Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Chemical analysis and enzyme inhibitory activities of essential oil obtained from *Allium proponticum* subsp. *proponticum,* an endemic species

Ceren EMİR [1], **Ahmet EMİR** [2*]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Ege University, İzmir, Turkey.
[2] Department of Pharmacognosy, Faculty of Pharmacy, Ege University, İzmir, Turkey.
* Corresponding Author. E-mail: ahmet.emir@ege.edu.tr (A.E.); Tel. +90-505-393 21 09.

Received: 01 November 2021 / Revised: 24 November 2021 / Accepted: 13 February 2022

ABSTRACT: *Allium* species belong to the Amaryllidaceae family and are represented with approximately 900 species, with 220 taxa found in Turkey. The aim of the present study is to analyze the chemical structure and to investigate the enzyme inhibitory activities linked to Alzheimer's Disease, skin disorders, and type 2 Diabetes Mellitus of essential oil obtained from *Allium proponticum* subsp. *proponticum*, which is an endemic species. The essential oil was obtained with hydrodistillation using a Clevenger-type apparatus from flower parts of the plant and the analysis was carried out by Gas Chromatography Mass Spectrometry. Inhibitory activities of samples against acetylcholinesterase, butyrylcholinesterase, tyrosinase, *a*-amylase, and *a*-glucosidase were realized in a 96-well microplate using an ELISA microplate reader. The yield of essential oils was 0.0095 %. A total of 17 components, 11 of sulfur-containing compounds (73.83 %), were identified, constituting 86.72 % of the total essential oil. IC_{50} values of enzymes inhibitory activities were 258.47 ± 3.28 for acetylcholinesterase, 522.81 ± 1.29 for butyrylcholinesterase, 38.22 ± 1.87 for tyrosinase, 28.50 ± 1.12 for *a*-glucosidase and 14.68 ± 0.78 for *a*-amylase. To conclude, the essential oil of *Allium proponticum* subsp. *proponticum* with its sulfur-containing compounds may be a promising natural product due to its activity in inhibition of tyrosinase, *a*-amylase, *a*-glucosidase.

KEYWORDS: *Allium;* essential oil; anticholinesterase; antityrosinase; antidiabetic.

1. INTRODUCTION

Clinical drug treatments of Alzheimer's disease (AD) are based on cholinesterase inhibitors (tacrine, donepezil, rivastigmine, galantamine) and antagonist of *N*-methyl-D-aspartic acid (NMDA) receptor (memantine). As neurotransmitter regulators, these drugs do not affect mortality but increase the quality of life and stabilize the disease stage. Thus, cholinesterase inhibitors are currently major drugs for the symptomatic treatment of AD [1]. Tyrosinase is a multifunctional, glycosylated, and copper-containing oxidase, which catalyzes the formation of melanin via steps of hydroxylation of tyrosine to L-Dopa and subsequent oxidation L-Dopa to dopaquinone. Melanin is a photoprotective pigment in human skin however, the excessive melanin deposition in different parts of the skin might give rise to melanoma and many other skin disorders [2]. Also, tyrosinase creates the enzymatic browning of fruits and vegetables, which negatively influences their color, taste, flavor, and nutritional value [3]. Diabetes mellitus (DM) is s a metabolic disorder characterized by hyperglycemia, which causes instability in the metabolism of fat, carbohydrates, and protein. The fast breaking down of starch into glucose leads to high blood glucose levels, known as postprandial hyperglycemia, an indicator for type 2 DM. *a*-amylase and *a*-glucosidase enzymes induce postprandial hyperglycemia due to the hydrolysis of starch and oligosaccharide. Starch and other complex polysaccharides are hydrolyzed by *a*-amylase to oligosaccharides such as maltose, maltotriose, etc., via the cleavage of *a*-1,4 glycosidic bonds. These fragments are subsequently transformed into the absorbable monosaccharide in the final step of the digestion by *a*-glucosidase located in the small intestine. Thus, the inhibition of these enzymes by pharmaceutical agents is an accepted clinical strategy for managing postprandial glycemia in patients affected by type 2 diabetes [4, 5].

Allium species belong to the Amaryllidaceae family and are represented with approximately 900 species with 220 taxa found in Turkey, distributed across the northern hemisphere [6]. While these species are rich in

Emir C, Emir A. Chemical analysis and enzyme inhibitory activities of essential oil obtained from Allium proponticum subsp. proponticum, an endemic species. J Res Pharm. 2022; 26(3): 574-580.

http://dx.doi.org/10.29228/jrp.155
ISSN: 2630-6344

carbohydrates, proteins, fats, minerals, and vitamins [7], they have a wide spectrum of secondary metabolites including mainly organosulfur compounds as well as polyphenolics, saponins, and alkaloids [8]. Accordingly, many cultivated and wild *Allium* species have been investigated for their anticarcinogenic, antimicrobial, antiplatelet, antithrombotic, antihyperlipidemic, antihypertensive, and antiasthmatic effects as well as immune-stimulatory properties [9].

While there are many studies on essential oils of aromatic plants, limited studies on essential oils obtained from *Allium* species have been reported. *A. proponticum* Stearn Et N. Özhatay has two endemic subspecies including *proponticum*, and *parviflorum*, also subsp. *proponticum* differs with 3.5-4 mm perianth segments from the other. *A. proponticum* subsp *proponticum*, has a flowering period between May and June, distributed in coastal cliffs, beaches, rocky slopes, vineyards, walls, ruins [10]. The core objective of this research was to determine the chemical compositions as well as cholinesterase, tyrosinase, *α*-amylase, and *α*-glucosidase inhibitory activities of essential oil obtained from flower parts of *A. proponticum* Stearn Et N. Özhatay subsp. *proponticum* Stearn Et N. Özhatay, for the first time to the best of our knowledge.

2. RESULTS and DISCUSSION

2.1. Essential oil analysis

Firstly, the whole plant was used to obtain essential oil by hydrodistillation, but the foam was formed with boiling due to secondary metabolites such as saponosides contained in the plant and the essential oil could not be obtained. Secondly, the bulb parts of the plant were tried, the foam did not ocur, but the essential oil could not be obtained sufficient for the analysis of experiments. Thirdly, the stem parts of the plant were tried however, the same problem occurred as in the first step. Lastly, the flower parts were used and the essential oil was obtained without the foaming problem by a yield of 0.0095 % (v/w). In the literature, essential oils were got from especially bulb and flower parts of wild *Allium* species with varied yields; from bulbs of *A. tuncelianum* (0.0046 %) [11], *A. macrochaetum* (0.25 %) [12], *A. macrostemon* (0.56 %) [13], and from flowers of *A. atroviolaceum* (0.7 %) [14], *A. sphaerocephalon* (0.0074 %) [15], and *A. roseum* (0.05 %) [16]. Essential oils, obtained from aromatic plants, are volatile, natural, complex compounds characterized by a strong odor and have terpenes as the main components. However, sulfur-containing molecules were found as major compounds which have low stability. Therefore, analyzes were performed as quickly as possible after essential oil isolation. The chemical composition of the oil was investigated by GC-MS and results are presented in Table 1, and also total ion chromatogram is given in Figure 1. 3,5-diethyl-1,2,4-trithiolane isomers were the main compounds. A total of 17 components, 11 of sulfur-containing compounds (73.83 %), were identified, constituting 86.72 % of the total essential oil. Similarly, sulfur compounds were determined as major components in the analysis of essential oils obtained from different *Allium* species. 3,5-diethyl-1,2,4-trithiolane isomer was a second dominant compound in the essential oil of *A. sphaerocephalon* [15], additionally, di-2-propenyl disulfide in *A. sativum*, dipropyl disulfide in *A. cepa* and *A. fistulosum* [17], diallyl disulfide in *A. tuncelianum* [11], and *A. macrochaetum* [12], methyl propyl disulfide in *A. macrostemon* [13] and allyl methyl trisulfide in *A. ursinum* [18] were the main compounds of essential oils. Although generally, polysulfides were the major molecules in these essential oils, methyl-propenyl disulfide isomer and dimethyl trisulfide were minor compounds in our sample. Basically, S-alkenyl-L-cysteine sulfoxides (ACSOs) are the main water-soluble molecules in crude *Allium* plants. Alliinase (EC 4.4.1.4) is released by mechanical processes such as crushing or chopping, and thiosulfinates are formed by an enzymatic reaction of ACSOs. Then, these quite unstable molecules convert to oil-soluble polysulfides [19]. So, the amount of polysulfides in the sample also depends on the diversity and quantity of ACSOs.

Table 1. The essential oil composition of *A. proponticum* Stearn Et N. Özhatay subsp. *proponticum* Stearn Et N. Özhatay

No	Compound	Retantion Time	Content %
1	Methyl-propenyl disulfide (isomer)	14.363	1.37
2	Dimethyl trisulfide	15.448	0.91
3	Nonanal	19.650	3.28
4	Ethyl-3-methyl thiopropionate	20.497	1.79
5	Decanal	22.724	1.49
6	3-ethyl-5-methyl-1,2,4-trithiolane (isomer)	23.835	4.83
7	3-ethyl-5-methyl-1,2,4-trithiolane (isomer)	24.049	5.52
8	3,5-diethyl-1,2,4-trithiolane (isomer)	26.677	19.61
9	3,5-diethyl-1,2,4-trithiolane (isomer)	26.905	23.38
10	3,4-bis(methylthio)-1,2,5-thiadiazole	28.282	1.01

http://dx.doi.org/10.29228/jrp.155
J Res Pharm 2022; 26(3): 574-580

11	N-methylpyrrolidine-2-thione	29.205	3.98
12	2,3-bis-methylthio-1,4-dithiane (isomer)	33.318	7.32
13	2,3-bis-methylthio-1,4-dithiane (isomer)	33.388	4.11
14	2-Pentadecanone, 6,10,14-trimethyl	38.413	0.78
15	Heneicosane	46.803	1.35
16	Pentacosane	50.943	2.28
17	Heptacosane	56.940	3.71
18	Sulfur-containing compounds		73.83
19	Hydrocarbons		7.34
20	Aldehydes		4.77
21	Ketones		0.78
	Total		86.72

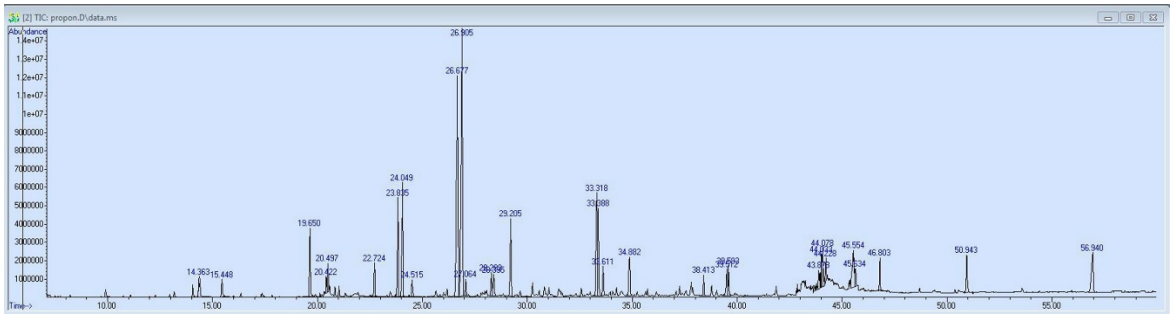

Figure 1. Total ion chromatogram of *A. proponticum* Stearn Et N. Özhatay subsp. *proponticum* Stearn Et N. Özhatay

2.2. Enzyme inhibitory activity

Inhibitory potentials of the essential oil against five enzymes including acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), tyrosinase, *a*-glucosidase, and *a*-amylase were investigated in 96-well microplates by an ELISA microplate reader. Galanthamine was used as a positive standard for AChE and BuChE inhibitory activities of the sample and IC_{50} values of galanthamine were calculated as 0.106 ± 0.01 and 1.04 ± 0.01 µg/mL, respectively. Low anticholinesterase activity of the sample was observed and IC_{50} values were determined as 258.47 ± 3.28 µg/mL (AChE) and 522.81 ± 1.29 µg/mL (BuChE). In contrast, in our previous studies, methanol extracts of different *Allium* species showed significant cholinesterase inhibitory activity due to their rich phenolic compounds [20, 21]. Also, a detailed structure-activity relationship has not been reported between sulfur compounds and cholinesterase enzymes. In our research, the sulfuric compounds were established as high amounts based on the peak area, and notwithstanding this point, lower values belonging to the essential oil samples for anticholinesterase activity were calculated. On the other hand, sulfur-containing molecules are considered as capable drug candidates for AD treatment because of possessing neuroprotective efficacy by modulating cellular redox homeostasis [22]. Regarding this property, numerous sulfuric constituents have antioxidant activity [23]. Among them, allyl sulfide derivatives, which were detected in the studied *Alliums*, such as diallyl disulfide and diallyl trisulfide displayed cytoprotective activities by prohibiting Aβ-induced cell death [24]. Moreover, allyl sulfides prevented Aβ aggregation in vitro thanks to different methods, including HPLC and thioflavin fluorescence [25]. Therefore, the mentioned molecules with small quantities in these *Allium* plants might play a role in antioxidant potential together with phenolics.

Tyrosinase inhibitory activity of the sample was carried out using *a*-kojic acid as a positive control, which demonstrated an IC_{50} value of 7.9 ± 0.02 µg/mL. The essential oil showed potent inhibitory activity with 38.22 ± 1.87 µg/mL IC_{50} value. As mentioned above, sulfur compounds were the main components in the essential oil sample, like other *Allium* essential oils notified in the literature. A high affinity to certain metal ions also makes S-containing molecules beneficial for competitive inhibitor design. The sulfur atom of the molecule binds to both copper ions in the active site of the tyrosinase and blocks enzyme activity [26]. So, many studies have revealed the competitive inhibitory property of S-containing compounds against the tyrosinase enzyme [26-28].

Acarbose was used as a positive standard to measure the *a*-amylase and *a*-glucosidase inhibitory potential of the essential oil, and the IC_{50} values were determined as 8.75 ± 0.97 µg/mL and 300.56 ± 6.65

http://dx.doi.org/10.29228/jrp.155
J Res Pharm 2022; 26(3): 574-580

µg/mL, respectively. Also, IC_{50} values of sample were calculated as 14.68 ± 0.78 µg/mL for *a*-amylase inhibitory and 28.50 ± 1.12 µg/mL for *a*-glucosidase inhibitory activities. Many *Allium* species have been used for the treatment or management of type 2 DM owing to their organosulfur compounds. These molecules could either behave as an insulin secretagogue or insulin-sensitizer to perform the antidiabetic activity [29, 30]. Schmidt et al. evaluated the *a*-glucosidase inhibitory activity of extracts of 35 different *Allium* species, and the activity was observed in all species and *A. nutans, A. pskemense, A. ramosum, A. schoenoprasum, A. cepa* were the most potent [31]. Also, extracts of six *Allium* species were investigated in terms of *a*-amylase inhibitor activity, and *A. akaka, A. sativum, A. porrum, A. cepa* showed significant inhibitory activity [32]. In addition, Rocchetti et al. studied both *a*-amylase and *a*-glucosidase inhibitory activities of aerial and bulb parts of nine *Allium* species. Remarkable inhibitory activities against both enzymes of all samples of *A. scabriflorum, A. atroviolaceum, A. hirtovaginatum, A. cappadocicum, A. trachycoleum, A. vineale and A. paniculatum* were reported [33].

3. CONCLUSION

In the present study, the essential oil obtained from flower parts of *A. Proponticum* subsp. *Proponticum* was investigated 577ülfür577 first time in terms of chemical profiling and determination of inhibitory activities against cholinesterases, tyrosinase, *a*-amylase and *a*-glucosidase. The GC-MS analysis of the essential oil suggested that the 577ülfür-containing compounds were dominant molecules. Also, the sample showed a significant inhibitory activity against enzymes, except cholinesterases. In this regard, this research revealed that this essential oil has the potential to be a therapeutic natural product due to related enzyme inhibition. The structure-activity relationship between sulfur compounds and tyrosinase, *a*-amylase, and *a*-glucosidase, needs to be further explored in detail.

4. MATERIALS AND METHODS

4.1. Plant material

A. proponticum subsp. *Proponticum* was collected from Spil mountain (Manisa/Turkey) in July 2018. The plant was identified by Dr. Hasan Yıldırım. Voucher specimen (No: 1623) was deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

4.2. Chemicals and reagents

The reference standards used in liquid chromatography–tandem mass spectroscopy (LC–MS/MS) analysis, acetylcholinesterase (AchE, from Electrophorus electricus), butyrylcholinesterase (BuChE, from equine serum), acetylthiocholine/butyrylthiocholine iodide, 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent), galanthamine, tyrosinase (from mushroom), L-Dopa, kojic acid, *a*-glucosidase (Type I, E.C.3.2.1.20), *p*-nitrophenyl glucopyranoside, *a*-amylase (Type VI-B, E.C.3.2.1.1) used for enzyme inhibitory activity, were purchased from Sigma Aldrich.

4.3. Isolation of the essential oil

The fresh flowers of the plant (300 g) were subjected to hydrodistillation for 4 h, in a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous sodium sulfate and essential oils were kept in airtight containers in a refrigerator at 4 °C for subsequent experiments.

4.4. GC-MS analysis

The qualitative analysis of essential oils was carried out according to Liu et al. [13] with modifications, using GC-MS (Agilent 5977A Series) equipped with a HP-5MS Ultra Inert (Agilent) capillary column (60 m x 0.250 m x 0.25 µm). The oven temperature was accomplished using a temperature-programmed route: accordingly, 40°C (hold for 2 min) was set as the initial temperature, then raised to 50 °C and increased to 260 °C (hold for 14 min) at the rate of 5 °C/min. Helium as a carrier gas at the flow rate of 1 mL/min. The split ratio was 1:10; ionization energy was 70 eV; scan time was 1 s; acquisition mass range was m/z 50–500. The compounds were identified by comparing the mass spectral fragmentation with standard reference spectra from the NIST 07 database (NIST Mass Spectral Database, PC-Version 7.0).

4.5. Cholinesterase inhibitory activity

The modified Ellman's method was applied for AchE and BuChE inhibitory activities of samples using a microplate reader. Galanthamine was used as the positive standard. The experiment was applied according

http://dx.doi.org/10.29228/jrp.155
J Res Pharm 2022; 26(3): 574-580

to Ellman et al. [34] with modifications. The steps of the method were explained in detail in our previous study [21].

4.6. Tyrosinase inhibitory activity

Antityrosinase activity of samples was carried out according to the modified dopachrome method [35]. Samples with seven different concentrations (1000, 750, 500, 250, 100, 10, and 1 µg/mL), and mushroom tyrosinase in 1/15 M potassium phosphate buffer (pH 6.8) (46 U/mL) were mixed in 96-well microplates. After incubating at 25 °C for 10 min, 2.5 mM L-Dopa solution was added to the mixture. Then the incubation was applied for 25 min and absorbance was measured at 475 nm. Kojic acid was a positive control.

4.7. *a*-Glucosidase inhibitory activity

a-Glucosidase inhibitory activity of samples was performed according to Feng et al. with slight modifications [36]. 0.1 U/mL enzyme, samples, which were prepared in different concentrations with 0.01 M PBS, and acarbose were added to 96-well microplates. The mixture was incubated at 37°C for 15 min, then *p*-nitrophenyl-*β*-D-glucopyranoside as substrate was put in. After incubation at 37 °C for 15 min, 0.2 M sodium carbonate was added as a reaction terminator. The absorbance of the yellow *p*-nitrophenol formed was measured in a microplate reader at 405 nm.

4.8. *a*-Amylase inhibitory activity

The assay was applied according to Nampoothiri et al. with slight modifications [37]. DNS reagent was prepared by dissolving 1 g of 3,5-dinitrosalicylic acid in 50 mL of distilled water, then adding 20 mL of 2 M NaOH and 28.2 g of sodium potassium tartrate tetrahydrate, also known as Rochelle's salt, and making it up to 100 mL with distilled water. According to the protocol created, 100 µL of starch solution and 100 µL of the sample were incubated for 10 minutes at room temperature. 100 µL of amylase enzyme solution was added and incubated at the same time and temperature. 200 µL of DNS reagent was added and the enzyme was allowed to break down the starch for 5 minutes in a shaker (100 °C). Subsequently, It was cooled at room temperature for a while. 50 µl of this mixture was taken and diluted with 200 µl distilled water, and measurements were taken at 540 nm.

Enzyme inhibitory potentials of sample were realized in a 96-well microplate using an ELISA microplate reader (Varioskan Flash Multimode Reader, Thermo Scientific, USA). The IC_{50} values of samples were calculated by the software GraphPad Prism V5.0 (GraphPad Software, San Diego, CA, USA).

Acknowledgements: Present study was financially supported by Ege University Scientific Research Projects Coordination Unit. Project Number: 20257. Special thanks are extended to Dr. Zinar Pınar Gümüş for performing the GC/MS analysis and Dr. Hasan Yıldırım for plant identification.

Author contributions: Concept – C.E., A.E.; Design – C.E., A.E.; Supervision – A.E.; Resources – A.E., C.E.; Materials – A.E., C.E.; Data Collection and/or Processing – A.E, C.E.; Analysis and/or Interpretation – A.E., C.E.; Literature Search – A.E., C.E.,; Writing – A.E.; Critical Reviews – A.E, C.E.

Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

REFERENCES

[1] Liu P, Xie Y, Meng X, Kang J. History and progress of hypotheses and clinical trials for Alzheimer's disease. Signal Transduct Target Ther. 2019; 4(29): 1-22. [CrossRef]

[2] Van Kempen LC, Redpath M, Robert C, Spatz A. Molecular pathology of cutaneous melanoma. Melanoma Management. 2014; 1(2): 151-164. [CrossRef]

[3] Martinez MV, Whitaker JR. The biochemistry and control of enzymatic browning. Trends Food Sci Technol. 1995; 6: 195-200. [CrossRef]

[4] Gray DM. Carbohydrate digestion and absorption-role of small intestine. N Engl J Med. 1995; 29: 1225-1230. [CrossRef]

[5] Dong H, Li M, Zhu F, Liu F, Huang J. Inhibitory potential of trilobatin from Lithocarpus polystachyus Rehd against *a*-glucosidase and *a*-amylase linked to type 2 diabetes. Food Chem. 2012; 130: 261-266. [CrossRef]

[6] Duman H, Ekşi G, Özbek F. Two new species of *Allium* L. sect. *Allium* (*Amaryllidaceae*) from Turkey. Plant Syst Evol. 2017; 303: 1271–1291. [CrossRef]

[7] Putnik P, Gabrića D, Roohinejad S, Barba FJ, Granatoe D, Mallikarjunan K, José M, Lorenzof, JM, Kovačević DB. An overview of organosulfur compounds from *Allium* spp.: From processing and preservation to evaluation of their bioavailability, antimicrobial, and anti-inflammatory properties. Food Chem. 2019; 276: 680-691. [CrossRef]

http://dx.doi.org/10.29228/jrp.155
J Res Pharm 2022; 26(3): 574-580

[8] Lanzotti V, Bonanomi G, Scala F. Compounds from Allium species with cytotoxic and antimicrobial activity. Phytochem Rev. 2014; 13: 769-791. [CrossRef]

[9] Block E. Biological activity of *Allium* compounds: Recent Results. Acta Hort. 2005; 688: 41-57. [CrossRef]

[10] Mill RR, Tan K. Liliaceae. In: Davis PH (Ed). Flora of Turkey and the East Aegean Islands, Volume 8, Edinburgh University Press, 1984, pp. 67–358.

[11] Karakaya S, Eksi G, Koca M, Demirci B, Kaymak HC, Kaplan ME, Aksakal O. Chemical and morphological characterization of *Allium tuncelianum* (Amaryllidaceae) and its antioxidant and anticholinesterase potentials. Anales. 2019; 76(2): e085. [CrossRef]

[12] Baser KHC, Kosar M, Koyuncu M. Composition of the essential oil of *Allium macrochaetum* Boiss. et Hausskn. from Turkey. J Essent Oil Res. 1997; 9(1): 125-126. [CrossRef]

[13] Liu XC, Qiyong Liu Q, Zhou L, Liu ZL. Evaluation of larvicidal activity of the essential oil of *Allium macrostemon* Bunge and its selected major constituent compounds against *Aedes albopictus* (Diptera: Culicidae). Parasites & Vectors. 2014; 7(184): 1-5. [CrossRef]

[14] Dehpour AA, Babakhani B, Khazaei S, Asadi M. Chemical composition of essential oil and antibacterial activity of extracts from flower of *Allium atroviolaceum*. Res J Med Plant. 2011; 5(16): 3667-3672. [CrossRef]

[15] Lazarević JS, Ethordević AS, Zlatković BK, Radulović NS, Palić RM. Chemical composition and antioxidant and antimicrobial activities of essential oil of *Allium sphaerocephalon* L. subsp. *sphaerocephalon* (Liliaceae) inflorescences. 2011; 91(2): 322-329. [CrossRef]

[16] Najjaa H, Neffati M, Zouari S, Ammar E. Essential oil composition and antibacterial activity of different extracts of *Allium roseum* L., a North African endemic species. CR Chimie 2007; 10: 820-826. [CrossRef]

[17] Pyun MS, Shin S. Antifungal effects of the volatile oils from *Allium* plants against *Trichophyton* species and synergism of the oils with ketoconazole. Phytomedicine. 2006; 13: 394-400. [CrossRef]

[18] Radulović NK, Miltojević AB, Stojković MB, Blagojević PD. New volatile sulfur-containing compounds from wild garlic (*Allium ursinum* L., Liliaceae). Int Food Res J. 2015; 78: 1-10. [CrossRef]

[19] Rose P, Moore PK, Whiteman M, Zhu Y. An Appraisal of developments in *Allium* sulfur chemistry: Expanding the pharmacopeia of garlic. Molecules. 2019; 24(4006): 1.17. [CrossRef]

[20] Emir C, Emir A. A comparative study of phenolic profiles and biological activities of *Allium sphaerocephalon* L. subsp. *sphaerocephalon* L. and *Allium sphaerocephalon* L. subsp. *trachypus* (Boiss. Et Spruner) K. Richter. J Res Pharm. 2020; 24(6): 893-900. [CrossRef]

[21] Emir C, Emir A. Phytochemical analyses with LC-MS/MS and in vitro enzyme inhibitör activities of an endemic species "*Allium stylosum* O. Schwarz" (Amaryllidaceae). S Afr J Bot. 2021; 136: 70-75. [CrossRef]

[22] Paul BD, Sbodio JI, Snyder SH. Cysteine metabolism in neuronal redox homeostasis. Trends Pharmacol Sci. 2018; 39: 513-524. [CrossRef]

[23] Butterfield DA, Halliwell B. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. Nat Rev Neurosci. 2019; 20: 148-160. [CrossRef]

[24] Griffin B, Selassie M, Gwebu ET. Effect of aged garlic extract on the cytotoxicity of Alzheimer β-amyloid peptide in neuronal PC12 cells. Nutritional Neurosci. 2000; 3: 139-142. [CrossRef]

[25] Gupta VB, Rao KSJ. Anti-amyloidogenic activity of S-allyl-L-cysteine and its activity to destabilize Alzheimer's beta-amyloid fibrils in vitro. Neurosci Lett. 2007; 429: 75-80. [CrossRef]

[26] Cai P, Xiong Y, Yao Y, Chen W, Dong X. Synthesis, screening and biological activity of potent thiosemicarbazone compounds as a tyrosinase inhibitor. New J Chem. 2019; 43: 14102-14111. [CrossRef]

[27] Mann T, Scherner C, Röhm KH, Kolbe L. Structure-activity relationships of thiazolyl resorcinols, potent and selective ınhibitors of human tyrosinase. Int J Mol Sci. 2018; 19: 690. [CrossRef]

[28] Venditti A, Mandrone M, Serrilli AM, Bianco A, Iannello C, Poli F, Antognoni F. Dihydroasparagusic acid: Antioxidant and tyrosinase inhibitory activities and improved synthesis. J Agric Food Chem. 2013; 61: 6848-6855. [CrossRef]

[29] Trio PZ, You S, He X, He J, Sakaoc K, Hou DX. Chemopreventive functions and molecular mechanisms of garlic organosulfur compounds. Food Funct. 2014; 5: 833-844. [CrossRef]

[30] Liu CT, Wong PL, Lii CK, Hse H, Sheen LY. Antidiabetic effect of garlic oil but not diallyl disulfide in rats with streptozotocin-induced diabetes. Food Chem Toxicol. 2006; 44: 1377-1384. [CrossRef]

http://dx.doi.org/10.29228/jrp.155
J Res Pharm 2022; 26(3): 574-580

[31] Schmidt JS, Nyberg NT, Staerk D. Assessment of constituents in *Allium* by multivariate data analysis, high-resolution *a*-glucosidase inhibition assay and HPLC-SPE-NMR. Food Chem. 2014; 161: 192-198. [CrossRef]

[32] Nickavar B, Yousefian N. Inhibitory effects of six *Allium* Species on *a*-amylase enzyme activity. IJPR. 2009; 8(1): 53-57. [CrossRef]

[33] Rocchetti G, Zhang L, Bocchi S, Giuberti G, Ak G, Elbasan F, Yıldıztugay E, Ceylan R, Picot-Allain MCN, Mahomoodally MF, Lucini L, Zengin G. The functional potential of nine *Allium* species related to their untargeted phytochemical characterization, antioxidant capacity and enzyme inhibitory ability. Food Chem. 2022; 368: 130782. [CrossRef]

[34] Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7: 88-95. [CrossRef]

[35] 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. [CrossRef]

[36] Feng J, Yang XW, Wang RF. Bioassay guided isolation and identification alpha-glucosidase inhibitors from the leaves of *Aquilaria sinensis*. Phytochemistry 2011; 72: 242-247. [CrossRef]

[37] Nampoothiri SV, Prathapan A, Cherian OL, Raghu KG, Venugopalan VV, Sundaresan A. *In vitro* antioxidant and inhibitory potential of *Terminalia bellerica* and *Emblica officinalis* fruits against LDL oxidation and key enzymes linked to type 2 diabetes. Food Chem Toxicol. 2011; 49: 125-31. [CrossRef]

http://dx.doi.org/10.29228/jrp.155
J Res Pharm 2022; 26(3): 574-580