a rate of 3 °C per minute, and it was then maintained at 250 °C for an additional four minutes. The carrier gas used in this experiment was helium (99.999%), flowing at a steady flow rate of 1 mL/min. After the ionization voltage was stabilized at 70 eV, the electronic impact mode was employed for detection. A mass acquisition in scan mode (40-450 m/z) was carried out. For identification, each volatile molecule was compared to its corresponding RI (concerning the C7–C30 alkane standards). Mass spectrum data were compared with those stored in the Wiley and NIST library of mass spectra, as well as the FFNSC1.2 and previous literatures[15]

Antimicrobial Activity

In this work, *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Klepsiella pneumonias*ubp. *pneumonia* ATCC 18883, *Pseudomonas auroginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193 *Candida tropicalis* ATCC 13803, and *Saccharomyces cerevisiae* RSKK 251 were selected as test microorganisms. Ampicillin (10 µg) for antibacterial activity and fluconazol (5 µg) for antifungal activity were used as standard drugs. Mueller Hinton agar and broth for gram (-) and gram (+) bacteria, potato dextrose agar for yeast-like fungi, BHI, and broth for *M. smegmatis* were used. In this study, antimicrobial activity was determined by applying some changes in the agar disc diffusion method.[16]

Tyrosinase (TYR) Inhibitory Effect

The TYR inhibitory effect of the plant was determined with the Masuda method.[17] Different concentrations of methanolic extract (25, 50, 100, and 500 µg/mL), TYR solution (46 U/mL), and phosphate buffer (0.2 M, pH 7.0) were prepared and transferred to the microplate. This mixture was incubated for 10 min at 23°C. Later, L-DOPA solution (2.5 mM) was added to all wells. After incubating for 10 min at 23°C, the absorbance of this mixture was read at 490 nm using a spectrophotometer. Kojic acid used as standard was prepared in various concentrations (25, 50, 100, and 500 µg/mL), and the TYR inhibition effect was determined with the same assay.

Cholinesterase Inhibitory Effect

The anticholinesterase activity of the methanol extract was examined using Ellman's assay with some modifications.[18] The methanolic extract (25, 50, 100, and 200 µg/mL), AChE (0.2 U/mL) BuChE (0.2 U/mL), and phosphate buffer (10 mM, pH 8) were respectively put in an Elisa plate and incubated for 15 min at 25°C. Then, DTNB (10 mM) and acetylthiocholine iodide (15 mM) or butyrylthiocholine iodide (15 mM) (substrate) were added to all wells and incubated for 10 min. The absorbance was measured spectrophotometrically at 412 nm. Galantamine in various concentrations (25, 50, 100, and 200 µg/mL) was used as standard in this experiment.

Data Analysis

All experiments were conducted at least three times. Statistical significance differences between the data were expressed as means ± standard deviations (SDs). GraphPad Prism 8.0.1 (244) was used for the calculation and to create graphs.

RESULTS

Chromatographic separation is based on the principle that first low molecular weight compounds are entrained in the column and then high molecular weight compounds are entrained. Qualitative analysis of the separated volatile compounds was made by comparing the index values obtained from the mass spectra with the library retention index values. Volatile components of the plant were determined for the first time in this study. Volatile components of the extract of *A. calvertii* were investigated by SPME-GC-MS analysis and results were presented in Table 1-3. Extract of *A. calvertii* was comprised of monoterpenes (12.00%), monoterpenes derivatives (12.92%), sesquiterpenes (37.71%), sesquiterpenes derivatives (10.02%), and sesquiterpenoid (5.85%) (Table 1). The most abundant volatile components found in the plant are o-cymene (10.60%) and sesquicineole (15.55%) (Table 2).

Additionally, a total of 18 volatile compounds identified as 2-hexenal, benzaldehyde (CAS), α- phellandrene, n-octylacetylene, o-cymene, *β*-phellandrene, α-

Table 1. The chemical class distribution of the essential oil components of *A. calvertii*

Compound Class	%Area	Number of compounds
Monoterpenes	12	2
Monoterpenes Derivatives	12.92	3
Sesquiterpenes	37.71	7
Sesquiterpenes Derivatives	10.02	3
Sesquiterpenoids	5.85	1
Others	12	3

Table 2. The major components in the chemical class distribution of the essential oil constituents of *A. calvertii.*

Compound class	Major component	% Area	RI
Monoterpenes	o-cymene	10.60	1026
Monoterpenes Derivatives	α- phellandrene	8.37	1007
Sesquiterpenes	Sesquicineole	15.55	1516
Sesquiterpenes Derivatives	Trans-*β*-caryophyllene	4.52	1426
Sesquiterpenoids	Copaene	8.65	1380
Others	Benzaldehyde	5.44	966

terpinene, copaene, 7-epi-sesquithujene, trans-*β*-caryophyllene, α-bergamotene, (E)-*β*-farnesene, *β*-cubebene, α-curcumene, *β*-bisabolene, sesquicineole, carotol and *β*-bisabolol were determined with SPME-GC -MS method (Table 3, Figure 1-2).

The antimicrobial activity of *A. calvertii* was specified using the agar disc diffusion method and the results were listed in Table 4. *A. calvertii* was found effective against bacteria of *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis* and *M. smegmatis*.

TYR inhibitory effect of methanolic extract of *A. calvertii* was investigated modified dopachrome method. Percentage (%) inhibitions of TYR of the kojic acid were found 31.25 ± 0.71, 43.13 ± 1.17, 67.5 ± 0.82, 98.13 ± 1.31 µg/mL for the 25, 50, 100, and 500 µg/mL, respectively (Table 5). Percentage (%) inhibitions of TYR of the methanolic extract of A. calvertii were found 26.88 ± 1.23, 51.75 ± 0.57, 65.63 ± 1.05, 83.75 ± 1.24 µg/mL for the 25, 50, 100, and 200 µg/mL. The methanolic extracts of the plant half-maximal inhibitory concentration (IC_{50})

Table 3. Major volatile components of *A. calvertii*based on SPME-GC/FID-MS analysis.

Retention Time	Compound Name	% Area[a]	Retention Index[b]	Compound Classification
10.27	2-Hexenal	3.40	861	alkyl aldehyde
14.29	Benzaldehyde (CAS)	5.44	966	aromatic aldehyde
15.98	α- phellandrene	8.37	1007	cyclic monoterpene
16.55	n-Octylacetylene	3.16	1020	alkyne
16.81	o-cymene	10.60	1026	monoterpene
17.05	*β*-phellandrene	4.55	1032	cyclic monoterpene
19.54	α-Terpinene	1.40	1090	monoterpene
31.70	Copaene	8.65	1380	sesquiterpenoid
32.12	7-Epi-sesquithujene	1.80	1390	sesquiterpene
33.54	Trans-*β*-caryophyllene	4.52	1426	bicyclic sesquiterpene
33.94	α-Bergamotene	3.33	1437	sesquiterpene
34.57	(E)-*β*-farnesene	5.85	1453	sesquiterpenoid
35.50	*β*-cubebene	2.93	1478	tricyclic sesquiterpene
35.69	α-Curcumene	1.28	1483	sesquiterpene
36.67	*β*-bisabolene	5.22	1508	sesquiterpene
36.93	sesquicineole	15.55	1516	sesquiterpene
40.18	Carotol	1.87	1605	sesquiterpene
42.58	*β*-bisabolol	2.57	1674	monocyclic sesquiterpene
Total		90.50		

a,: % Area obtained by FID peak-area normalization; b,: RI calculated from MS, retention times relative to that of n-alkanes (C6-C30) on the nonpolar Restek Rxi-5MS column.

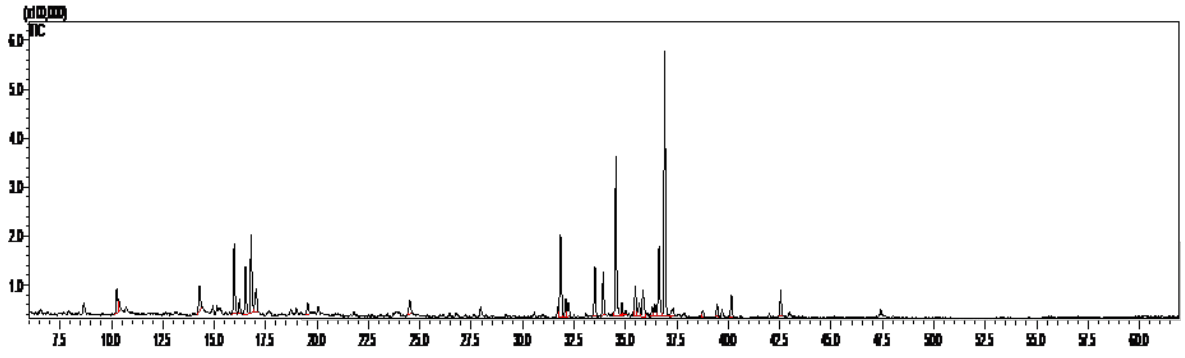

Figure 1: MS Spectrum of *A. calvertii*

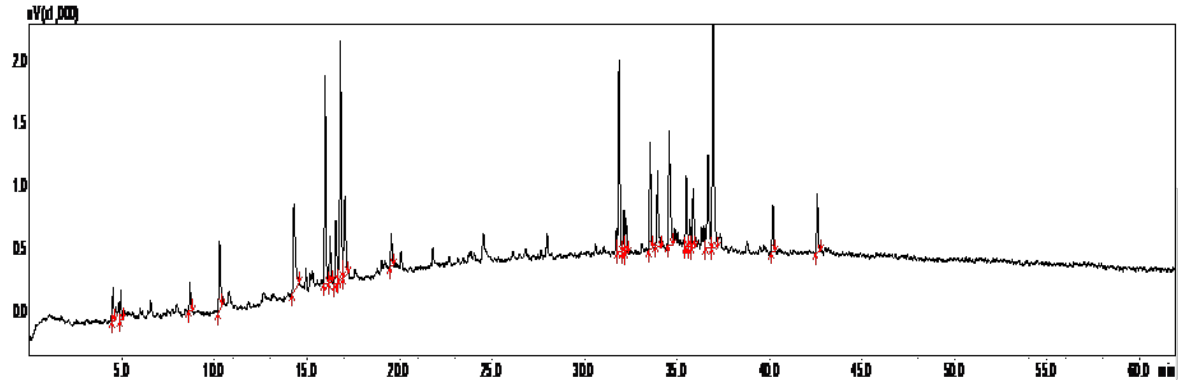

Figure 2: FID Spectrum of *A. calvertii*

Table 4. Antimicrobial activities of methanolic extract of *A. calvertii*(mm).

Tested Compounds	Microorganisms and Inhibition Zone (mm)										
	Gram-negative				Gram-positive			No gram	Yeast Like Fungi		
	Ec	Yp	Kp	Pa	Sa	Ef	Bc	Ms	Ca	Ct	Sc
The methanolic extract of *A. calvertii*	10	6	6	10	10	12	6	10	6	12	6
Ampicillin	10	10	10	18	35	10	15				
Streptomycin								35			
Fluconazole									25	25	25

Ec,:*E. coli* ATCC 25922, **Yp,:** *Y. pseudotuberculosis* ATCC 911,**Kp,:** *K. pneumonia*subp. *pneumonia* ATCC 18883, **Pa,:** *P. aeruginosa* ATCC 27853, **Sa,:** *S. aureus* ATCC 25923, **Ef,:** *E. faecalis* ATCC 29212, **Bc,:** *B. cereus* 702 Roma, **Ms,:** *M. smegmatis* ATCC607, **Ca,:** *C. albicans* ATCC 60193, **Ct,:** *C. tropicalis* ATCC 13803, **Sc,:** *S. cerevisiae* RSKK 251

value (60.12 ± 1.75 µg/mL) was similar to kojic acid (57.41 ± 1.03 µg/mL).

Cholinesterase inhibitory activity of the methanolic extract was examined in this study. The AChE percentage inhibition of the methanolic extract and galantamine is given in Figure 3. IC_{50} values of the methanolic extract of *A. calvertii* and galantamine were found 576.88 ± 2.35 and 8.07 ± 1.15 µg/mL, respectively. According to the BuChE inhibition test, IC_{50} values of the galantamine and the methanolic extract of *A. calvertii* were detected as 29.25 ± 2.35 µg/mL and 111.54 ± 1.71 µg/mL, respectively.

DISCUSSION

Most plants possess peculiar smell and aromatherapeutic properties because of their qualified volatile contents.[9,19] Many researchers have focused on plants' volatile contents which have many functional features such as antimicrobial, antiviral, antioxidant, and anticancer.[20,21] In this present study, volatile components of *A. calvertii* plant were identified by SPME-GC-MS. As stated in Table 1, the classification of the volatile components from *A. calvertii* was consisted of monoterpenes, sesquiterpene, and sesquiterpenoid. The major volatile components of *A. calvertii* were determined as *o*-cymene and sesquicineole. Terpenes provide lots of protective functions for the organism. In addition, terpenes possess significant biological activities such as antimicrobial, antifungal, and antimalarial properties.[11,22,23] Furthermore, volatile terpenoids has been proven to be as potential drug leads in Alzheimer's disease by ChE inhibition[24]. So *A. calvertii*, which includes

Table 5.TYR inhibitory effects of (% inhibition) of methanolic extract of *A. calvertii*

Samples	25 µg/mL	50 µg/mL	100 µg/mL	500 µg/mL	IC_{50} (µg/mL)
The methanolic extract of *A. calvertii*	26.88 ± 1.23	51.75 ± 0.57	65.63 ± 1.05	83.75 ± 1.24	60.12 ± 1.75
Kojic acid	31.25 ± 0.71	43.13 ± 1.17	67.5 ± 0.82	98.13 ± 1.31	57.41 ± 1.03

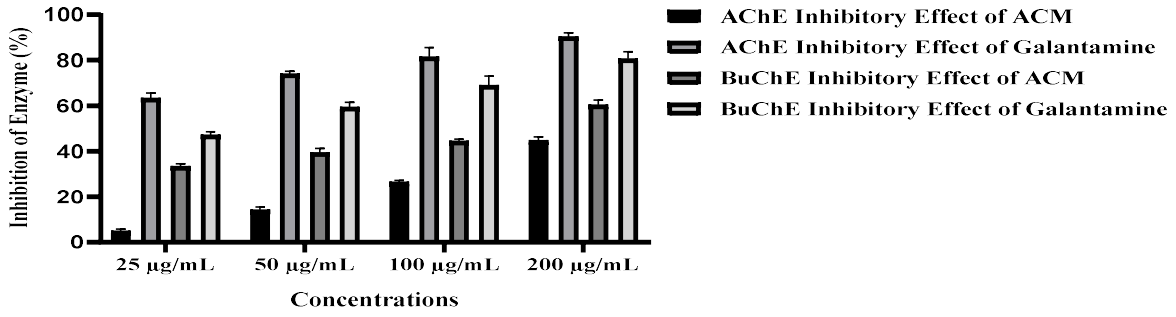

Figure 3: AChE and BuChE inhibitory effect (% inhibition) of methanolic extract of *A. calvertii* (ACM)

volatile components that belong to terpenes classes, can be a natural source of medicine for human health. It is the first study about SPME-GC-MS analysis of *A. calvertii*, but there are several studies about other *Alcea* species. A total of 28 terpenes were determined by GC-MS in the study on *Alcea nudiflora*.[25] Also, 24 terpenes and 29 terpenes were detected by GC-MS analysis for *Alcea pallida* and *Alcea apterocarpa*, respectively.[26]When the previous studies are compared with the present study, common volatile components were not detected between the *Alcea* species. The volatile compound differences of *Alcea* species can be related to typical properties or climatic, seasonal, and geographical factors. Other GC-MS analyses of *Alcea* species such as *Alcea rosea*, *Alcea pallida* are generally about fixed oil of the species.[2,27]

Nowadays, the importance of antimicrobial research has increased due to the rapid spread of antibiotic-resistant bacteria and microorganisms. Besides, the success rate against multiple antibiotic resistance in treating infection is decreasing gradually. Medicinal plants as an alternative to synthetic drugs continue to be important for antimicrobial treatments.[28,29] For this purpose, the antimicrobial activity of *A. calvertii* was determined in the current study. According to the data given in Table 4, the methanolic extract of aerial parts of *A. calvertii* showed significant antimicrobial activities against some microorganisms. The growth inhibition zones ranged from 6 to 12 mm against microorganisms. The methanolic extract of aerials of *A. calvertii* exhibited antimicrobial activity against bacteria of *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, and *M. smegmatis*. Compared with the effect of ampicillin, the plant showed moderate antimicrobial activity against *E. faecalis*. Activity on yeast fungus of the methanolic extract was observed against *C. tropicalis*. It has been reported that most of the subspecies of *Alcea* showed antimicrobial and antiviral activity in various studies.[5] In the previous study, *Alcea pallida* and *Alcea apterocarpa* inhibit the growth of *C. albicans*, *E. coli*, *Streptococcus pyogenes*, *S. aureus,* and *P. aeruginosa*.[26] The methanolic extract of flowers of *A. calvertii* was found to be effective on *Listeria monocytogenes*, *E. coli*, *B. subtilis,* and *C. albicans*.[17] Different effects on some microbial organisms of the same species are probably related to the selections of different parts of the plants. Also, climatic, seasonal, or geographical factors can be efficacious for this situation. Similarly, discrepancies between different *Alcea* species can be due to climate, seasonal, or geographical factors. It can also be said that the significant antimicrobial activity of *A. calvertii* is due to its volatile components.

Terpenes with antimicrobial qualities, or those that enable them to eradicate or inhibit the growth of microorganisms, are commonly used in both traditional and modern medications.[30] Studies on the antibacterial activity of β-caryophyllene have been actively conducted on its effects. According to the presented study, β-caryophyllene is the most abundant component belonging to sesquiterpenes derivatives in *A. calvertii*. Research about the antimicrobial activity of β-caryophyllene has been proven to affect both Gram-positive and Gram-negative aerobic bacteria, including *E. coli* and *S. aureus*.[31] Copaene is the most common essential component belonging to sesquiterpenes in *A. calvertii* have been proven to possess antimicrobial activity against Gram-positive and Gram-negative human pathogens.[32] Therefore, in particular, to β-caryophyllene and Copaene, all terpene components may contribute to the antimicrobial activity of *A. calvertii*.

The main task of melanin is to protect the skin from the harmful effects of ultraviolet light. TYR enzyme plays a key role in melanin synthesis. Many studies have been reported to be effective on the TYR enzyme of plant extracts.[33-36] In this study, the IC_{50} value of the *A. calvertii* extract and kojic acid were found 60.12 ± 1.75 and 57.41 ± 1.03 µg/mL. These findings have indicated that *A. calvertii* exhibited potent TYR inhibition. In the previous study about enzyme inhibition of *A. rosea*, the IC_{50} value of *A. rosea* extract was calculated as 0.38 mg/mL. *A. calvertii* is more effective than *A. rosea* for TYR enzyme inhibition compared with IC_{50} values.[16]

As mentioned before, *A. calvertii* was shown to possess high concentrations (≥4%) of *β*-caryophyllene, benzaldehyde, o-cymene, *β*-bisabolene, and sesquicineole components based on the presented study. *β*-Caryophyllene has proven to inhibit melanogenesis by downregulating tyrosinase, TRP-1, TRP-2, and MITF expression, which would lower the amount of melanin in the skin.[37] Benzaldehyde and its derivates have been evidenced to have the potential for tyrosinase inhibition.[38] Numerous herbal extracts one of the main constituents of their essential oils was determined as o-cymene have been shown to have tyrosinase inhibitory properties.[39,40] The tyrosinase inhibitory effect potential of a some plants-derived extracts which include *β*-bisabolene as marker components in their essential oil was revealed.[41] It has been demonstrated that a herbal essential oil source with high concentrations of sesquicineole and β-Caryophyllene constituents also has a strong inhibitory impact on tyrosinase at a concentration of 200 µg/mL (IC_{50}=63.30 ± 2.35 µg/mL).[42] Therefore, it may follow that these components contributed to *A. calverti*'s tyrosinase inhibitory property.

Interest in cholinesterase inhibition activity due to its importance for the treatment of Alzheimer's disease is increasing day by day. Synthetic drugs are being developed, but significant research is being continued to discover natural products that can be used for this purpose. Some studies show that some plants have traditionally been used to improve and alleviate other cognitive functions and symptoms associated with Alzheimer's disease.[12] BuChE inhibitor activity (% Inhibition) of the *A. calvertii* was found 33.25 ± 1.23, 39.26 ± 2.05, 44.35 ± 1.03 and 60.39 ± 2.16 µg/mL for the 25, 50, 100 and 200 µg/mL, respectively. AchE inhibitor activity (% inhibition) of the *A. calvertii* extract was found 4.86 ± 0.97, 14.21 ± 1.22, 26.43 ± 0.81 and 44.73 ± 1.61 µg/mL for the 25, 50, 100 and 200 µg/mL, respectively. According to the previous study, AchE inhibitory effect (% Inhibition) of the *A. pallida* and *A. apterocarpa* methanolic extracts were found 53.26 ± 1.24 and 57.07 ± 0.37 for 200 µg/mL, respectively.[26] The methanolic extracts of *A. pallida* and *A. apterocarpa* are similar but more effective than the methanolic extract of *A. calvertii* in terms of AChE inhibition. Consequently, the presented results revealed that *A. calverti* showed limited AChE and BuChE inhibitory effects like other *Alcea* species.

CONCLUSION

This study is a preliminary study to uncover the therapeutic potential of *A. calvertii* which includes abundant volatile content. It is also an original study in terms of the first SPME-GC-MS analysis, antityrosinase, and anticholinesterase activity screening studies on the plant. The plant has the potency to be used for hyperpigmentation treatments because of its inhibitory effect on the TYR enzyme. The study ensured to creation of preliminary data for using of *A. calvertii* in the treatment of various global diseases because of its cholinesterase inhibitory and antimicrobial effects. However, further studies about the determination of the compounds that are responsible for the activities, and detection of the underlying mechanism of the activities are needed to be clarified to benefit the therapeutic effects of *A. calvertii*.

Ethics Committee Approval: This study was not required ethics committee approval due to its scope.

Informed Consent: The relevant research does not require approval.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept-SOS; Design-SOS, SK, MB; Supervision-RA, UO; Resources-SOS, MB, SAK, AK; Materials-SOS, SK, MB, NUC; Data Collection and/or Processing-SOS, SK; Analysis and/or Interpretation-SOS, SK, MB, NUC, SAK; Literature Search- SOS, NUC; Writing Manuscript- SOS, SK; Critical Review- RA, UO.

Declaration of Interests: The authors declare that there is no conflict of interest.

Funding: This research did not receive support from any funding agency/industry.

Acknowledgements: We would like to thank University of Health Sciences, Karadeniz Technical University, Recep Tayyip Erdogan University, Erzincan Binali Yildirim University for providing substructure and infrastructure opportunities.

Etik Komite Onayı: Bu çalışma, kapsamı gereği etik kurul onayı gerektirmemektedir.

Bilgilendirilmiş onam: İlgili çalışma onam gerektirmemektedir

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir- SÖŞ; Tasarım-SÖŞ, ŞK, MB; Denetleme-RA, UÖ; Kaynaklar- SÖŞ, MB, ŞAK, AK; Malzemeler-SÖŞ, ŞK, MB, NUÇ; Veri Toplanması ve/veya işlenmesi-SÖŞ, ŞK; Analiz ve/veya yorum-SÖŞ, ŞK, MB, NUÇ, ŞAK; Literatür taraması-SÖŞ, NUÇ; Yazıyı yazan - SÖŞ, ŞK; Eleştirel inceleme-RA, UÖ.

Çıkar Çatışması: Yazarlar arasında herhangi bir çıkar çatışması bulunmamaktadır.

Finansal Destek: Bu araştırma herhangi bir finansman kuruluşundan/sektörden destek almamıştır.

Teşekkür: Sağlık Bilimleri Üniversitesi, Karadeniz Teknik Üniversitesi, Recep Tayyip Erdoğan Üniversitesi, Erzincan Binali Yıldırım Üniversitesi'ne alt yapı olanakları sağladıkları için teşekkür ederiz.

REFERENCES

1. Bat Özmatara M. The effect of extraction methods on antioxidant and enzyme inhibitory activities and phytochemical components of *Galium aparine* L. *Trak Univ J Nat Sci*. 2021;22(1):17-22. doi:10.23 902/trkjnat.772976
2. Korkmaz M, Karakurt E. Medicinal plants sold in Kelkit (Gümüshane) herbalists. *J Appl Nat Sci.* 2014;18(3):60-80.
3. Shad AA, Shah HU, Bakht J. Ethnobotanical assessment and nutritive potential of wild food plants. *J Anim Plant Sci*. 2013;23(1):92-97.
4. Dursun BY. Determination of monoamine oxidase inhibitions and antioxidant effects of various herbal teas. *Trak Univ J Nat Sci*. 2017;18(2):105-113. doi:10.23902/trkjnat.302152
5. Azab A. *Alcea*: Traditional medicine, current research and future opportunities. *Eur Chem Bull.* 2016;5(12):505-514. doi:10.17628/ ECB.2016.5.50 5
6. Azaizeh H, Saad B, Khalil K, Said O. The state of the art of traditional Arab herbal medicine in the Eastern region of the Mediterranean: A review. *Evid Based Complement Alternat Med*. 2006;3 (2):229-235. doi:10.1093/ecam/nel034
7. Ali R, Sheikha IA, Jabirb NR, Kamal MA. Comparative review of decade's research on cholinesterase inhibition. *Am J Neuroprot Neuroregen*. 2012;4(2):136-144. doi:10.1166/ajnn.2012.1051
8. Chang TS. An updated review of tyrosinase inhibitors. *Int J Mol Sci*. 2009;10(6):2440-2475. doi:10.3390/ijms10062440
9. Gholamhoseinian A, Razmi Z. Screening the methanolic extracts of some plants for tyrosinase inhibitory activity. *Toxicol Environ Chem*. 2012;94 (2):310-318. doi:10.1080/02772248.2011.644794
10. Zolghadri S, Bahrami A, Hassan-Khan MT, et al. A comprehensive review on tyrosinase inhibitors. *J Enzyme Inhib Med Chem*. 2019;34(1):279-309. doi:10.1080/14756366.2018.1545767
11. Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. *Food Control*. 2014;46:412-429. doi:10.1016/j.foodcont.2014.05.047
12. Saeed F, Afzaal M, Tufail T, Ahmad A. Use of Natural Antimicrobial Agents: A Safe Preservation Approach. Active Antimicrobial Food Packaging. London: IntechOpen; 2019: 92. doi:10.5772/ intecho pen.80869
13. Erdoğan EA. Use of plant essential oils and possible genetic effects. Mersin University Faculty of Medicine Lokman Hekim Journal of Medical History and Folkloric Medicine. 2012;2(2):21-24.
14. Gherghel S, Morgan RM, Arrebola-Liébanas J, et al. Development of a HS-SPME/GC–MS method for the analysis of volatile organic compounds from fabrics for forensic reconstruction applications. *Forensic Sci Int*. 2018;290:207-218. doi:10.1016/j.forsciint. 2018.07.015
15. Bicchi C, Liberto E, Matteodo M, et al. Quantitative analysis of essential oils: A complex task. *Flavour Fragr J*. 2008;23:382-391. doi:10.1002/ ffj.1905
16. Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar well diffusion method. *Acta Biologiae Experimentalis.* 1990;15(1):113-115.
17. Masuda T, Yamashita D, Takeda Y, Yonemori S. Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from *Garcinia subelliptica*. *Biosci*

Biotechnol Biochem. 2005;69(1):197-201.
18. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol.* 1961;7(2):88-90.
19. Carrapiso AI, Ventanas J, García C. Characterization of the most odor-active compounds of Iberian ham headspace. *J Agric Food Chem*. 2002;50 (7):1996-2000. doi:10.1021/jf011094e
20. Gonzalez-Burgos E, Gomez-Serranillos MP. Terpene compounds in nature: a review of their potential antioxidant activity. *Curr Med Chem*. 2012;19(31):5319- 5341. doi:10.2174/092986712803833 335
21. Göger G, Akçal Çomoğlu B, Işcan G, Demirci F. Evaluation of anticandidal effects of essential oils of commercial *Lavender* (*Lavandula angustifolia* Miller) in combination with ketoconazole againts some *Candida* strains. *Trak Univ J Nat Sci*. 2020;21(1):13-19. doi:10.23902/trkjnat.590839
22. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. *J Appl Microbiol*. 1999;86(6):985-990. doi:10.1046/j.1365-2672.1999.00780.x
23. Ebel R. Terpenes from marine-derived fungi. Mar Drugs. 2010;8(8):2340-2368. doi:10.3390/md80 82340
24. Wojtunik-Kulesza, KA, Targowska-Duda K, Klimek K, et al. (2017). Volatile terpenoids as potential drug leads in Alzheimer's disease. *Open Chem.* 2017;15(1):332-343. doi:10.1515/chem-2017-004 0
25. Hanif M, Mehmood MH, Ishrat G, et al. Pharmacological basis for the medicinal use of *Alcea rosea* in airways disorders and chemical characterization of its fixed oils through GC-MS. *Pak J Pharm Sci*. 2019;32(5):2347-2355.
26. Keser S, Keser F, Tekin S, et al. *In vitro* antiradical, antimicrobial and antiproliferative activities and phytochemical compositions of endemic *Alcea calvertii* (Boiss) Boiss. flowers. *Duzce University Journal of Science and Technology*. 2020;8(1):693 -701. doi:10.29130/dubited.595147.
27. Khidyrova NK, Rakhmatova MZ, Kukina TP, Shakhidoyatov RK, Shakhidoyatov KM. Polyprenols and triterpenoids from leaves of *Alcea nudiflora*. *Chem Nat Compd*. 2012;48(2):180-184.
28. Lee KG, Shibamoto T. Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. *J Agric Food Chem*. 2002;50(17):4947-4952. doi:10.1021/jf025568
29. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A comprehensive review on medicinal plants as antimicrobial therapeutics: Potential avenues of biocompatible drug discovery. *Metabolites*. 2019;9(11):258-271. doi:10.3390/ metabo9110258
30. Cox-Georgian D, Ramadoss N, Dona C, Basu C. Therapeutic and medicinal uses of terpenes. *Medicinal Plants*, 2019;2019:333-359. doi:10.1007/978-3-030-31269-5_15
31. Yoo HJ, Jwa SK. Inhibitory effects of β-caryophyllene on *Streptococcus mutans* biofilm. *Arch. Oral Biol*. 2018;88:42-46 doi:10.1016/j.arc horalbio.2018.01.009
32. Martins CDM, Nascimento EAD, de Morais SA, et al. Chemical constituents and evaluation of antimicrobial and cytotoxic activities of *Kielmeyera coriacea* Mart. & Zucc. essential oils. *Evid Based Complement Alternat Med.* 2015;2015:1-9. doi:10.1155/2015/ 842047
33. Baurin N, Arnoult E, Scior T, Do QT,Bernard P. Preliminary screening of some tropical plants for anti-tyrosinase activity. *J Ethnopharmacol*. 2002;82:155-158. doi:10.1016/S0378-8741(02) 00174-5
34. Tengamnuay P, Pengrungruangwong K, Pheansri I, Likhitwitayawuid K. *Artocarpus lakoocha* heartwood extract as a novel cosmetic ingredient: Evaluation of the *in vitro* anti-tyrosinase and *in vivo* skin whitening activities. *Int J Cosmet Sci*. 2006; 28: 269-276. doi:10.1111/j.1467-2494.2006.00339.x
35. Murray A, Faraoni M, Castro M, Alza N, Cavallaro V. Natural AChE Inhibitors from plants and their contribution to Alzheimer's Disease therapy. *Curr Neuropharmacol*. 2013;11(4):388-143. doi:10.2174/ 1570159X11311040004
36. Namjoyan F, Jahangiri A, Azemi ME, Arkian E, Mousavi H. Inhibitory Effects of *Physalis alkekengi* L., *Alcea rosea* L., *Bunium persicum* B. Fedtsch. and *Marrubium vulgare* L. on mushroom tyrosinase. *Jundishapur J Nat Pharm Prod*. 2015;10(1):1-6. doi:10.17795/jjnpp-23356
37. Yang, CH, Huang YC, Tsai ML, et al. Inhibition of melanogenesis by β-caryophyllene from lime mint essential oil in mouse B16 melanoma cells. *Int J Cosmet Sci.* 2015;37(5):550-554. doi:10.1111/ics. 12224
38. Maghsoudi S, Adibi H, Hamzeh M, et al. Kinetic of mushroom tyrosinase inhibition by benzaldehyde derivatives. *J. Rep. in Pharm. Sci.* 2013;2(2):156-164.
39. Samai Z, Toudert N, Djilani SE, et al.Chemical Composition and in vitro Antioxidant, Anti-Alzheimer, Anti-Diabetic, Anti-Tyrosinase, and Antimicrobial Properties of Essential Oils and Extracts Derived from Various Parts of the Algerian *Calendula suffruticosa* Vahl subsp. *boissieri* Lanza. *Chem. Biodiversity.* 2023;20(1):1-10. doi:10.1002/cbdv.20220 0620
40. Pintatum A, Laphookhieo S. Volatile constituents of *Amomum argyrophyllum* Ridl. and *Amomum dealbatum* Roxb. and their antioxidant, tyrosinase inhibitory and cytotoxic activities. *Arab. J. Chem.* 2022;15(10):1-12. doi:10.1016/ j.arabjc.2022.104148
41. José-Rita BJ, Bertin GK, Ibrahime SK, et al. Study of the chemical and in vitro cytotoxic activities of essential oils (EOs) of two plants from the Ivorian flora (*Lippia multiflora* and *Zingiber officinale*) and their antiviral activities against non-enveloped viruses. *S Afr J Bot.* 2022:151;387-393. doi:10.1016/j.sajb.2022.03.053
42. Adewinogo SO, Sharma R, Africa CW, et al. Chemical Study and Comparison of the Biological Activities of the Essential Oils of *Helichrysum petiolare*,

H. cymosum, and *H. odoratissimum*. *Plants.* 2022;11(19):1-16. doi:10.3390/plants11192606.