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Monofloral Features of Turkish Honeys According to Melissopalynologic, Total Phenolic and Total Flavonoid Content

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Abstract

The main objective of this study was to evaluate the plant composition of honey from different part of Turkey. Within this scope, melissopalynological characterizations, total phenolic and total flavonoid content were determined of 28 honey samples. The study was carried out with six kinds of honey; sunflower, canola, chestnut, clover, citrus and rhododendron. The total phenolic content varied from 75.72±0.02 to 312.61±0.19 mgGAE/kg and the total flavonoid content varied from 9.67±0.02 to 42.63±0.17 mgQE/kg. According to these contents, the highest values obtained from chestnut honeys. Botanical similarity of all honey samples was found 62.6%.

1. INTRODUCTION

Turkey is a home to 12.476 native plant species, nearly 450 species of which are known as honey plants and these are important for beekeeping [1,2]. Therefore, Turkey has one of the highest potentials in the world for apiculture, due to its climate, rich botanical diversity and status as a transitional region between the European and Asian continents. It is in the second place in the world after China with about 103 thousand tons honey production per year and about 7 million bee hives [3].

Honey can be classified as monofloral or unifloral when it is composed predominantly from a single botanical origin or multifloral, commonly called wildflower when it derives different plant varieties blooming at the same time. Because of their unique flavour, taste and odor, monofloral honeys are often regarded as better quality and are preferred by consumers, thus attaining higher market values [4].

Melissopalynological characterization is used for the classification of honey according to its floral content [5]. The determination of the botanical origin is based on the relative frequencies of nectariferous species' pollen types. The frequency classes of pollen grains were given as predominant (>45%), secondary pollen (16-44%), important minor pollen (4-15%) and minor pollen (<3%) [2,6].

Usually, a honey is considered as unifloral if the pollen frequency of that plant is >45% [7]. It is considered honeydew if the ratio "Honeydew Elements/Pollen Grains (HDE/PG)" exceeds 3. These are general guidelines but many pollen types are underrepresented (*Robinia pseudoacacia*, *Citrus* spp., *Tilia* spp., *Lavandula* spp., *Rosmarinus* spp.) or over-represented (*Castanea sativa* Mill, *Eucaliptus* spp.). For unifloral honey with under-represented pollen, the minimum percentage of the taxon that gives the honey

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name is 15% for acacia, 10% for citrus while honey with overrepresented pollen, a content of 70-90% of monofloral pollen is required to classify chestnut and eucalyptus honey, honey as unifloral [6-8].

The components in honey responsible for its antioxidative effect are flavonoids, phenolic acids (caffeic, coumaric, ferrulic, ellagic, chlorogenic), ascorbic acid, catalase, peroxidase, carotenoids and products of the Maillard reaction. Many researchers have studied the phenolic and flavonoid contents of honey to find if there is a correlation with floral origin [9-12].

The aim of the study to investigate the pollen composition of monofloral honeys. Total phenolic and total flavonoid contents of the honey samples were also evaluated.

2. EXPERIMENTAL

2.1. Collection of Honey Samples

Total 28 honey samples were collected from 8 different districts [Bartın (n=9); Kastamonu (n=1); İstanbul (n=1); Artvin (n=1); Tekirdağ (n=4); Edirne (n=3); Bingöl (n=5); Mersin (n=2)] of Turkey in 2015 (Figure 1).

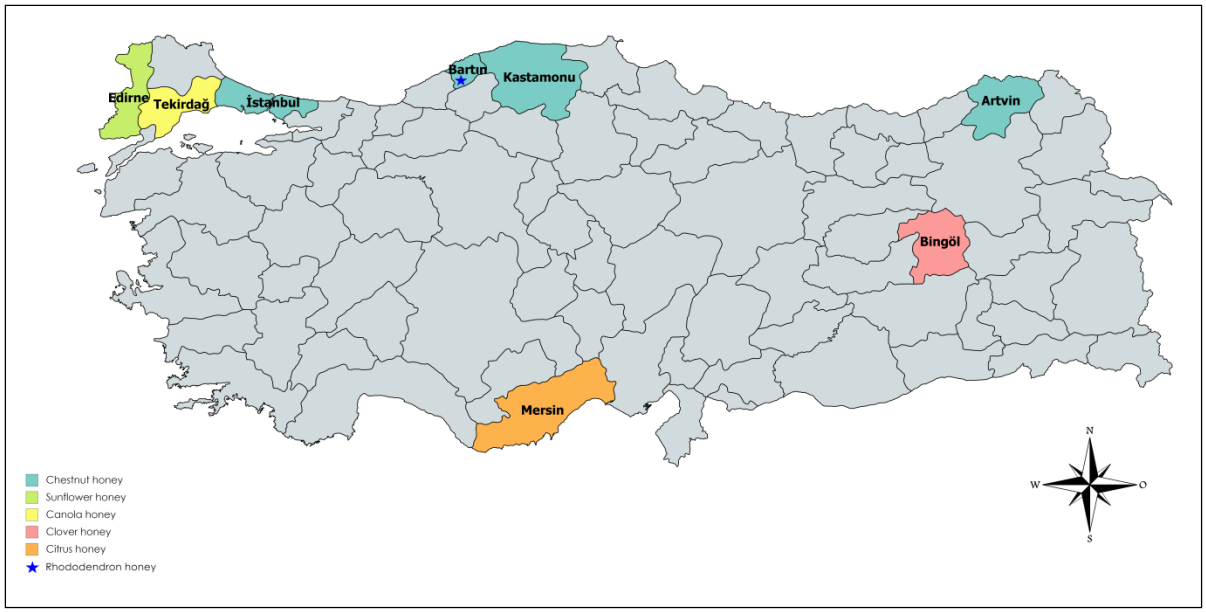

Figure 1. *Total 28 monofloral honey samples collected different regions of Turkey*

2.2. Melissopalynological Analysis

Qualitative microscopic analysis and frequency determination of the classes of pollen grains in the honey samples were done as described [2,13] by Nikon Eclipse E400 microscope. Relevant sources were used in the identification of the pollen were from Persano Oddo & Piro, Özkök Tüylü & Sorkun and Sorkun [2,14,15] as well as reference preparats.

2.3. Total Phenolic Content Analysis

The total phenolic content of honey samples was determined by the Folin-Ciocalteu method [16,17].

For the detection of total phenolics, extracts were prepared by 5 g of honey sample dissolved in 50 mL of 70% methanol. One mL of each methanolic honey solution was transferred to a test tube. Next, 5 mL of a 10% aqueous dilution of Folin-Ciocalteu reagent was added and mixed well using vortex mixer for about 1 min. After 3 min to before 8 min, 4 mL of a 75 g/L anhydrous Na_2CO3 solution was added. The

mixture was mixed thoroughly for another 1 min and incubated in water bath at 45°C for 15 min. After cooling, the absorbance was recorded at 765 nm against a zero absorbance blank at UV-Spectrophotometer (Genesys 10S UV-VIS Spectrophotometer). The total phenolic content of each sample was determined by means of a calibration curve prepared using gallic acid and expressed as milligrams of gallic acid equivalents (GAE) per kilogram of d.m. Stock solution of gallic acid was prepared in methanol at a concentration of 250 mg/L. All calibration solutions (25, 50, 75 and 100 mg/L) were prepared by serial dilution of the stock solution with methanol. The correlation coefficients were obtained using the linear regression model in Excel (R^2=0.99448).

2.4. Total Flavonoid Analysis

The total flavonoid content of honeys was estimated by aluminium chloride ($AlCl_3$) colorimetric method [16,17].

For the detection of total flavonoids, extracts were prepared by 5 g of honey sample dissolved in 25 mL of 80% ethanol. One mL of each ethanolic honey solution was transferred to a test tube. Next, 3 mL of 95% ethanol, 0.2 mL of a 10% aqueous dilution of $AlCl_3$ reagent, 0.2 mL of 1 M potassium acetate (CH_3COOK), and 5.6 mL of distilled water was added. The mixture was mixed thoroughly by vortex mixer for about 30 s and allowed to stand at room temperature for 30 min. Absorbance readings were taken by a UV/Visible Spectrophotometer (Genesys 10S UV-VIS Spectrophotometer) at 415 nm. The total flavonoid content was expressed as milligrams of quercetin equivalents (QE) per kilogram of d.m. All calibration solutions of quercetin (25, 50, 75 and 100 mg/L) were prepared by serial dilution of the stock solutions (250 mg/L) with ethanol. The correlation coefficients were obtained using the linear regression model in Excel (R^2=0.99925).

2.5. Cluster Analysis (CA)

Cluster analysis were carried out by using the MultiVariate Statistical Package (MVSP) ver. 3.22 software for Windows [18] and based on Gower's (1971) [19] general coefficient similarity [20]. In this research honey types are grouped in clusters in term of botanical similarity.

2.6. Statistical Analysis

Total phenolic and total flavonoid analyzes were made in three replicates and the averages and standard deviations were calculated by using Microsoft Excel (2010).

3. RESULTS AND DISCUSSION

3.1. Microscopic Analysis of Honeys

The honey samples were organised by the melissopalynological analysis regarding the authenticity. Thanks to palynological analysis we get information about pollen diversity in the honey consequently the vegetation of melliferous plants.

The investigated honey samples were classified into six groups according to their predominant pollen source. So, the honey samples are categorized as monofloral honey and classified as sunflower, canola, chestnut, clover, citrus and rhododendron honey. The results of the microscopical analysis of the honeys used in this work are briefly summarized. Percentages refer to pollen frequency. Among these groups, every sample was summarized with stacked column charts using Microsoft Excel (2010) (Figures 2-7).

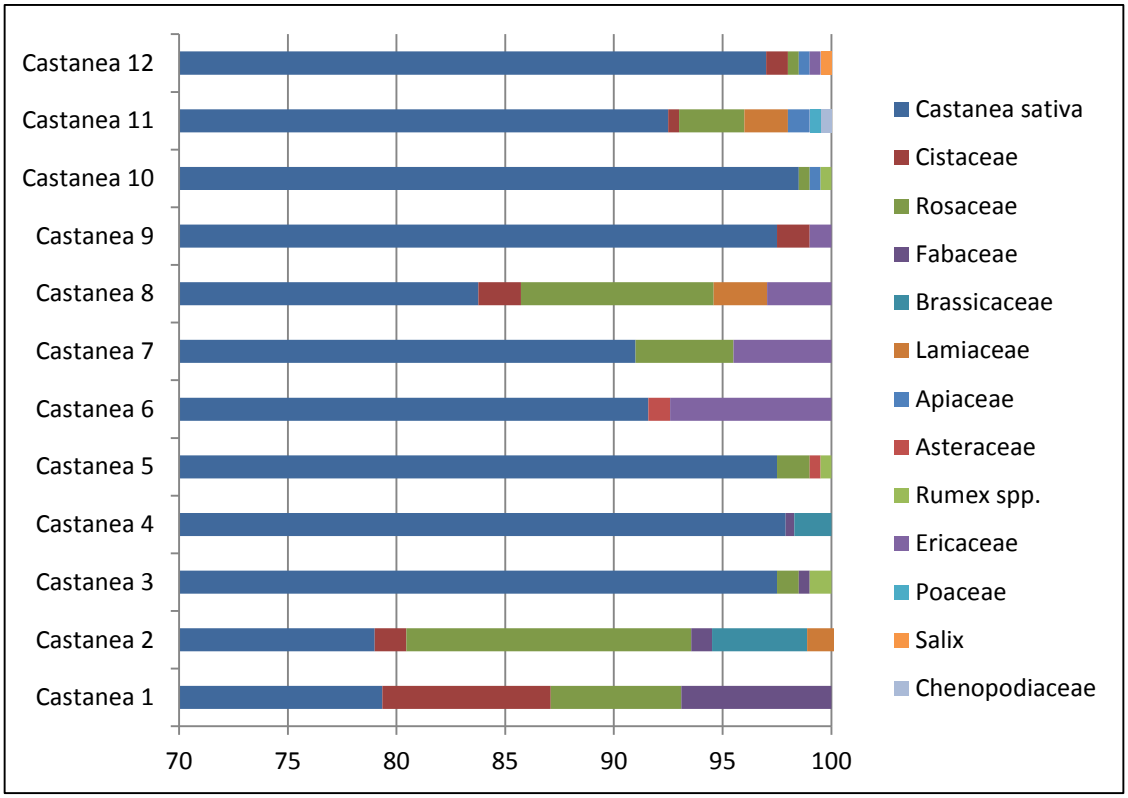

Figure 2. *Pollen composition of chestnut honey samples*

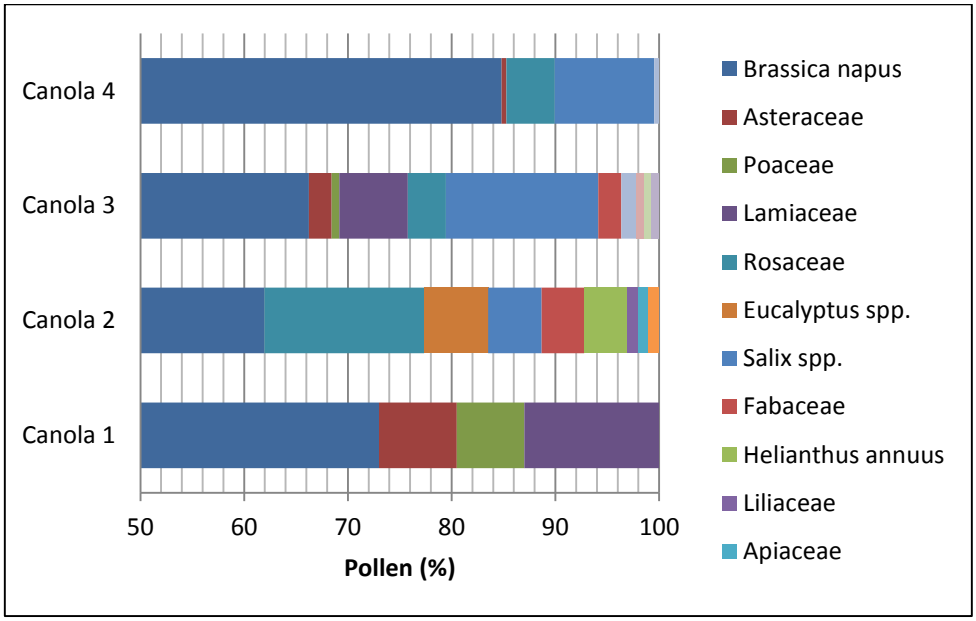

Figure 3. *Pollen composition of canola honey samples*

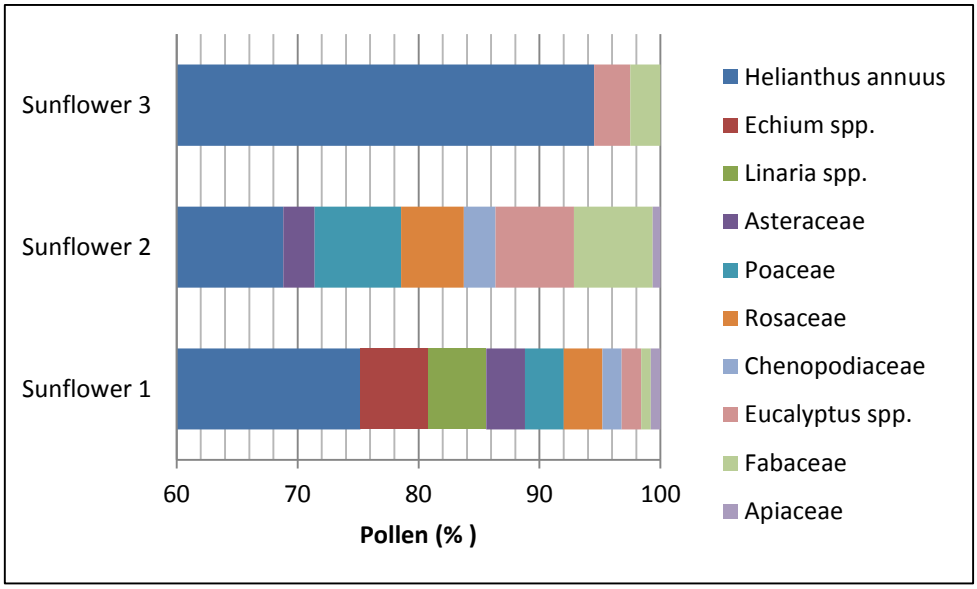

Figure 4. *Pollen composition of sunflower honey samples*

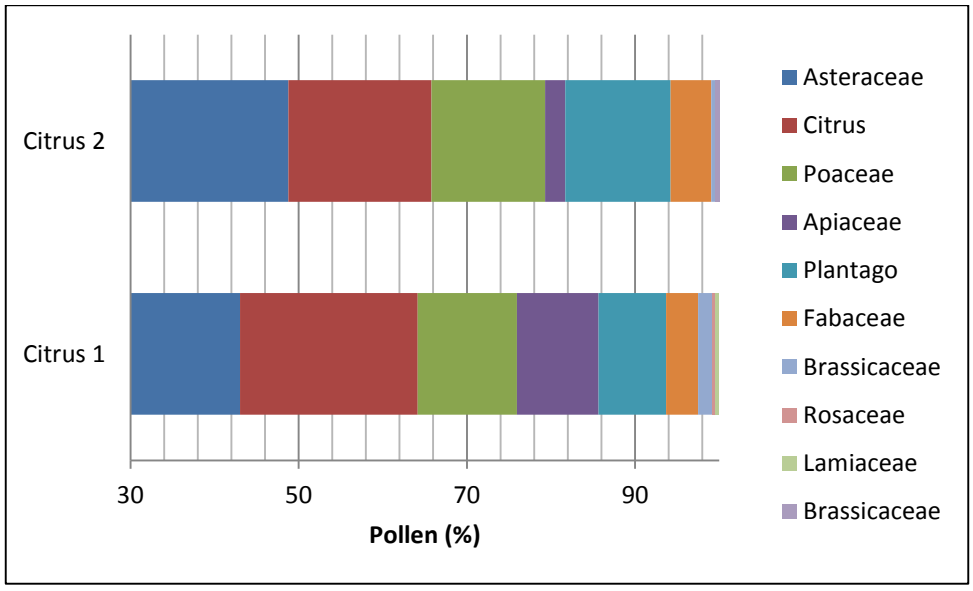

Figure 5. *Pollen composition of citrus honey samples*

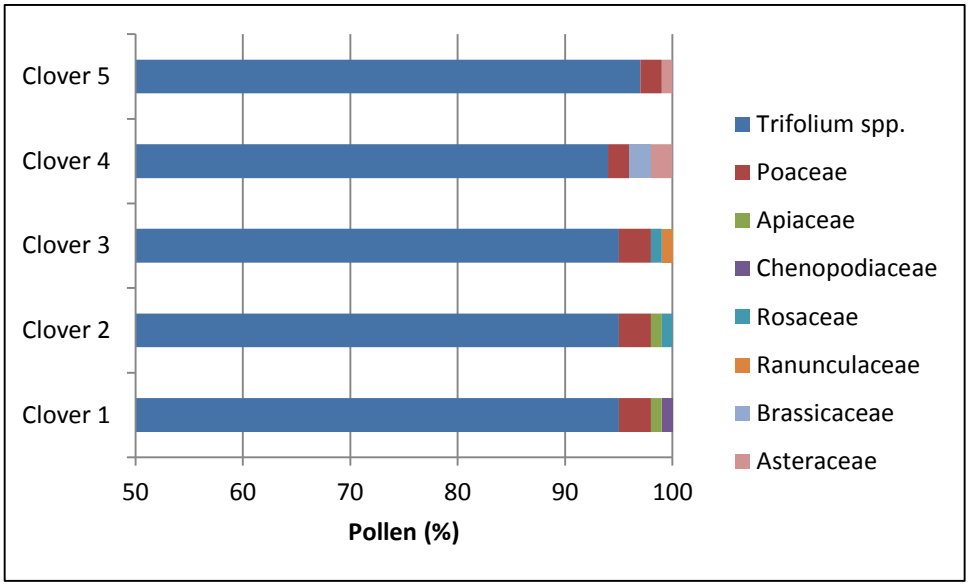

Figure 6. *Pollen composition of clover honey samples*

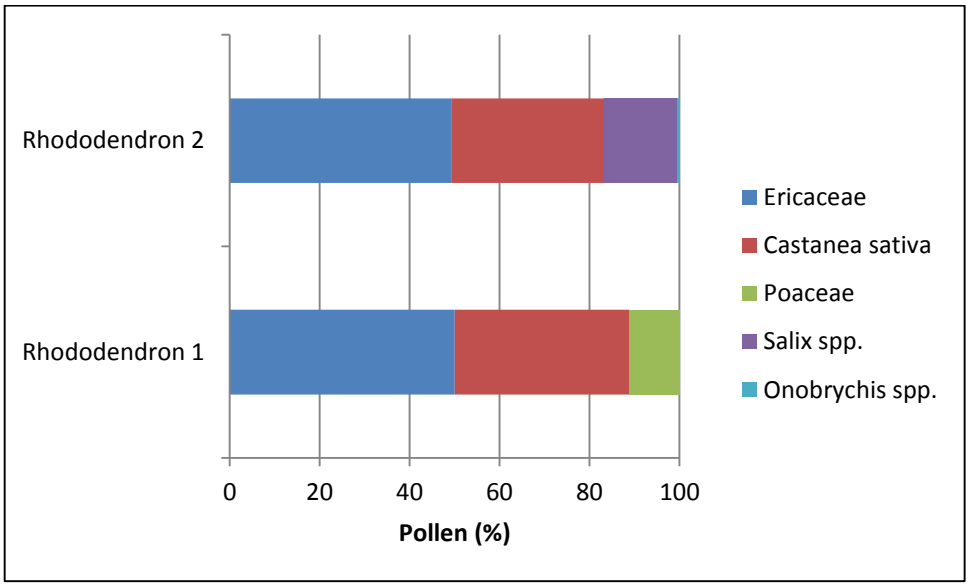

Figure 7. *Pollen composition of Rhododendron honey samples*

3.2. Total Phenolic and Total Flavonoid Contents

Total phenolic content varied from 75.72±0.02 to 312.61±0.19 mgGAE/kg using the standard curve of gallic acid (R^2 = 0.99448). Using the standard curve generated by quercetin (R^2 = 0.99925), the total flavonoid content of honey samples were found in this study to vary from 9.67±0.02 to 42.63±0.17 mgQE/kg (Table 1).

Table 1. *Total Phenolic and Total Flavonoid content of monofloral honey samples*

Sample no and honey type	**Location**	**Predominant Pollen name**	**Predominant Pollen % (>45%)**	**Total Phenolic (mgGAE/kg)**	**Total Flavonoid (mgQE/kg)**
Sunflower 1	Edirne	*Helianthus annuus* L.	75.2	90.54 ±0.13	25.79± 0.05
Sunflower 2	Edirne	*Helianthus annuus* L.	68.8	87.7±0.12	23.25±0.03
Sunflower 3	Edirne	*Helianthus annuus* L.	94.5	103.65±0.03	24.32±0.04
Canola 1	Tekirdağ	*Brassica napus* L.	73	97.98±0.15	25.03±0.08
Canola 2	Tekirdağ	*Brassica napus* L.	61.8	111.38±0.2	34.27±0.04
Canola 3	Tekirdağ	*Brassica napus* L.	66.2	96.35±0.17	32.22±0.19
Canola 4	Tekirdağ	*Brassica napus* L.	83.7	151.73±0.05	26.96±0.76
Chestnut 1	Artvin	*Castanea sativa* Mill.*	79.3	153.93±0.06	34.32±0.04
Chestnut 2	Bartın	*Castanea sativa* Mill.*	77.2	121.44±0.05	38.93±0.09
Chestnut 3	Bartın	*Castanea sativa* Mill.*	97.5	312.61±0.19	42.63±0.17
Chestnut 4	Bartın	*Castanea sativa* Mill.*	97.7	199.08±0.12	29.47±0.18
Chestnut 5	Bartın	*Castanea sativa* Mill.*	97.5	167.93±0.07	20.08±0.19
Chestnut 6	Bartın	*Castanea sativa* Mill.*	91.5	116.54±0.14	11.82±0.06
Chestnut 7	Bartın	*Castanea sativa* Mill.*	90	100.99±0.02	9.67±0.02
Chestnut 8	Bartın	*Castanea sativa* Mill.*	83.7	137.55±0.03	15.3±0.11
Chestnut 9	İstanbul	*Castanea sativa* Mill.*	97.5	219.13±0,16	30.15±0.3
Chestnut10	Kastamonu	*Castanea sativa* Mill.*	98.5	233.53±0.08	29.43±0.12
Chestnut 11	Bartın	*Castanea sativa* Mill.*	92.5	158.32±0.08	28.55±0.13
Chestnut 12	Bartın	*Castanea sativa* Mill.*	97	187.49±0.41	24.73±0.05
Clover 1	Bingöl	*Trifolium* spp. L.	95	72.94±0.04	24.12±0.03
Clover 2	Bingöl	*Trifolium* spp. L.	95	81.68±0.1	25.02±0.51
Clover 3	Bingöl	*Trifolium* spp. L.	95	76.44±0.08	22.28±0.04
Clover 4	Bingöl	*Trifolium* spp. L.	94	77.17±0.02	21.84±0.05
Clover 5	Bingöl	*Trifolium* spp. L.	97	86.94±0.04	22.04±0.02
Citrus 1	Mersin	*Citrus* spp.** L.	21.09	75.72±0.02	21.39±0.01
Citrus 2	Mersin	*Citrus* spp.** L.	17	78.7±0.04	20.81±0.08
Rhododendron 1	Bartın	*Rhododendron ponticum* L.	50	142.69±0.1	16.25±0.19
Rhododendron 2	Bartın	*Rhododendron ponticum* L.	48.4	190.96±0.03	26.03±0.08

*Chestnut honey pollen content is overrepresented and should be 70% for unifloral honey.
**Citrus honey pollen content is underrepresented and should be at least 10% for unifloral honey.

3.3. Cluster Analysis

Cluster analysis including 17 different taxa as variables describes the overall nearness between honey samples. In our study for the CA, the dataset was treated by Gower General Similarity Coefficient as measure of similarity. The results obtained, presented as a dendogram (Figure 8), showed the presence of honey clusters. Generally, at a similarity level of 62.6%, the samples clustered into two groups, comprising of honey samples corresponding to botanical origin of the honey.

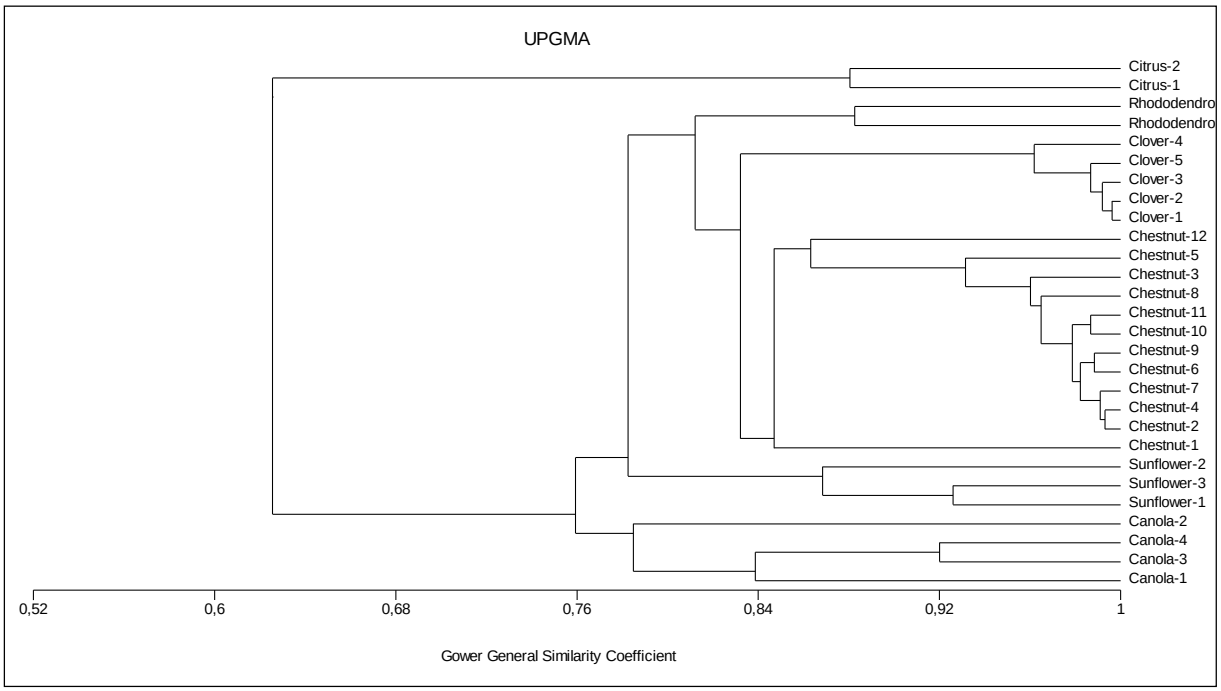

Figure 8. *Dendogram of cluster analysis of honey samples*

The main objectives of this study were evaluate the melissopalynological similarities of 28 monofloral honey samples and effects to total phenolic and total flavonoid content. Total phenolic content varied from 75.72±0.02 to 312.61±0.19 mg GAE/kg using the standard curve of gallic acid (R^2 = 0.99448). It has been reported that Australian unifloral honeys total phenolic contents ranged from 14 to 195.96 mgGAE/kg [16]. Meda et al. [12], determined that total phenolic content varied from 32.59 to 114.75 mg mgGAE/100g. Using the standard curve generated by quercetin (R^2 = 0.99925), the total flavonoid content of honey samples were found in this study to vary from 9.67±0.02 to 42.63±0.17 mgQE/kg. It has been determined that Australian unifloral honeys total flavonoid contents ranged from 8.81 to 45.04 mgQE/kg [16]. Meda et al. (2005) [12], determined lower ranges of total flavonoid content that varied from 0.17 to 8.35 mgQE/100g.

Castanea sativa Miller pollen was always predominant (77.2-98,5%) in chestnut honeys according to the reported overrepresenting presence of this pollen type and the Rosaceae, Ericaceae, Cistaceae, Brassicaceae and Fabaceae were the minor groups in chestnut honey samples (Figure 2). Chestnut honeys have the highest phenolic content. Total phenolic contents were determined between 100.99±0.02 and 312.61±0,19 mgGAE/kg; total flavonoid contents were determined between 9.67±0.02 and 42.63±0.17 mgQE/kg in chestnut honeys (Table 1). Can et al. (2015) [21] found total phenolic content 98.26 mgGAE/100 g and total flavonoid content 8.10 mgQE/100g in chestnut honeys and Bertoncelj (2007) [11] found total phenolic content 199.9±34.1 mgGAE/kg in chestnut honey.

Canola honeys contained 61.8-83.7% pollen of *Brassica napus* L. The minor group's pollen taxa in canola honeys were Rosaceae, *Salix* spp., Fabaceae, Lamiaceae, *Helianthus annuus* L., *Eucalyptus* spp. L. and Lamiaceae (Figure 3). Total phenolic contents were found between 96.35±0.17 and 151.73±0.05 mgGAE/kg; total flavonoid contents were found between 25.03±0.08 and 34.27±0.04 mgGAE/kg in canola honeys (Table 1).

Sunflower honeys contained 68.8–94.5% pollen of *Helianthus annuus* L. as dominant pollen and the minor pollen types were *Eucalyptus*, Rosaceae, Poaceae, Asteraceae, Fabaceae, *Linaria* spp. and *Echium* spp. (Figure 4). Total phenolic content was found between 87.7±0.12 and 103.65±0.03 mgGAE/kg; total flavonoid contents were found between 25.79±0.05 and 23.25±0.03 mgGAE/kg in sunflower honeys (Table 1).

Citrus spp. L. pollen was usually low (17–21.09%) in citrus honeys considering the under-representation of this pollen in the spectra. Asteraceae pollens were in secondary group in citrus honey samples and Poaceae, Apiaceae, Plantago spp. and Fabaceae were the minor pollen types (Figure 5). Total phenolic contents were determined between 75.72±0.02 and 78.7±0,04 mgGAE/kg; total flavonoid contents were determined between 20.81±0.08 and 21.39±0.01 mgQE/kg in citrus honeys (Table 1).

Dominancy of *Trifolium* spp. L. pollen in clover honey were 95-97% and the minor group consisted of Poaceae pollen grains (Figure 6). Total phenolic contents were determined between 72.94±0.04 and 86.94±0,04 mgGAE/kg; total flavonoid contents were determined between 21.84±0.05 and 25.02±0.51 mgQE/kg in clover honeys (Table 1). Can et al. [21] found total phenolic content 25.53 mgGAE/100 g and total flavonoid content 0.65 mgQE/100g in clover honeys.

Rhododendron honeys contained 48.4–50% *Rhododendron ponticum* L. pollen as dominant pollen and the minor pollen types were *Onobrychis* spp., *Salix* spp., and Poaceae (Figure 7). *Rhododendron ponticum* L. honeys' total phenolic contents were found 142.96±0.10 and 190.96±0.03 mgGAE/kg±SD; total flavonoid contents 16.25±0.19 and 26.03±0.08 mgQE/kg±SD (Table 1). Can et al. [21] found total phenolic content 23.55 mgGAE/100 g and total flavonoid content 0.92 mgQE/100g in rhododendron honeys. Also Silici et al. (2010) [22], studied with fifty *Rhododendron* honey samples and determined total phenolic content 0.24 between 141.83 mg GAE/100 g.

We obtained the first cluster enclosing citrus honey samples, and the second one composed of chestnut honey, sunflower honey, rhododendron honey, clover and canola honey. But with the following parameters we could observe that the second cluster clearly creates five separate subgroups consisting of chestnut honey, sunflower honey, rhododendron honey, clover and canola honey. All the chestnut samples are aggregated in two close clusters with a similarity level above 80%, one of them including the majority of the chestnut samples (11 of 12). On the other hand, all the canola honeys are grouped in one close cluster but one of them grouped closer to sunflower honey because it contains minor *Helianthus annuus* L. and *Eucalyptus* spp. pollen's similar as sunflower honey.

4. CONCLUSION

According to this study results floral sources are very important for the honey composition and shows variability from honey to honey. At the same time total antioxidant capacity varies according to plant type and generally it is increasing when the monofloral plant pollen percentage increase in this study. Also we found chestnut honeys antioxidant capacity are higher than other honey types. So this can be helpful for the human comsumption and health.

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CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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