Determination of fatty acids and elements in *Allium nemrutdaghense* Kit Tan & F. Sorger endemic plant

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Abstract. In this study, we performed first literaturally known fatty acid and trace element analyses of the plant *Allium nemrutdaghense*. The fatty acid and trace element analyses were determined by using GC and ICP-OES. Among fatty acid levels, myristic acid, palmitic acid, oleic acid, and linoleic acid levels were determined as 19.420%, 17.240%, 5.169%, and 39.397%, respectively. The plant bulb was found containing Ni, Ba, Al, Cu, Fe, Mn, Zn and Cr with respective quantities of 0.83, 10.25, 6.91, 2.23, 34.75, 16.04, 21.16, 0.45 µg/g, and K, Na at 14.70, 0.04 mg/g (dry matter) quantities, respectively. In particular, this study reports the biochemical parameters of the *Allium nemrutdaghense* endemic plant for the first time. Thus, it could be important for the future medical studies.

Key words: Allium nemrutdaghense, fatty acids, trace element, endemic plant.

Introduction

Allium nemrutdaghense is an endemic plant type and grown on the Mountain Nemrut in the city of Adiyaman, Turkey. People of the Nemrut region consume this plant as food. One of the local recipes of the region known as "Hıtap" is made by cooking in the oven the leaves and the bulb of this plant inside of a dough spread. Allium nemrutdaghense endemic plant's Liliacaeae family was defined as Allium L. genus. Plant systematics of this plant, features of which were given in the Figure 1, has been defined (Davis 1965–1988).



Figure 1. View of Allium nemrutdaghense endemic plant.

Elements are important biochemical substances for biological systems. They are needed for the development and health ofplant and animal metabolisms. Insufficient or excessive quantities of elements in a metabolism cause metabolic anomalies (Arzani et al. 2007, Nikolov et al. 2009, Koichi & Masayoshi 2003). Trace elements play important role in the formation of active chemical compounds present in the plants used for medical purposes. One of the reasons for their medical use is that they contain elements (Karak & Bhagat 2010, Kremer et al. 2012). Trace elements have been

reported as significant with their biochemical impacts on immunologic reactions, various enzymatic activities, and healing effect on skin diseases, immune system, and serious infection cases (Chaturvedi et al. 2004, Selvaraju et al. 2009, Emsley et al. 2011).

Lipids are important biochemical substances found in the plant and animal metabolism. Being as components of lipids, fatty acids are classified as saturated and unsaturated fatty acids. Among unsaturated fatty acids, monounsaturated and polyunsaturated fatty acids are very important for metabolism. Polyunsaturated fatty acid compounds contain omega-6 (n-6) and omega-3 (n-3) fatty acids and most common linoleic acid (18:2 n6) and linolenic acid (18:3 n3) synthesis are done by the plants. These types of derived fatty acids are known as essential fatty acids as they cannot be synthesized by animal metabolisms. For this reason, animals need to take essential fatty acids through their diet (Siddiqui et al. 2008) and the investigation and the analysis of the elemental, and essential fatty acid compositions of the plants are very crucial and important.

In this study, we measured biochemical parameters, fatty acid and element levels of *Allium nemrutdaghense* endemic plant for the first time, and explained their significance for the metabolism.

Materials and methods

Plant material

Allium nemrutdaghense plants were collected from Adıyaman/Nemrut mountain. The plant material was identified by Dr. Ahmet Zafer Tel and Dr. Isa Gokler (Tel et al. 2010). After the plants were picked up, they were naturally dried in a shady.

Fatty acid analysis

We weighed 0.5 g's of *Allium nemrutdaghense* plant's bulb samples, and homogenized them in a hexane/ isopropanol (3:2 v/v) mixture. We centrifuged and filtered lipid extracts for 5 minutes and at 10000 revolutions. Solvents were evaporated at 40 °C in evaporator. Lipid extract was stored at -25 °C until the analysis. We repeated this procedure 5 times for each sample. Fatty acids in the lipid extract were treated with 2% sulphuric acid (v/v) in methanol and transformed

into methyl esters. Fatty acid methyl esters were extracted with hexane. After transforming the fatty acids in the lipid extract to methyl esters (Hara & Radin 1978, Christie 1990). We analyzed them with SHIMADZU GC 2025 gas chromatography (GC). For that analysis, we used technocrome capillary TRCN 100 colons at 60 m length with 0.20 internal diameters and 25 micron film thickness. During the analysis, colon, injection and detector temperatures were kept at 80-240 °C, 240 °C and 250 °C respectively. Colon temperature program was set as 3 °C/min from 80 °C to 140 °C. We waited one min. We heated it from 140 °C to 240 °Cat 3 °C/min and ended the heating after waiting for 5 min. We established a method with a total duration of 61 min. We used helium for carrier gas. During the analysis, before analyzing the fatty acid methyl esters of the samples, we determined the retention time for each fatty acid by injecting the mixtures of standard fatty acid methyl esters. After this treatment, by making necessary programming, we analyzed the fatty acid methyl ester mixtures of samples. Fatty acid analyses of the samples were made by USKIM (University-Industry-Public Enhancement of Cooperation, Application and Research Center/Kahramanmaras-Turkey). Results were determined as a percentage quantity of each fatty acid among total fatty acids. Calculations were made by using GC Solution 2.3 program.

Determination of trace elements

The samples of bulb (1.00 g) were taken into polytetrafluorethylen bombs. 4 mL HNO3 (65%, w/w) solution was added to each sample and digested with a microwave system. Also, the blank samples were prepared according to this method. Then, sample solutions were analyzed by Inductively Coupled Plasma Atomic Emission Spectrometer (Ciftci et al. 2009).

Statistical analysis

All the measurements were repeated for three times, and results were presented as mean \pm standard error mean.

Results and Discussion

Element and fatty acid levels of Allium nemrutdaghense's bulb samples are given in Table 1 and Table 2. Considering the fatty acid compositions, the highest percentages of fatty acids were found as 19.420% myristic acid(14:0), 17.240% palmitic acid(16:0), 5.169% oleic acid(18:1n9c), and 39.397% linoleic acid (18:2n6c). Also, total saturated fatty acid (SFA) level of 42.226%, total unsaturated fatty acid (∑USFA) level of 57.731%, mono unsaturated fatty acid (SMUFA) level of 7.624% and polyunsaturated fatty acid level (SPUFA) of 50.107% was observed. To date, myricstic acid fatty acid level the Allium cepa (onion)have been presented in very low levels or have not been reported. In our study, the reason why the myricstic acid fatty acid level wasobserved as high (17.240%) could be due to differences of the environmental, climatic, and ecological conditions. On the other hand, the limited number of fatty acids in the analysis of Allium cepa onion have been reported up to know(Bello et al. 2013, Tsiaganis et al. 2006). Apart from the literature, we have observed the higher number of fatty acids. The reason behind this could be the variation in the methodological differences in the identification of fatty acids in GC during their analy-

The results of the elemental composition analysis of *Allium nemrutdaghense* bulb revealed that Ni, Ba, Al, Cu, Fe, Mn, Zn and Cr were at respective levels of 0.83, 10.25, 6.91, 2.23, 34.75, 16.04, 21.16, 0.45 μg /g whereasK, Nalevels were at 14.70 and 0.04 mg/g (dry matter), respectively. Bello et al.

Table 1. Mineral composition of *Allium nemrutdaghense* plant bulb samples.

Metals	Bulb
Ni μg / g	0.83±0.04
Baµg/g	10.25±0.71
Alμg/ g	6.91±0.42
Cuµg/g	2.23±0.18
Feµg/g	34.75±2.78
Mnµg/g	16.04±2.57
Znµg/g	21.16±1.98
Crµg/g	0.45±0.03
K mg/g	14.70±1.61
Na mg/g	0.04±0.02

Table 2. Fatty acid composition of *Allium nemrutdaghense* plant bulb samples (%).

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Fatty acids	Bulb
8:0(Caprylic Acid)	0.027±0,01
10:0(Capric Acid)	-
11:0(Undecanoic Acid)	-
12:0(Lauric Acid)	0.102±0.02
13:0(Tridecanoic Acid)	0.130±0.03
14:0(Myristic Acid)	19.420±2.21
15:0(Pentadecanoic Acid)	0.401±0.05
16:0(Palmitic Acid)	17.240±1.15
17:0(Heptadecanoic Acid)	0.489 ± 0.04
18:0(Stearic Acid)	3.225±0.32
20:0(Arachidic Acid)	0.168±0.03
21:0(Heneicosanoic Acid)	0.216±0.04
23:0(Tricosanoic acid)	0.332±0.06
24:0(Lignoceric Acid)	0.514±0.05
∑SFA	42.226±2.98
14:1n5(Myristoleic Acid)	-
15:1n5(Cis-10-Pentadecanoic Acid)	0.212±0.03
16:1n7(Palmiteloic Acid)	0.699±0.04
18:1 n9t(Elaidic acid)	0.477±0.03
18:1n9c(Oleic Acid)	5.169±1.12
20:1n9(Cis-11-Eicosenoic Acid)	0.128±0.02
22:1n9(Erucic Acid)	0.330±0.01
24:1n9(Nervonic Acid)	0.609±0.02
∑MUFA	7.624±0.95
18:2n6t(Linolelaidic acid)	0.189±0.01
18:2n6c(Linoleic Acid)	39.397±2.21
18:3n6(Gama-Linolenic Acid)	0.197±0.01
20:2n6(Cis-11,14-Eicosadienoic Acid)	0.141±0.01
20:3n6(Cis-8,11,14-Eicosatrienoic Acid)	0.677±0.02
20:4n6(Arachidonic Acid)	0.509±0.01
18:3n3(Linolenic Acid)	3.833±0.15
20:3n3(Cis-11,14,17-Eicosatrienoic Acid)	3.143±0.12
20:5n3(cis-5,8,11,14,17-Eicosapentaenoic Acid)	1.306±0.09
22:6n3(cis-4,7,10,13,16,19-Docosahexaenoic Acid)	0.715±0.02
ΣPUFA	50.107±2.25
ΣUSFA	57.731±2.78

(2013) reported K, Ca, Mn, Fe, and Cu levels in the *Allium cepa* onion bulb as 2.98, 1.22, 0.05, 0.04 and 0.13 g/100g, respectively. On the other hand, Galdonet al. (2008) reported Na, K, Fe, Cu, Zn and Mn as 162, 1136, 1.41, 0.37, 1.21, and

0.88 mg/kg, respectively. The reason for the difference of the elemental levels of *Allium nemrutdaghanse* bulb and *Allium cepa* onion could be depended on the genetics and ecological essentials.

We have found that most noteworthy elements found in the Allium nemrutdaghense bulb are Ba, Cu, Fe, Mn, Zn and K. Of these elements, especially Fe, Cu, Mn and Zn are crucial trace elements for human metabolism. Allium nemrutdaghense endemic plant's bulb are consumed as food by the local people of the Nemrut Mountain region. Fe is one of the most essential elements for the human and animal metabolism. It is especially needed in the formation of hemoglobin, immune system and energy generation (Ullah et al. 2012). Mn, Zn and Cu are found in the antioxidant enzymes which play an important role in defending against the free radicals (Ayodele & Bayero 2010, Bhowmik et al. 2010). Insufficiency of these elements causes many diseases on metabolism (Saracoglu et al. 2009, Linder & Hazegh-Azam 1996). Since these elements, as suggested by the literature, are important for the metabolism, we are of the view that Allium nemrutdaghense endemic plant is important from medicinal point of view.

Fatty acids are essential biomolecules for human metabolism. Humans get their need of these molecules from plants or animal products. Unsaturated fatty acids have important functions in organizing the immune system, cholesterol metabolism, regulation of membrane structure and brain functions. Furthermore, unsaturated fatty acids have been reported with positive effects for regulation of low-density lipoprotein, against various diseases such as skin diseases (Steffen & Wirth 2005), arthritis (Kremer et al. 1988), nephritis (Thais et al. 1987), asthma (Lands 1986), lupus erythematosus (Kelley et al. 1985), cardiovascular diseases and cancer (Li & Hu 2009), inflammatory and autoimmune disease (Calder 2006), coronary heart disease (De Lorgerilet al. 1999), hypertension (Appel et al. 1994), ulcerative colitis (Stenson et al. 1992) and multiple sclerosis (Bates et al. 1989). Since Allium nemrutdaghense endemic plant's containing unsaturated fatty acids 18:2 n6c, 18:3 n3, 20:3 n3, 18:1n9c, 16:1 n7 in its fatty acid composition, we believe that this plant is important from the health point of view. Especially in its bulb, PUFA, USFA and MUFA fatty acid percentages are high which makes it's bulb even more valuable for the health purposes.

Conclusions

As a conclusion; in this study, we presented trace elements and fatty acid levels of *Allium nemrutdaghense* endemic plant, among its biochemical parameters firstly to the literature and we think that these parameters may be important for the future medicinal studies.

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References

- Appel, L.J., Miller, E.R., Seidler, A.J., Whelton, P.K. (1993): Does supplementation of diet with "fish oil" reduce blood pressure? A metaanalysis of controlled clinical trials. Archives of Internal Medicine 153(12): 1429-1438.
- Arzani, A., Zeinali, H., Razmjo, K.(2007): Iron and magnesium concentrations of mint accessions (*Mentha* spp.). Plant Physiology and Biochemistry 45(5): 323–329
- Ayodele, J.T., Bayero, A.S. (2010): Manganese concentrations in hair and fingernail of some Kano inhabitants. Journal of Applied Sciences and Environmental Management 4(1): 17-21.
- Bates, D., Cartlidge, N., French, J.M., Jackson, M.J., Nightingale, S., Shaw, D.A., Smith, S., Woo, E., Hawkins, S.A., Millar, J.H. (1989): A double blind controlled trial of long chain n-3 polyunsaturated fatty acids in the treatment of multiple clerosis. Journal of Neurology, Neurosurgery & Psychiatry 52(1): 18-22.
- Bello, M.O., Olabanji, I.O., Abdul-Hammed, M., Okunade, T.D. (2013): Characterization of domestic onion wastes and bulb (Allium cepa L.): fatty acids and metal contents. International Food Research Journal 20(5): 2153-2158.
- Bhowmik, D., Chiranji, B.C., Kumar, K.P.S. (2010): Apotential medicinal importance of zinc in human health and chronic disease. Journal of Pharmaceutical and Biomedical Sciences 1: 5-11.
- Calder, P.C. (2006): n3 Polyunsaturated fatty acid,inflammation, and inflammatory diseases. The American Journal of Clinical Nutrition 83(6): 1505–1519.
- Chaturvedi, U.C., Shrivasta, R., Upreti, R.K. (2004): Viral infections and trace element: a complex interaction. Current Science 87(11): 1536-1554.
- Christie, W.W. (1990): Gas Chromatographyand Lipids. The Oily Press. Glasgow, UK.
- Ciftci, H., Ozkaya, A., Kariptas, E. (2009): Determination of fatty acids, vitamins and trace elements in *Pistaciaterebinthus coffee*. The Journal of Food, Agriculture and Environment 7(3-4): 72-74.
- Davis, P.H. (1965–1988): Flora of Turkey and the East Aegean Islands, 1–10University Press, Edinburgh.
- De Lorgeril, M., Salen, P., Martin, J.L., Monjaud, I., Delaye, J., Mamelle, N. (1999): Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction. Final report of the lyon diet heart study. Circulation 99(6): 779–785.
- Emsley, J. (2011): Nature's building blocks: an A-Z guide to the elements. Oxford, UK: Oxford University Press. 2011, pp.1-699.
- Galdon, B.R., Gonzalez, R.O., Rodriguez, E.R., Romero, C.D. (2008). Comparison of mineral and trace element contents in onion cultivars (*Allium cepaL.*). Journal of the Science of Food and Agriculture 88: 1554-1561.
- Hara, A., Radin, N.S. (1978): Lipid extraction of tissues with a low toxicity solvent. Analytical Biochemistry 90(1): 420–426.
- Karak, T., Bhagat, R.M. (2010): Trace elements in tea leaves, made tea and tea infusion: A review. Food Research International 43(9): 2234–2252.
- Kelley, V.E., Ferretti, A., Izui, S., Strom, T.B. (1985): A fish oil diet rich in eicosapentaenoic acid reduses cyclooxygenase metabolites and suppresses lupus in MLR-lpr mice. The Journal of Immunology 134(3): 1914–1919.
- Koichi, W., Masayoshi, K. (2003): Trace elements in the environ-ment the elements analysis of the tissue section of the lung disease caused by environmental pollution by means of electron probe microanalysis. Biomedical Researchon Trace Elements 14: 275–278.
- Kremer, D., Edith Stabentheiner, E., Dunkic, V., Müller, I.D., Vujiv, L., Kosalec, I., Ballian, D., Bogunic, F., Bezic, N. (2012): Micromorphological and Chemotaxonomical Traits of *Micromeriacroatica* (Pers.) Schott. Chemistry& Biodiversity 9(4): 755-768.
- Kremer, J.M., Lawrence, D., Jubiz, W. (1988): Different doses of fish oil fatty acid supplementation on rheumatoid arthritis. A prospective double-blinded randomized study. Arthritis & Rheumatology 31: 530-536.
- Lands, W.E.M. (1986): Fish and human health. Academic Press, Orlando, FL., 170p.
- Li, D., Hu,X. (2009): Fish and its multiple human health effects in times of threat to sustainability and affordability: are there alternatives? Asia Pacific Journal of Clinical Nutrition 18(4): 553–563.
- Linder, M.C., Hazegh-Azam, M. (1996): Copper biochemistry and molecular biology. The American Journal of Clinical Nutrition 63(5): 797-811.
- Nikolov, I.G., Mozar, A., Drueke, T.B., Massy, Z.A. (2009): Impact of disturbances of calcium and phosphate metabolismon vascular calcification and clinical outcomes in patients with chronic kidney disease. Blood Purification 27(4): 350–359.
- Saracoglu, S., Tuzen, M., Soylak, M. (2009): Evaluation of trace element contents of dried apricot samples from Turkey. Journal of Hazardous Materials 167(1-
- Selvaraju, R., Raman, R.G., Narayanaswamy, R., Valliappan, R., Baskaran, R. (2009): Trace element analysis in hepatitis B affected human blood serum by

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- inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Romanian Journal of Biophysics 19: 35-42.
- Siddiqui, R.A., Harvey, K.A., Zaloga, G.P. (2008): Modulation of enzymatic activities by n-3 polyunsaturated fatty acids to support cardiovascular health. The Journal of Nutritional Biochemistry 19(7): 417–437.
- Steffens, W., Wirth, M. (2005): Fresh water fish-an important source of n-3 polyunsaturated fatty acids: a review. Archives of Polish Fisheries 13: 5–16.
- Stenson, W.F., Cort, D., Rodgers, J., Burakoff, R., Deschryver-Kecskemeti, K., Gramlich, T.L., Beeken, W. (1992): Dietary supplementation with fish oil in ulcerative colitis. Annals Internnal Medicine 116(8): 609–614.
- R. Ullah Khader, J.A., Hussain, I., Abdelsalam, N.M., Talha, M., Khan, N. (2012): Investigation of macro and micro-nutrients in selected medicinal plants. African Journal of Pharmacy and Pharmacology 6: 1829-1832.
- Tel, A.Z., Tatli, A., Varol, O. (2010): Phytosociological structure of Nemrut Mountain (Adiyaman/Turkey). Turkish Journal Botany 34(5): 417-434.
- Thais, F., Stahl, R.A. (1987): Effect of dietary fish oil on renal function in immune mediated glomerular injury. pp.123–126. In Lands, W.E.M. (ed.), AOAC short course on polyunsaturated fatty acid and eicosanoids. American Oil Chemists Society Press, Champaign, IL.
- Tsiaganis, M.C., Laskari, K., Melissari, E. (2006): Fatty acid composition of Allium species lipids. Journal of Food Composition and Analysis 19: 620-627.