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An Important Value of Turkey: Lavender (Lavandula stoaches L.) Honey

Türkiye'nin Önemli Bir Değeri: Karabaş Otu (Lavandula stoaches L.) Balı

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Abstract

Lavandula stoechas L., belonging to the Lamiaceae family, is locally known as "karabaşotu, karan, gargan, keşişotu" and is used in central nervous system diseases (epilepsy and migraine), in the treatment of various wounds and in reducing blood sugar. This plant is also used as analgesic, antiseptic, sedative, expectorant, cardiotonic and to heal eczema. *L. stoechas* is also a well-known good nectar source for honeybees. In this study, melissopalynological, total phenolic, and chemical compound analysis of *L. stoechas* honey produced in Kemalpaşa-İzmir was examined. As far as we know, this is the first study in which the chemical compounds of Turkish *L. stoechas* honey were investigated via GC-MS. As a result of the study, 10 plant taxa were determined in the honey and *L. stoechas* pollen was found to be dominant with 41.37%. *Sanguisorba* spp., Cistaceae, Ericaceae, Rosaceae, *Trifolium* spp., Asteraceae, Brassicaceae, Chenopodiaceae and *Echium* spp. plant taxa, respectively, were detected in the honey in important minor and minor proportions. In addition to melissopalynological analysis, total phenolic and chemical compound analyses were performed in the honey. As a result of total phenolic analysis 385.72±2.25 mgGAE/L was found as a total phenolic amount. On the other hand, acetic acids and esters (5.87%), aldehydes (3.05%), alcohols (1.59%), carboxylic acids and esters (18.10%), fatty acids and esters (27.22%), hydrocarbons (7.96%), ketones (14.98%), terpenes (2.56%) and other chemical compounds (6.63%) were determined by the GC-MS analysis. The most abundant volatiles were found pyruvic acid (16.22%), myristic acid (5.74%), stearic acid (17.95%), which belong to carboxylic acids, fatty acids, and their esters. As a result, carboxylic acids (18.10%) and fatty acids (27.22 %) were detected most abundant compounds in *L. stoaches* honey. These detected components can be used as markers of *L. stoaches* honey.

Keywords: Honey, Chemical Compounds, *Lavandula stoaches* L., Pollen Analysis, Türkiye

Özet

Lamiaceae familyasına ait olan *Lavandula stoechas* L., yöresel olarak "karabaşotu, karan, gargan, kesişotu" adlarıyla bilinmekte olup merkezi sinir sistemi hastalıklarında (epilepsi ve migren), çeşitli yaraların tedavisinde ve kan şekerinin düşürülmesinde kullanılmaktadır. Bu bitki aynı zamanda ağrı kesici, antiseptik, sakinleştirici, balgam söktürücü, kardiyotonik ve egzamayı iyileştirmek için de kullanılmaktadır. *L. stoechas* aynı zamanda bal arıları için iyi bir nektar kaynağı olarak da bilinmektedir. Bu çalışmada Kemalpaşa-İzmir'de üretilen *L. stoechas* balının melissopalinolojik, toplam fenolik ve kimyasal bileşik analizi incelenmiştir. Bildiğimiz kadarıyla bu çalışma, Türk *L. stoechas* balının kimyasal bileşiklerinin GC-MS ile araştırıldığı ilk çalışmadır. Çalışma sonucunda balda, 10 bitki taksonu belirlenmiş ve *L. stoechas* poleninin %41,37 ile baskın olduğu tespit edilmiştir. *Sanguisorba* spp., Cistaceae, Ericaceae, Rosaceae, *Trifolium* spp., Asteraceae, Brassicaceae, Chenopodiaceae ve *Echium* spp., taksonları balda sırasıyla önemli minör ve minör oranlarda bitkiler tespit edilmiştir. Melissopalinolojik analizlerin yanı sıra toplam fenolik ve kimyasal bileşik analizleri de yapılmıştır. Toplam fenolik analiz sonucunda toplam fenolik içeriği 385,7223±2,253298101 mgGAE/L olarak bulunmuştur. Diğer taraftan asetik asitler ve esterler (%5,87), aldehitler (%3,05), alkoller (%1,59), karboksilik asitler ve esterler (%18,10), yağ asitleri ve esterler (%27,22), hidrokarbonlar (%7,96), ketonlar (%14,98), terpenler (%2,56) ve diğer kimyasal bileşikler (%6,63) GC-MS analizi ile belirlenmiştir. En çok bulunan uçucu maddeler ise karboksilik asitler, yağ asitleri ve bunların esterlerine ait pirüvik asit (%16,22), miristik asit (%5,74) ve stearik asit (%17,95) olarak bulunmuştur. Sonuç olarak *L. stoaches* balında en fazla bulunan bileşiklerin karboksilik asitler (%18.10) ve yağ asitleri (%27.22) olduğu saptanmıştır. Tespit edilen bu bileşenler *L. stoaches* balının belirteçleri olarak kullanılabilir.

Anahtar Kelimeler: Bal, Kimyasal Bileşenler, *Lavandula stoaches* L., Polen Analizi, Türkiye

1. INTRODUCTION

Lamiaceae family is represented by 236 genera and 7280 species worldwide and is a plant family that is especially widespread in the temperate zone. These plants, which are generally found in the form of herbs, rarely shrubs and trees, have a quadrangular stem and a circular (verticillate) leaf arrangement extending along the stem. Its flowers are dioecious and zygomorphic. Their pollen usually has three or six colpus. Family plants, due to their essential oils, it is included in the composition of cosmetic products and is used in the preparation of various foods and beverages. Also, plant products have been used as a natural medicine against many different diseases for years The best known and economically important species are *Lavandula angustifolia*, *Lavandula stoechas*, *Lavandula latifolia*, and *Lavandula* x *intermedia* hybrid (Angiosperm Phylogeny Group, 2016; Çelik & Arslantürk, 2007; Çinbilgel & Kurt, 2019; Ez Zoubi et al., 2020).

Turkey is an important gene center of the Lamiaceae family. Lamiaceae family is represented by 45 genera, 565 species, and 735 taxa in the flora of Turkey. Also Lamiaceae

family is one of the highest endemic families in Turkey. The endemism rate is 45% (Gedik et al., 2016).

Lavandula stoechas L., belonging to the Lamiaceae family, is locally known as "karabaşotu, karan, gargan, keşişotu" (Küçük et al., 2019) and is used in central nervous system diseases (epilepsy and migraine), in the treatment of various wounds and in reducing blood sugar (Çelik & Arslantürk, 2007). It is also used as analgesic, antiseptic, sedative, expectorant, cardiotonic and to heal eczema (Küçük et al., 2019).

L. stoechas is spread over three continents (Africa, Europe, and Asia). It is growing around the Mediterranean basin, including in Morocco, Algeria, Tunisia, Spain, Greece, France, Italy, and Turkey. It is also found in Saudi Arabia and Iran (Ez zoubi et al., 2020). *L. stoechas* is also a well known good nectar source for honeybees and *L. stoechas* honey is especially produced in Portugal and Spain (Guyot-Declerck et al., 2002). In Turkey, this honey is produced in Kemalpaşa, Izmir, on the Aegean coast.

In this study, melissopalynological, total phenolic, and chemical compound analysis of *L. stoechas* (lavender) honey produced in Kemalpaşa-İzmir was examined. As far as we know, this is the first study in which the chemical compounds of Turkish *L. stoechas* honey were investigated via GC-MS.

2. MATERIAL AND METHODS

2.1. Collection of Samples

Pool sample material was collected in a glass jar from hives, which is in Kemalpaşa district of Izmir. It was sent to Hacettepe University Bee and Bee Products Center (HARÜM) for melissopalynological, total phenolic, and chemical compounds analyses.

Kemalpaşa is a district of Izmir province, located in the Aegean region in western Turkey, with a population of approximately 112,049 (Figure 1). Kemalpaşa district is adjacent to İzmir's Bornova, Buca, Torbalı, and Bayındır districts and Manisa's Yunusemre, Şehzadeler and Turgutlu districts. The distance to the city center is approximately 24 km. The district's latitude and longitude coordinates are 38.427679 and 27.418462, respectively. Its surface area is 681 square kilometers and its altitude value, which represents its height above sea level, is ~ 197 meters (Anonymus, 2021).

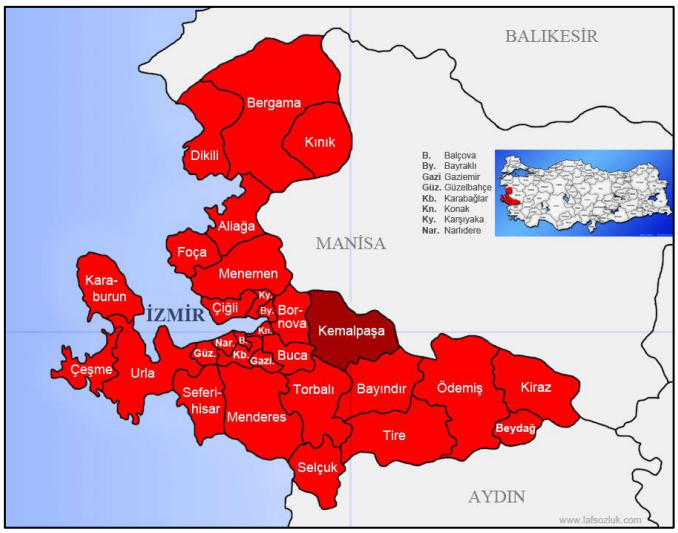

Figure 1. Kemalpaşa-İzmir region. Anonymus, (2021).

2.2. Melissopalynological Analysis

Melissopalynological analysis, which also means microscopic pollen analysis in honey, was carried out according to the method suggested by Sorkun, (2008) and Louveaux et al., (1978). Accordingly, 10 g of the honey sample, mixed well with the help of a glass drumstick, was weighed into a centrifuge tube, and 20 mL of distilled water was added and kept in a 45°C water bath for 10-15 minutes to ensure that the honey dissolved in water and became homogeneous. This solution, which then became homogeneous, was centrifuged at 3500 rpm for 45 minutes and then the supernatant was removed. Then, some sediment remaining at the bottom of the tube was treated with basic-fuchsine glycerin-gelatin and transferred to the slide. Finally, the slide was heated on the heating table at 30-40°C to melt the basic fuchsin-containing glycerin-gelatin. An 18x18 mm^2 coverslip was covered on this melted mixture. The inverted preparation was ready for examination after approximately 12 hours. Honey preparations were examined under a Nikon Eclipse E400 microscope. During the identification of pollen grains, pollen preparation libraries prepared from herbal references in the laboratory where the research was conducted, along with different reference sources were used (Sorkun 2008, Anonymus, 2000).

Starting from the upper left corner of the preparation, a total of 200 plant pollen grains were counted and identified. An immersion objective (x100) was used to identify the pollens, x20 and x40 size lenses were used to easily distinguish small-sized pollens during counting, and a microscopic picture of the *L. stoechas* pollen grains observed in honey was taken.

2.3. Total Phenolic Content

The total phenolic content of *L. stoechas* honey sample was determined by the Folin-Ciocalteu method (Özkök et al., 2010).

The stock was prepared by dissolving 25 mg gallic acid in 100 ml 70% methanol. Concentrations of 0, 25, 50, 75, and 100 mg/L were prepared from this stock. Standards were prepared by diluting 10 ml of the prepared concentrations with 70% methanol. 5 g of each honey sample was taken and dissolved in 50 ml of 70% methanol. 1 ml of each honey sample and the prepared standards were taken and placed in a test tube. 5 ml of 10% Folin Ciocalteu Reagent was placed on it. It was mixed with vortex. After 3 minutes, 4 ml of 75 g/l Na2CO3 solution was added within 8 minutes. The mixture was stirred for 1 minute. 15 min at 45°C was incubated in a water bath. Samples and standards were read on a UV Spectrophotometer at 765 nm.

2.4. Chemical Compounds Analysis

The chemical compounds of *L. stoechas* honey sample was determined by Gas Chromatography-Mass Spectrometry (GC–MS) analysis. For this analysis, Agilent 6890N GC system coupled with a mass selective detector MS5973 was used. Honey was dissolved in methanol and 1 µl of honey extract was injected into the GC-MS system to screen the chemical compounds. For the GC-MS experimental conditions Temiz et al., (2011) method followed. According to this, a DB 5MS capillary column (30 m x 0.25 mm x 0.25 µm) was used and the flow rate of the mobile phase (He) was set at 0.7 mL/min. In the gas chromatography part, the temperature was kept at 50° for 1 min. After this period, the temperature was increased to 150°C with a 10°C/min heating ramp and then kept at 150°C for 2 min. Finally, the temperature was increased to 280°C with a 20°C/min heating ramp and then kept at 280°C for 30 min.

Chemical compounds were identified by computer search using a reference to The Wiley Registry/NIST Mass Spectral Library, which is available in the data acquisition system of GC-MS.

3. RESULTS AND DISCUSSIONS

As a result of the study, 10 plant taxa were determined at the pool honey sample. In melissopalynological analysis, it was determined which plants the pollen found in the honey belonged to, and classification was made as dominant pollen (>%45), secondary pollen (%16-45), important minor pollen (%3-15) and minor pollen (<3%) according to the frequency of pollen grains. Generally, honey with more than 45% pollen from a plant is called unifloral or monofloral honey, and the honey is named after that plant. For example, astragalus, canola, clover, sainfoin honey. However, this is not the case for some plant species. Because while some plants can produce plenty of pollen, some plants can produce plenty of nectar. In this case,

when searching for monofloral pollen in honey, classification is made by taking into account the nectar and pollen production potential of the plant. For example, *Robinia* spp., *Citrus* spp., *Tilia* spp., *Lavandula* spp., and *Rosmarinus* spp. These species are plants that contain plenty of nectar but low pollen, and therefore, in order to be monofloral honey, it may be sufficient to have 10-20% pollen in the honey. On the other hand, *Castanea sativa*, and *Eucalyptus* spp. species produce abundant pollen, and in order to be monofloral honey, 70-90% of pollen must be present in the honey. If there is no dominant pollen in the honey and honey consists of secondary, minor, and trace pollens, such honey is called multifloral or mixed flower honey. (Louveaux et al., 1978; Terrab et al., 2003).

In our study, *L. stoechas* pollen was found to be dominant with 41.37% in the honey. Under normal conditions, 10 to 20% lavender pollen in lavender honey is sufficient for that honey to be dominant, but since the region is dense with *L. stoechas* plants, the pollen was detected at 41.37%. On the other hand, pollen grains belonging to *Sanguisorba* spp., Cistaceae, Ericaceae, Rosaceae, *Trifolium* spp., Asteraceae, Brassicaceae, Chenopodiaceae and *Echium* spp. plants, respectively, were detected in the honey at important minor and minor proportions (Table 1).

Table 1. Lavender honey melissopalynological and total phenolic acid content results

Honey type	Plant species in honey*	Total phenolic acid content (mgGAE/L)	Region
Lavender	*Lavandula stoechas*: 41.37 (D)** *Sanguisorba* spp.: 13.79 (IM) Cistaceae: 12.06 (IM) Ericaceae: 8.62 (IM) Rosaceae: 7.75 (IM) *Trifolium* spp.: 6.03 (IM) Asteraceae: 5.17 (IM) Brassicaceae: 2.58 (M) Chenopodiaceae: 1.72 (M) *Echium* spp.: 0.86 (M)	385.72±2.25	Kemalpaşa-İzmir

*>45%Dominant (D), 16-45% Seconder (S), 3-15% Important Minor (IM), <3% Minor (M)
**For lavender honey, 10-20% lavender pollen amount is sufficient for dominance.

At the same time, microscopic photographs of *L. stoechas* pollen, which is dominantly found in honey, were taken in equatorial and polar positions. Accordingly, *L. stoechas* pollen has medium-sized (26-50 µm), hexacolpate, stephanocolpate aperture and punctitegillate exine ornamentation (Anonymus, 2024) (Figure 2).

Figure 2. Plant photographs of *L. stoechas* L and Microscopic photographs of *L. stoechas* pollen (A.x20, B-C.x40, D-E.x100 Polar view, F-G.x100 Equatorial view) in lavender honey.

In addition to melissopalynological analysis, total phenolic and chemical compound analyses were performed. As a result of total phenolic analysis 385.72±2.25 mgGAE/L was found (Table 1). On the other hand, acetic acids and esters (5.87%), aldehydes (3.05%), alcohols (1.59%), carboxylic acids and esters (18.10%), fatty acids and esters (27.22%), hydrocarbons (7.96%),

ketones (14.98%), terpenes (2.56%) and other chemical compounds (6.63%) were determined by the GC-MS analysis in the *Lavandula stoaches* honey (Table 2).

Table 2. Chemical compounds of *Lavandula stoaches* honey

CHEMICAL COMPOUNDS	*Lavandula stoaches*
Acetic acids and esters (%)	
Lauryl acetate	0.42
Ethyl acetoacetate	3.87
n-Propyl acetate	0.16
Hexyl acetate	0.42
n-Butyl acetate	0.35
Nonyl acetate	0.65
Total	***5.87***
Aldehydes (%)	
Citral	0.04
Phenylacetaldehyde dimethyl acetal	0.90
2,4-Dimethylbenzaldehyde	0.19
2-Methyl-2-pentenal	1.15
2-Methylbutyraldehyde	0.77
Total	***3.05***
Alcohols (%)	
1-Decanol	0.33
Furfuryl alcohol	1.26
Total	***1.59***
Carboxylic acids and esters (%)	
Benzoic acid	0.53
Propionic acid	0.43
Pyruvic acid	16.22
2-Methyl-2-pentanoic acid	0.77
4-Methylpentanoic acid	0.07
3-Hexenoic acid	0.08
Total	***18.10***
Fatty acids and esters (%)	
Ethyl oleate	0.28
Myristic acid	5.74
Stearic acid	17.95
Lauric acid	0.61
Nonanoic acid	2.05
Isobutyl butyrate	0.59
Total	***27.22***
Hydrocarbons (%)	
n-Eicosane	0.43
n-Nonane	0.90
n-Undecane	6.63
Total	***7.96***
Ketones (%)	
3,4-Dimethyl-1,2-cyclopentadione	0.58
3,5-Dimethyl-1,2-cyclopentadione	0.52

CHEMICAL COMPOUNDS	*Lavandula stoaches*
Homofuronol	11.60
1-Methyl-2,3-cyclohexadione	0.36
6-Methyl-3,5-heptadien-2-one	0.28
5-Methyl-2,3-hexanedione	0.10
2-Octanone	1.1
Geranylaceton(isomer 2)	0.44
Total	***14.98***
Terpenes (%)	
Isoborneol (isomer 1)	2.56
Total	***2.56***
Others (%)	
Hexyl formate	0.31
Isoamyl propionate	0.81
2-Methoxy-3-methylpyrazine	1.68
5-Methylquinoxaline	1.83
Methyl-2-furoate	0.34
ɣ-Butyrolactone	0.50
5-hydroxy-2-decenoic acid delta-lactone	1.10
Propyl isovalerate	0.06
Total	***6.63***

Studies investigating the palynological and chemical properties of *L. stoaches* honeys are limited in the literature. Although Louveaux et al., (1978) say 10-20% lavender pollen is sufficient for monofloral lavender honey, the results of this study showed that more than 40% lavender pollen could be detected in lavender honey, depending on the density of lavender in the region. Castro-Vázquez et al., (2014) also found *L. angustifoliaxlatifolia* honey separetly from *L. latifolia* pollen grains in the honey samples but they didn't indicate the pollen percentage. On the other hand, Can et al., (2015) detected the highest percentage of *L. stoechas* pollen in honey as 53%.

Phenolic acids, also known as aromatic carbonic acids, are phytochemicals that have a basic antioxidant effect in honey (Özkök et al., 2010). Karabagias et al., (2019) found a total phenolic amount of 217 mgGAE/L, at the methanolic plant extract of *L. stoaches*. In our study, we determined 385.7 mgGAE/L in the *L. stoaches* honey. Because *L. stoaches* honey's total phenolic amount rate may be higher than just *L. stoaches* plant extract. As a matter of fact, similar to our study, Can et al., (2015) found the total phenolic amount 533.9 mgGAE/L in the *L. stoaches* honey.

Also, Karabagias et al., (2019) found 50 volatile compounds belonging to alcohols, aldehydes, ketones, norisoprenoids, and numerous terpenoids by the GC-MS at the methanolic

plant extract of *L. stoaches*. In our study, unlike Karabagias et al., (2019) work, we determined 45 volatile compounds by the GC-MS at the methanolic honey extract of *L. stoaches*. Acetic acids and esters, aldehydes, alcohols, carboxylic acids and esters, fatty acids and esters, hydrocarbons, ketones, terpenes, and other chemical compounds were found by the GC-MS analysis. The most abundant volatiles were pyruvic acid (16.22%), myristic acid (5.74%), stearic acid (17.95%), which belong to carboxylic acids, fatty acids, and their esters. As a result, carboxylic acids (18.10%) and fatty acids (27.22 %) were found most abundant compounds in *L. stoaches* honey. Özkök, (2019) also, found carboxylic acids (14.49%) and fatty acids (12.63%) in the mullein (*Verbascum* spp.) honey. Besides this, Karabagias et al., (2019) determined mostly essential oils in the *L. stoaches* plant extract. Ez zoubi et al., (2020) also emphasized the essential oil content of *L. stoechas* plants which is used for the preparation of traditional meals, herbal teas, and cosmetic purposes. On the other hand, Guyot-Declerck et al., (2002) compared the aromatic profiles of French (*L. angustifolia* and *L. angustifoliaxlatifolia*) and Portuguese (*L. stoechas*) lavender honey samples and they showed that there are major qualitative and quantitative differences between the honey types. They didn't find any marker specific to *L. stoechas* honey, but Portuguese lavender honey showed much lower concentrations of n-hexanal, n-heptanal, n-hexanol, and heptanoic acid than French lavender honey samples. In our study, we also determined 3-hexenoic acid, which is a carboxylic acid like heptanoic acid, in the *L. stoechas* honey. Castro-Vázquez et al., (2014) revealed the chemical composition of *L. angustifoliaxlatifolia* and *L. latifolia* by SPE/GC-MS, they found ɣ-nonalactone, farnesol, acetovanillone, 1- heptanol, decanal, 4-methoxyacetophenone and dehydrovomifoliol at the *L. angustifoliaxlatifolia* honey separetly from *L. latifolia* honey. In our study we couldn't determine these compounds at the *L. stoechas* honey.

4. CONCLUSION

In conclusion, lavender pollen frequency may vary depending on the lavender plant density of the region and the dominance can be determined to be over 20%. The total phenolic acid amount was found 385.7 mgGAE/L., and carboxylic acids, fatty acids, and their esters were determined mostly in the *L. stoaches* honey. The most abundant volatiles were found pyruvic acid (16.22%), myristic acid (5.74%), stearic acid (17.95%), which belong to carboxylic acids, fatty acids, and their esters. As a result, carboxylic acids (18.10%) and fatty acids (27.22 %) were detected most abundant compounds in *L. stoaches* honey. These detected components can be

used as markers of *L. stoaches* honey. Further extensive studies should be done about *L. stoaches* plant and it's honey to determine more specific characteristics.

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