The Pharmaceutical and Chemical Journal, 2018, 5(3):72-79

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Essential Oil Composition and Polen Morphology of Local Endemic *Allium tuncelianum* from Munzur Valley in Turkey

Ebru Yuce Babacan[1], Gülden Dogan[2], Azize Demirpolat[3], Eyüp Bagci[2]

[1]Munzur University, Pertek Sakine Genç Vocation School, Food Processing Department, Tunceli, Turkey
[2]Firat University, Science Faculty, Biology Department, Elazig, Turkey
[3]Bingöl University, Department of Animal and Vegetable Production, Bingöl, Turkey

Abstract Tunceli is a province that has different kind plants and large biodiversity and also there are more endemic and local endemic species. It has also consist of more medicinal and aromatic plants naturally grown. The essential oil from plants has more widely used for different purposes. The essential ois of wild-growing *Allium tuncelianum* from Munzur Valley in Turkey was obtained by hydrodistillation and analyzed by GC and GC-MS. In the oil of the *Allium tuncelianum* were characterized 1-propene 3,3- thiobis (21.2%), disulfide di-2-propenyl (12.9%), trisulfide di-2-propenyl (21.2%), diallyl tetrasulphide (17.35%) and linoleic acid ethyl ester (6.2%) as the main constituents. The light and scanning electron microscope results showed that the pollen grain *Allium tuncelianum* is monads, oblate (P/E= 1.39), monosulcate. The exine sculpturing is rugulate-perforate. The sulcus extends to the proximal side of pollen.

Keywords *Allium tuncelianum*, GC-MS, Munzur Valley, Essential oil, pollen morphlogy

Introduction

Turkey is very rich in plant species due to it location on Euro- Siberian, Mediterranean and Iran-Turan phytogeographic regions. It has 12054 plant species, out of which about 32.56% species are endemic to Turkey. The plants belonging to *Allium* species have special place among these. The genus *Allium* comprises around 750 species according to Stearn [1]. This is in fair agreement with the current online version of the World Checklist of Selected Plant Families maintained by Royal Botanic Gardens, KEW, which recognizes 860 species. The latest intrageneric classification divides the genus *Allium* into 15 subgenera and 72 sections [2]. The subgenus *Allium* is the largest, comprising around 280 species [3], 114 of which compose its largest section, *Allium* [4]. This section includes economically important species, such as garlic (*A. sativum* L.) and leek (*A. ampeloprasum* L.), as well as other minor crops of local importance, such as great headed garlic (GHG), and kurrat [5].

The genus *Allium* comprises of 700 species of bulbous perennials and biennials that occur in temperate regions of the northern hemisphere [6] and 164 of which are available in the Turkish flora; 65 of them being endemic [7-8]. As far as being beneficial to human health, *Allium* plants are already well known. For example, garlic (*A. sativum*) is of particular interest owing to its prophylactic and therapeutic actions. Anectodal evidence supports the important roles of the members of this genus in the prevention and treatment of pathogenic infections, tumors and cardiovascular diseases. Antioxidative activity of some *Allium* species has been reported elsewhere [9-10]. This ability has mainly been attributed to a variety of sulphur-containing compounds and their precursors [11].

Garlic (*Allium sativum* L.) has been cultivated since the ancient times and its progenitor species has been suggested but not yet identified. Fritsch and Friesen [12] have suggested that if there is a wild ancestor species of garlic, it should grow in the region from Mediterranean to south Central Asia, based on their taxonomic studies. Since ancient time, many *Allium* species, such as onion, garlic, leek, and chives, have been used as foods, spices, and herbal remedies in widespread areas of the world, especially in the northern hemisphere [13]. In Iran cultivation and consumption of garlic has a long history and areas under its cultivation is estimated about 10.000 ha. At present six pharmaceutical garlic products exist in the Iranian markets under license of the health ministry. The constituents of garlic are divided into two main groups: sulfur-containing compounds and non-sulfur-containing compounds. Most of the medicinal effects of garlic are referable to a sulfur compound known as allicin [14]. The intact garlic clove does not contain allicin but rather its precursor, the non-protein amino acid alliin. Alliin is converted to allicin, pyruvate and ammonia by the enzyme allinase, when the bulb is cut or crushed [15]. The amount of allicin in fresh garlic is highly variable [14]. It has been known that allicin content, which released from garlic samples from various regions, is very variable [16-17,14]. Furthermore agronomic parameters also cause variations in phytomedical levels [18]. According to British pharmacopoeia [19], the minimum allicin content to ensure pharmaceutical and economical viability of garlic powder products should be 4.5 mg/g. Hence it is important to standardize garlic, i.e. breeding a garlic clone with suitable content of alicin and agronomical traits which are needed for the large-scale culture and drug production [20].

It is known that diet rich in vegetables and fruits has the potential to lower the risk of cancer [21]. Recently, there has been a great deal of interest about the anti-mutagenic and anti-carcinogenic potential of compounds derived from plants and natural food stuff [22]. Garlic which is produced and consumed worldwide has drawn attention for its protective effects against a number of disorders 1,3. Epidemiological studies showed that garlic contains many biologically and pharmacologically active compounds. It has been used for medical purposes since the ancient times, and its use for cancer treatment dates back to 3500 years ago [23]. Furthermore, antitoxic effects of garlic are also attributed to its active components including sulphydyril groups and organosulfur compounds as diallyl sulphide, diallyl disulphide, ajoene, allixin, allyl mercaptans and allyl methylsulphides found in the plant [24].

A. tuncelianum is originally named as *A. macrochaetum* Boiss and Haussk subsp. *tuncelianum* Kollmann [25]. Although, it is native to "Tunceli" province especially at Platos of Munzur Mountains in Ovacik district of Turkey, it naturally grows in the limited region located between Sivas and Erzurum provinces. Due to its resemblance to common garlic, it is locally called as "Tunceli garlic" or "Ovacik garlic" in the region. *A. tuncelianum* usually forms single cloved white bulb, unlike garlic which has a multiple cloved bulb. The flower scape of *A. tuncelianum* coils early in its elongation, which is typical characteristic of some garlic types. While *A. tuncelianum* forms non-bulbiferous inflorescences with fertile flowers, all flowering garlic genotypes have bulbils formation in their inflorescences along with the flowers. Bulbil formation in the garlic inflorescence has been suggested as a cause of garlic sterility [26]. *Allium tuncelianum* (Kollman) Ozhatay, Matthew & Siraneci is grown in the eastern part of Turkey, and it is an endemic genus which is peculiar to this region [27]. Its plant architecture resembles garlic (*Allium sativum* L.) and it has mild garlic odor and flavor. Because of these similarities between two species, *A. tuncelianum* has been locally called "garlic" [28]. *A. tuncelianum* is placed among vulnerable plant species in the Red Book of Turkish Plants [29].

Mathew suggested that *A. tuncelianum* which is utilized as garlic in Eastern region of Turkey might be the wild ancestor of garlic [4]. The plants of three species, *A. sativum, A. longicuspis*, and *A. tuncelianum* share some common characteristics such as odor, coiling of the flower stem before anthesis, pale colored, small, glabrous, rather narrow perianth segments, and glabrous filaments with very long lateral cusps [4,25].

A. tuncelianum usually forms single cloved white bulb, unlike garlic which has a multiple cloved bulb. The flower scape of *A. tuncelianum* coils early in its elongation, which is a typical characteristic of some garlic types. While *A. tuncelianum* forms non-bulbiferous inflorescences with fertile flowers, all flowering garlic genotypes have bulbils formation in their inflorescences along with the flowers [28].

Cytological studies on *A. tuncelianum* has demonstrated that its genome is diploid with 2n =16 chromosomes, which is the same number of chromosomes with most of the widely cultivated edible *Allium* species, except with leek that

has predominantly tetraploid genome [4,30]. Ozkan and his colleagues study, ethanol extract of *A. tuncelianum* significantly reduced the chromosomal aberration rate as compared with the culture treated with MMC. The resultst could also indicate that *A. tuncelianum* could be protective against mutagenic and carcinogenic compounds when consumed through the diet [31].

According to phylogenetic analyses, *A. sativum* L., *A. porrum* L., and *A. ampeloprasum* L. that are morphologically similar species to *A. tuncelianum* have been classified under subgenus *Allium* section *Allium* [32-33].

Allium species show a large morphological diversity, and therefore many taxonomical problems remainun solved [34]. However, pollen morphology studies conducted on Allium [35-41] have been restricted to geographical areas not covering Iran and Turkey. Among the published work, the most detailed papers on the palynologyof Allium [37,42] underline the possible use of pollen morphology asanaid when answering taxonomic questions in the genus, especially for separating A. sect. Codonoprasum from sect. *Allium.*

The main aim of the present study is to perform a detailed pollen morphological survey of *Allium tuncelianum* distributed in Tunceli, in order to elucidate the usefulness of pollen characters for the systematics of the genus. Because the pollen morphology of only *A. tuncelianum* species has up to expectation been studied, the application of palynological characters in the systematics of the genus and for delimitation of its species has not been evaluated. And examine the possible use of these characters for solving certain taxonomical problems.

Material and Methods

Plant Materials: Samples of *A. tuncelianum* used in the study were collected from rural areas in Ovacik district of Tunceli province, Turkey in September (2012). The plants were dried in a room without receiving direct sun light. Voucher specimens are kept at the Firat University Herbarium (FUH).

Extraction of the essential oil: The essential oil was extracted by hydrodistillation using a modified Clevenger apparatus coupled to a 2 L round-bottom flask. A total of 100 g of fresh plant material (aerial parts) and 1 L of water were used for the extraction. The extraction was performed over 3 hour period. Subsequently, the hydrolate was collected and centrifuged at 10,000 rpm for 10 minutes. The organic phase was removed with the aid of a Pasteur pipette, and subsequently transferred to an black coloured vials, wrapped in parafilm and aluminum foil and 4^{o}C under refrigeration until analysis. The yields of oils were calculated on the basis of the dry mass.

Gas chromatography (GC) analysis: The essential oil was analysed using HP 6890 GC equipped with FID detector and HP- 5 MS (30 m x 0.25 mm *i.d.*, film tickness 0.25 µm) capillary column was used. The column and analysis conditions were the same as in GC-MS expressed as below. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors.

Gas chromatography / Mass spectrometry (GC-MS) analysis: GC-MS analyses of the oils were performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with Hewlett Packard 5973 mass spectrometer system equipped with a HP 5-MS capillary column (30 m x 0.25 mm id, film thickness 0.25 µm). The oven temperature was programmed from 70-240^{o}C at the rate of 5^{o}C/ min. The ion source was set at 240^{o}C and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 mL/min. Scanning range was 35 to 425 amu. Diluted oil in *n*-hexane (1.0 µL) was injected into the GC-MS.

The identification of constituents was performed on the basis of retention indices (RI) determined by co-injection with reference to a homologous series of *n*-alkanes, under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST 98 Libraries (on ChemStation HP) and Wiley 7th Version. The relative amounts of individual components were calculated based on the GC (HP-5MS column) peak area (FID response) without using correction factors. The identified constituents of the essential oils are listed in Table 1.

Pollen Investigations: Pollen grains of specimens for light microscope investigations were prepared according to the methods of WODEHOUSE [43]. Pollen dimensions of this species were measured in such amounts that the resulting data followed Gaussian curves. These measurements were not acetolysed pollen and on SEM micrographs (Tab. 1). Light microscope studies were made by using a "Olympus BX51" microscope and the following parameters were measured for pollen grains (Fig.1).

For SEM investigations, the pollen grains were put on the stubs, sputter-coated with gold plate, and examined under a Jeol JSM – 840A scanning electron microscope. Ulthrathin sections of the pollen grains were obtained with a glass knife in a microtome. Post-staining was done with lead citrate for 5 minutes [44], and the sections were examined under a Zeiss EM9. The clearest lighy microscope and SEM and photographs representing each pollen type and the main pollen features were selected for this paper (Fig.2). The terminologies for pollen morphology proposed by Walker, Faegri & Iversen and Punt et al. were followed [45-48].

Table 1: Characteristic features of the investigated pollen in species of *Allium tuncelianum*

Species	P	E	P/E	Pollen shape	Clg	Clt	Exine Thickness
Allium tuncelianum	23±1.6	16.5±1.6	1.39	Prolate	14.5±0.7	3.2±04	1.6±0.34

P=Polar axis, E= equatoral axis, Clg= Colpus lenght, Clt= Colpus width.

Table 2: Constituents of the essential oil from *Allium tuncelianum*

No	Compounds	RI	%
1	1-Propene-3,3-thiobis (Allyl sulfide)	967	**21.2**
2	n- Propyl trans 1- propenyl sulfide	976	2.4
3	Disulfide di-2-propenyl	1131	**12.9**
4	Benzene-1-methyl-4	1141	2.6
5	Isopinocarveol	1174	1.3
6	Trisulfide di-2-propenyl (Perilla alchol)	1296	**21.2**
7	3H-1,2,4-triazole-3-thione,2,4,-dihydro-4-methyl	1307	4.1
8	5-Methyl –tetrathia-cyclohexane	1354	2
9	Diallyl tetrasulphide	1472	**17.35**
10	Hexadeconoic asit ethyl ester	1710	3.1
11	Cyclooctasulfur	1749	5.3
12	Linoleic acid ethyl ester	1823	**6.2**
Total			**99.65**

RI: Retention Indices

Figure1: Light microscope micrographs of pollen grains in Allium tuncelianum

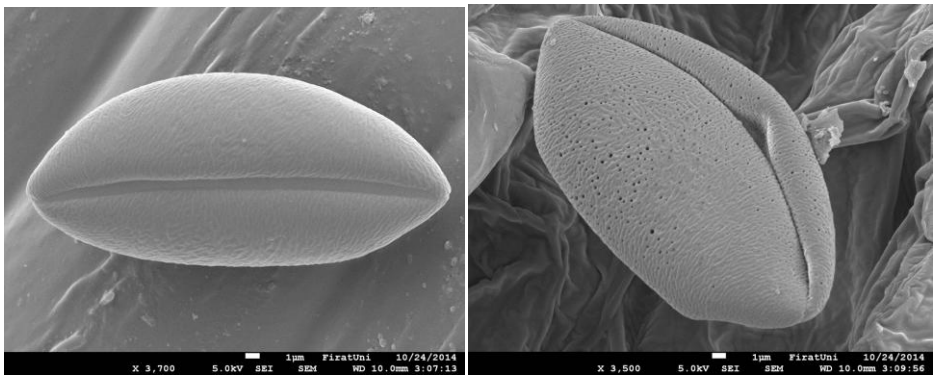

Figure 2: SEM micrographs of pollen grains in Allium tuncelianum

Results and Discussion

The chemical composition of essential oils obtained by hydrodistillation of *Allium tuncelianum* was investigated by GC and GC-MS. The composition of the oil of *A. tuncelianum* is listed in Table I, in which the percantage and retention indice of components are given. The essential oils yield is 0.3 (v/w) of *A. tuncelianum*. Twelve constituents were comprised the 99.65 % of the total essential oil extracted from the *A. tuncelianum*. The predominant compounds of *A. tuncelianum* were determined as 1-propene 3,3- thiobis (21.2%), disulfide di-2-propenyl (12.9%), trisulfide di-2-propenyl (21.2%), diallyl tetrasulphide (17.35%) and linoleic acid ethyl ester (6.2%) as the main constituents.

The main palynogical features of Turkish *A. tuncelianum* examined are summarized in Table 1. A general description can be given as follows; the light and scanning electron microscope results showed that the pollen grain *A. tuncelianum* is monads, oblate (P/E= 1.39), monosulcate. The exine sculpturing is rugulate- perforate. The sulcus extends to the proximal side of pollen.

In the study of Yumrutas et al. showed that, was designated to evaluate in vitro antioxidant activity of MeOH extracts of *A. tuncelianum* and to determined total phenolic content (TPC) of MeOH extract of this plant. The sample was subjected to screening for their possible antioxidant activity by using 2,2-diphenyl-1-picriylhydrazyl (DPPH) and β-carotene/linoleic acid assays. The MeOH extract was found to posses weak antioxidant activity. In the case of linoleic acid system, oxidation of the linoleic acid was moderately inhibited by the methanol extract (51.1 ± 5.5 %). Also, in the MeOH extract of *A. tuncelianum*, we have determined TPC in value of 4.8 ± 1.30 mg/g. Their results showed that MeOH extract of *A. tuncelianum* was exhibited more weak antioxidant activity than the synthetic antioxidant butylated hydroxytoluene (BHT), curcumin, and ascorbic acid [49]. Our analysis results study were showed similarity with the Yumrutas's findings, because the linoleic acid ethyl ester (6.2%) was important component in our sample.

Ozkan et al. reported that, the effect of ethanol extract of *A. tuncelianum* at concentrations of 0.10, 0.15, 0.20, 0.25 µl/mL were screened for chromatid and chromosome breaks, chromosome exchange as well as chromatid union and polyploid cells against negative acetone and positive control Mitomycine C.A significant decrease in the frequency of chromosomal aberration was observed for all treatments with *A. tuncelianum* ethanol extract at 24 h. In conclusion, ethanol extract of *A. tuncelianum* significantly reduced the chromosomal aberration rate as compared with the culture treated with MMC. The results could also indicate that *A. tuncelianum* could be protective against mutagenic and carcinogenic compounds when consumed through the diet [50].

In the study of Ipek et al. [28], amplified fragment length polymorphisms (AFLP) markers and nucleotide sequence analysis of the internal transcribed spacer region (ITS) were used to assess genetic and phylogenetic relationships among *A. tuncelianum*, garlic and some other *Allium* species. AFLP analysis demonstrated that *A. tuncelianum* and garlic are genetically distinct and they are likely different species. Phylogenetic analyses based on the nucleotide sequence of ITS suggested that *A. tuncelianum* and garlic are distinct species and placed *A. tuncelianum*, garlic, *Allium ampeloprasum* and *Allium scorodoprasum* into the same clade in the neighbor joining dendrogram and in the consensus tree of parsimony analysis. However, *A. tuncelianum* was phylogenetically less related to garlic than either *A. ampeloprasum* or *A. scorodoprasum*, suggesting that *A. tuncelianum* may not be the immediate wild ancestor species of garlic. Further studies to generate hybrid progeny between *A. tuncelianum* and garlic (if possible) could provide more information on the homology between the chromosomes of *A. tuncelianum* and garlic and genetic relationships between these two species.

The yield and the composition of the essential oils from garlic (*A. sativum*) obtained by SE (solvent extraction) were determined, and compared with those obtained by the supercritical fluid extraction (SFE). The essential oils were analyzed by gas chromatography–mass spectrometry (GC–MS). Major essential oil components were 3-vinyl-4H-1,2-dithiin (31.89%), diallyl trisulfide (13.31%), diallyl sulfide (2.22%), dially disulfide (6.87%), propyl allyl disulfide (13.89%), and dimethyl disulfide (7.05%). The compositions of garlic essential oil obtained by SE and SFE methods were compared. Although main compositions of the essential oils obtained by SE and SFE are basically similar, their minor compositions do differ quantitatively. In addition, extraction yield of SE was slightly higher than that obtained by SFE. However, comprehensively considering various factors, it can be concluded that the SE

method offers obvious advantages over SFE. Therefore, SE is considered as the optimum process for btaining garlic essential oil with high quality [51]. Our results were showed partially similarity with Li's findings, because they found diallyl trisulfide (13.31%), diallyl sulfide (2.22%), dially disulfide (6.87%) as major components while our results showed diallyl tetrasulfide (17.35%) as major components.

The main palynogical features of Turkish *A. tuncelianum* examined are summarized in Table 1. A general description can be given as follows; the light and scanning electron microscope results showed that the pollen grain *A. tuncelianum* is monads, oblate (P/E= 1.39), monosulcate. The exine sculpturing is rugulate- perforate. The sulcus extends to the proximal side of pollen.

Güler and Pehlivan were investigated under light microscopy and by scanning electron microscopy of pollen morphology of 14 *Allium* L. species grown in Turkey. The genera Allium homogeneous in both aperture type and exine ornamentation. It is suggested that some palynological characters, such as aperture type and the presence of an operculum, could be of taxonomic value at the section level. Species show that their pollen apertures are monosulcate and monosulcate-operculate [42]. These results show that there are several pollen characters of taxonomic significance in *Allium*.

The common characteristics of the pollen grains of *Allium* have been investigated. We have found that the *Allium* pollen is monosulcate and exine sculpturing is rugulate- perforate. These results are similar to former studies [38, 40-42].

Conclusion

Consequently, the major compounds identified in *A. tuncelianum* in our study support the presence of high levels of sulphydyril groups and organosulfur compounds in this genus. and that founded the *Allium* pollen is monosulcate and exine sculpturing is rugulate- perforate.

References

1. Stearn, W.T. (1992). How many species of *Allium* are known Kew Magazine 9, Swofford, 180–182.
2. Friesen, N., Fritsch, R.M., Blattner, F.R. (2005). Phylogeny and new intrageneric classification of Allium L. (Alliaceae) based on nuclear rDNA ITS sequences. *Aliso*, 22: 372-395.
3. Hanelt, P., Schultze-Motel, J., Fritsch, R., Kruse, J., Maass, H.I., Ohle, H., Pistrick, K. (1992). Infrageneric Grouping of Allium. the Gatersleben Approach. In: Hanelt P, Hammer K, Knupffer (Eds) the genus Allium. Taxonomic Problems and Genetic Resources, 107-123.
4. Mathew, B. (1996). A Review of Allium Section Allium. Royal Botanic Gardens, Kew Publishing, Richmond.
5. Hirschegger, P., Jakše, J., Trontelj, P., Bohanec, B. (2010). Origins of Allium ampeloprasum horticultural groups and a molecular phylogeny of the section Allium (Allium: Alliaceae) *Molecular Phylogenetics and Evolution*, 54: 488–497.
6. Konemann, K. Botanica. Hong Kong: Gordon Cheers Publication, 1020p.
7. Davis, P. (1984). Flora of Turkey and the East Aegean Islands Edinburgh: Edinburgh University Press. (Vol. 8).
8. Guner, A., Ozhatay, N., Ekim, T., Baseri, KHC. (2010). Flora of Turkey and the East Aegean islands (Vol. 11) (supplement-II). Edinburgh: Edinburgh University Press.
9. Gabriella, G., Papetti, A., Daglia, M., Bertè, F., Gregotti, C. (1998). Protective activity of water soluble components of some common diet vegetables on rat liver microsome and the effect of thermal treatment. *Journal of Agricultural and Food Chemistry*, 46: 4123- 4127.
10. Yin, M.C., Cheng, W.S. (1998). Antioxidant activity of several Allium members. *Journal of Agricultural and Food Chemistry*, 46, 4097-4101.
11. Kim, S.M., Kubota, K., Kobayashı, A. (1997). Antioxidative activity of sulfur-containing flavor compounds in garlic. *Bioscience, Biotechnology and Biochemistry*, 61: 1482-1485.

12. Fritsch, R.M., Friesen, N. (2002). Evolution, domestication, and taxonomy. In: Rabinowitch, H.D., Currah, L. (Eds.), Allium Crop Science: Recent Advances. CABI Publishing, New York, pp. 5–30.
13. Guohua, H., Yanhua, L., Rengang, M., Dongzhi, W., Zhengzhi, M. (2009). Aphrodisiac properties of Allium tuberosum seeds extract. *Journal of Ethnopharmacology*, 122 579–582
14. Schulz, V. Garlic. In: Hansel, R., Tayler, V.E. (1998). (Eds.), Rational Phytotherapy. A Physicians' Guide to Herbal Medicine. 3rd ed. Springer, Berlin, pp. 107–125.
15. Rabinkov, A., Zhu, X.Z., Grafi, G., Galili, G., Mirelman, D. (1994). Alliin lyase (Allinase) from garlic (Allium sativum). *Appl. Biochem. Biotechnol.* 48, 149–171.
16. Iberl, B., Winkler, G., Muller, B., Knobloch, K. (1990). Quantitative determination of allicin and alliin from garlic by HPLC. *Planta Med*. 56, 320–326.
17. Ueda, Y., Kawajiri, H., Miyamura, N., Miyajima, R. (1991). Content of some sulfur containing components and free amino acids in various strains of garlic. *Nippon Shokukin Kogyo Gokashi*, , 38, 429–434.
18. Mayeux, P.R., Agrawal, K.C., Tou, J.S.H., King, B.T., Lippton, H.L., Hyman, A.L., Kadowiz, P.J., McNamara, D.B. (1998). The pharmacological effects of allicin, a constituent of garlic oil. *Agents Actions*, 25, 182–190.
19. British pharmacopoeia. (1995). Vol 1 (International edition and addendum). London, Her Majesty's Stationery Office,
20. Baghalian, K., Seyed A. Z., Naghavi, M.R., Badi, H.N, Khalighi, A. (2005). Evaluation of allicin content and botanical traits in Iranian garlic (*Allium sativum* L.) ecotypes. *Scientia Horticulturae*, 103: 155–166.
21. Belloir C., Singh, V., Daurat, C., Siess, M.H. (2006). Protective effects of garlic sulfur compounds against DNA damage induced by direct- and indirect-acting genotoxic agents in HepG2 cells. *Food Chem Toxicol*, 44: 827-834.
22. Bhuvaneswari, V., Suresh K. A., Siddavaram N. (2005). Combinatorial antigenotoxic and anticarcinogenic effects of tomato and garlic through modulation of xenobiotic-metabolizing enzymes during hamster buccal pouch carcinogenesis. *Nutrition*, 21: 726-731.
23. Shukla, Y., Kalra, N. (2007). Cancer chemoprevention with garlic and its constituents. *Cancer Lett*, 247: 167-181.
24. Assayed, M.E., Khalaf, A.A., Salem, H.A. (2010). Protective effects of garlic extract and vitamin C against in vivo cypermethrin-induced teratogenic effects in rat offspring. *Food Chem Toxicol*, 48: 3153-3158.
25. Etoh, T., Simon, P.W. (2002). Diversity, fertility and seed production of garlic. In: Rabinowitch, H.D., Currah, L. (Eds.), *Allium* Crop Science: Recent Advances. CABI Publishing, New York, pp. 101–117.
26. Koul, A.K., Gohil, R.N. (1970). Causes averting sexual reproduction in *Allium sativum* Linn. *Cytologia*, 35: 197-202.
27. Ipek, M., Ipek, A., Simon, P.W. (2003). Comparison of AFLPs, RAPD markers, and isozymes for diversity assessment of garlic and detection of putative duplicates in germplasm collections. *J. Am. Soc. Hort. Sci.*, 128: 246–252.
28. Ipek, M., Ipek, A., Simon, P. (2008). Genetic characterization of *Allium tuncelianum*: An endemic edible Allium species with garlic odor. *Sci Hortic-Amsterdam*, 115 (4): 409-415.
29. Içgil, Y.D. (2012). Tunceli sarımsağının (Allium tuncelianum (Kollman), N. Özhatay, D. Matthew, Ş. Şiraneci) in vitro mikroçoğaltımı. Dicle Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek lisans tezi.
30. Ozhatay, N. (2002). Diversity of bulbous monocots in Turkey with special reference. Chromosome numbers. *Pure Appl. Chem*. 74: 547–555.
31. Ozkan, O., Aydin, H., Bagcigil, F. (2009), In vitro evaluation of antimicrobial activities of Salvia verticillata and Phlomis pungens. *Kafkas Univ Vet Fak Derg*, 15 (4): 587-590.
32. Mes, T.H.M., Fritsch, R.M., Pollner, S., Bachmann, K. (1999). Evolution of the chloroplast genome and polymorphic ITS regions in Allium subgenus Melanocrommyum. *Genome*, 42: 237–247.
33. Ricroch, A., Yockteng, R., Brown, S.C., Nadot, S. (2005). Evolution of genome size across some cultivated Allium species. *Genome*, 48: 511–520.

34. Gurushidze, M. et al.. (2007). Phylogeny of *Allium* subgenusMelanocrommyum evidence from molecular data. In Symp. 7th plant life of south west Asia, Turkey, 1-20.
35. Nair, P.K.K., (1965). Sharma, M. Pollen morphology of Liliaceae. *J. Palynol*. 1: 38-61.
36. Thunert, K. (1967). Beitrge zur Pollenmorphologie und Taxonomie der Gattung *Allium.* Staats examens arbeit.Univ. of Jena, Germany,
37. Kuprianova, L. A. and Aliev, T. A. (1979). Palynological data on thetaxonomy of the genus Allium *L. Bot. Zh*., 64: 12731284.
38. Schulze, W. (1980). Beitrage zur Taxonomie der Liliiflorae, V.Alliaceae. Wiss. *Z. Univ. Jena,* 29: 595-606.
39. Baktir, I. (2005). Tunceli Sarımsağı'nın (*Allium tuncelianum)* in vitro koşullarında coğaltılması. In: Proceeding of GAP IV. Tarim Kongresi, Turkey.
40. El-Sadek, L. et al.. (1994). Cytology and palynology of commonmonocots in Mariut Egypt. I. Common species of the familiesAlliaceae and Liliaceae. Quatar Univ. *Si. J.*, 14: 270-280.
41. Tolgor, Z.Y. (1995). Pollen morphology of Allium and itstaxonomic significance. *J. Jilin Agr. Univ*., 17: 36-40.
42. Guler, U., Pehlivan, S. (2006). Pollen morphology of somespecies belonging to Codonoprasum and Allium sections ofAllium (Liliaceae, Alliaceae). *Biologia*, 61: 449-455.
43. Wodehouse, R.P. (1935). Pollen grains, McGraw-Hill, New York.
44. Reynold, E.S. (1963). The use of lead citrate at high ph as on electron opaque stain in electron microscopy. *Stain Technol*. 43: 139–144.
45. Walker, J.W. (1974a). Evolution of exine structure in the pollen of primitive angiosperms. *Amer. J. Bot*., 61: 891–902.
46. Walker, J.W. (1974b). Aperture evolution in the pollen of primitive angiosperms. *Amer. J. Bot*., 61: 1112–1136.
47. Faegri, K., Iversen, J. (1989). Textbook Of Pollen Analysis, 4th Ed. In: Faegrı, K.,Kalland, P.E.&Krzywınskı, K. (Eds), J.Wiley & Sons, Chiester, New York, Brisbane, Toronto, Singapore.
48. Punt, W., Blackmore, S., Nilsson, S., Le Thomas, A. (1994). Glossary of pollen and spore terminology. LPP Foundation, Contributions Series No 1., Utrecht..
49. Yumrutaş, Ö., Demirörs, S., Doğan, M. (2009). The in vitro antioxidant activity of Allium tuncelianum. An endemic. Journal of Applied Biological Sciences, 3(3): 61-64.
50. Özkan, O., Süleyman, G., Kart A., Çiçek, B.A., Kılıç, K. (2013). In Vitro Antimutagenicity of Allium tuncelianum Ethanol Extract Against Induction of Chromosome Aberration by Mutagenic Agent Mitomycine C. *Kafkas Univ Vet Fak Derg.,* 19 (2): 259-262.