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Compositional Characterization and Bioactive Potential of Two Bee Formulas with the Addition of Natural Oils

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RESEARCH ARTICLE

Abstract

Bee products and natural oils are appreciated due to their beneficial effects on health, which are strongly related to several pharmacological and biological activities. The present study aimed to assess the bioactive potential of two bee products with the addition of natural oils, the first product consisting of honeydew honey, soft propolis extract and *Thymus sp.* (thyme) essential oil, whereas the second product comprises honeydew honey and *Hippophae rhamnoides* L. (sea buckthorn) oil. In order to reach the proposed goal, the following analyses were carried out: determination of the physico-chemical parameters of the honeydew honey sample, used as a common basis for the two products; compositional analysis of sea buckthorn oil (total carotenoids, fatty acids, tocopherols, sterols) and thyme essential oil (organic compounds); evaluation of the total polyphenols and flavones/flavonols content; evaluation of the antioxidant capacity (DPPH and FRAP methods). The results highlighted the superior quality and compliance with the legal framework of the honeydew honey sample and natural oils. Moreover, the two investigated products exhibited a rich content of bioactive compounds as well as good antioxidant activity.

Keywords: honeydew honey; propolis; thyme essential oil; sea buckthorn oil; bioactive compounds.

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INTRODUCTION

Over time, numerous researchers in the field of bio-medical sciences and beyond have been concerned with discovering as many bioactive properties of bee products as possible (Barta et al., 2022). Other natural products that are gaining more and more ground in the medical field due to their multiple benefits and uses are natural oils (Tamas-Krumpe et al., 2020). Generally, there are two categories of bioactive compounds in honey: antibacterial and antioxidant (Ramanauskiene et al., 2012; Wieczorek et al., 2014; Tamas-Krumpe et al., 2019). However, the two classes of compounds are interdependent and by their association, honey acquires more therapeutic properties (Tamas-Krumpe et al., 2020). A diversity of compounds can also be found in the propolis, and its biologically active properties are considered to be mainly due to flavonoids, phenolic acids and esters (Castaldo and Capasso, 2002; Boke Sarikahya et al., 2022). On the other hand, the sea buckthorn (*Hippophae rhamnoides* L.) and thyme (*Thymus* sp.) oils are also

considered significant sources of bioactive compounds (Wang et al., 2022; Moldovan et al., 2023). In this regard, different studies attest almost 200 substances in sea buckthorn oil, which confers multiple therapeutic qualities (Orr et al., 2013; Yang and Kallio, 2002b; Kallio et al., 2002a). With regard to the thyme essential oil, its chemical compounds are believed to be responsible for the bioactive properties (Burt et al., 2005; Sacchetti et al., 2005; Babotă et al., 2023).

The conducted investigations were part of the research objectives included in the following doctoral thesis: "Assessment of the biologically active potential of some apitherapeutic products with addition of natural oils in experimental skin wounds in rats" (Negrea Tamas-Krumpe, 2021).

MATERIALS AND METHODS

Biological material

The biological material subjected to this study was represented by two natural formulas and by each component that made up these therapeutic formulas. Product I was obtained by mixing honeydew honey (95%) and sea buckthorn oil (5%), per 100 g. Product II was obtained by mixing honeydew honey (89%), soft propolis extract (10%) and thyme essential oil (1%), per 100 g. These two formulas were developed and provided by a private commercial company with the aim of being tested further from the point of view of bioactive potential but also for future studies regarding their regenerative effect in experimentally induced wounds in animals. Before carrying out the pursued objectives, we also performed the compositional analysis of the honeydew honey used as a common base for the two final formulas and of the two natural oils, sea buckthorn and thyme respectively, with the aim of verifying and certifying their quality and compliance with the standardized norms. All samples were kept in sealed glass containers, and stored in dry spaces protected from direct sunlight for preserving their composition until further investigations.

Quality parameters of honeydew honey

Melissopalynological analysis, described by (Louveaux et al., 1978) was used for this analysis. The microscopic slide was examined with an Olympus BX 51 optical microscope, and all photos were taken with a UC30 camera. An Olympus Stream Basic software was later used for processing the obtained photos.

Electrical conductivity was measured by using a HANNA-type conductivity meter according to the procedure mentioned by (Bogdanov et al., 2002) with slight modifications (Bobis et al., 2013). The results were finally expressed in $\mu S\ cm^{-1}$.

Water content was determined using an ABBE digital refractometer (Bogdanov et al., 2002; Bobis et al., 2013). The measured refractive index was read at 20°C, and the results were expressed in percentage.

pH, free acidity, lactones and total acidity. To evaluate the acidity, the free acids in honey were neutralized with a NaOH (0.05M) solution to the pH equivalence point. The total acidity was calculated as the sum of the free and lactone acidity (Bogdanov et al., 2002; Bobis et al., 2013). The results were given in meq NaOH/kg honey fresh weight (fw), which is the amount of sodium hydroxide required to neutralize one kilogram of honey.

Hydroxymethylfurfural (HMF) was determined using an HPLC (High-performance liquid chromatography) system with a photodiode array detection according to the procedure described by (Bogdanov et al., 2002; Bonta et al., 2020). The results were expressed in mg HMF/kg honey fw.

Diastase index was determined according to (Megazyme International Ireland, 2014; Bonta et al., 2020). The procedure consisted of the amylase test, characteristic of α-amylase. The results were expressed as diastase number (DN) in Schade units fw.

Sugar content was measured according to the method described by (Bogdanov et al., 2002; Bonta et al., 2007). A SHIMADZU HPLC apparatus with an IR detector (model LC–10AD VP, Shimadzu, Kyoto, Japan) was used. A calibration curve was also made for each sugar with a linear regression factor (R^2) higher than 0.998. The results were expressed in g/100 g honey fw;

Total proteins were determined according to the Kjeldhal method described by (Campos et al., 2008). Distillation was performed with a Büchi distillation unit, KjelFlex K-360, whereas the titration was performed with an automatic titrator TitroLine Easy (Schott). The total nitrogen was calculated according to the general formula mentioned in the literature.

Total lipids were done using the Soxhlet method (Almeida-Muradian et al., 2005) and expressed as percentages.

Tetracycline residue determination was carried out using the HPLC method described by the Association of Official Agricultural Chemists International (1995) (AOAC., 1995) and (Bonta et al., 2007). The sample preparation involved a solid-phase extraction of oxytetracycline and tetracycline residues by MCAC (metal-chelate affinity chromatography) using an ALLTECH extraction system. The high-performance liquid chromatography system was the SHIMADZU VP series.

Compositional analysis of sea buckthorn oil

The content of total carotenoids. It was carried out by the spectrophotometric method following the protocol

described in the literature (Pintea et al., 2003).

Individual carotenoids were determined with a Shimadzu LC20 AT system with an SPD-M20A photodiode array detector. An HPLC-PDA (high-performance liquid chromatography-photodiode array) was used for the separation of carotenoids (Pintea et al., 2003). The sample was analyzed in triplicate and the results were expressed as the mean of three determinations ± standard deviation as mg/100 g.

Gas-chromatographic analysis of fatty acids. The fatty acid methyl esters (FAMEs) were obtained by acid-catalyzed trans-esterification (Christie, 1989). The FAME profile in the sea buckthorn oil sample was determined using a gas chromatography (GC), coupled with a mass spectrometer (MS), PerkinElmer Clarus 600 T GC-MS following the method described by (Pintea et al., 2003; Dulf et al., 2016). The results were expressed as the mean of three determinations ± the standard deviation as a percentage.

The tocopherol content was determined by a liquid chromatography/mass spectrometry method described in literature (Pop et al., 2021; Vlase et al., 2022; Rusu et al., 2020). The HPLC system was an 1100 Series chromatograph Agilent Technologies, equipped with a binary pump, automatic injector, and thermostat, coupled with a Brucker Ion Trap SL spectrometer. The separation was performed on a Zorbax SB-C18 chromatographic column, 100 × 3.0 mm i.d., 3.5 µm (Agilent Technologies). The sample was analyzed in triplicate, and the results were the mean of three determinations ± standard deviation as mg/100 g.

The sterol content was performed by liquid chromatography coupled with mass spectrometry (LC/MS) according to (Toiu et al., 2018; Rusu et al., 2019; Vlase et al., 2022). The equipment consisted of an Agilent 1100 Series HPLC system. Chromatographic separation was done using a Zorbax SB-C18 100 mm x 3.0 mm i.d., 5 µm analytical column (Agilent Technologies). Four standards were used for the quantitative determination, such as β-sitosterol, stigmasterol, campesterol and ergosterol. The results were expressed as the mean of three determinations ± the standard deviation as mg/100 g.

Compositional analysis of thyme essential oil

The GC-MS analysis was determined using a Trace GC Ultra gas chromatograph coupled with a Therm Electron Polaris Q mass spectrometer according to (Marincaş and Feher, 2018). The organic compounds were separated by using a DB-5MS capillary column and helium as carrier gas. The results represented the mean of three determinations ± standard deviation, expressed as percentage.

Determination of the bioactive compounds of both products and honeydew honey sample

Total polyphenol content was carried out following the Folin-Ciocâlteu spectrophotometric method (Folin and Ciocalteu, 1927, Singleton, 1999), and adapted by (Stanciu, 2009). The samples' absorbance was read at a wavelength of 760 nm with a Biotek Synergy HT multi-detection spectrophotometer. The total polyphenols content was quantified based on a gallic acid calibration curve. The results were expressed in mg GAE (gallic acid equivalents)/100 g sample.

Flavones-flavonols content was carried out according to the described spectrophotometric method (Arvouet-Grand A, 1994, Kim et al., 2003, Meda et al., 2005) adapted by (Bobiş et al., 2011) which uses 5% $AlCl_3$ as a specific reagent. Because the aluminum ion Al (III) and the carbonyl and hydroxyl groups of the flavonoid create a complex, the quantification of flavonoids using aluminum chloride is only precise for flavones and flavonols (Meda et al., 2005). The samples' absorbance was measured using a Biotek Synergy HT multi-detection spectrophotometer at a wavelength of 415 nm. A calibration curve was created using quercetin as the reference substance. The results were given as mg QE/100 g of sample (quercetin equivalents).

Evaluation of the antioxidant activity of both products and honeydew honey sample

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay was determined using the spectrophotometric method described by (Velázquez et al., 2003; Stanciu, 2009), using a multichannel spectrophotometer Microplate reader BioTek Synergy HT. The samples' absorbance was assessed at a wavelength of 517 nm. Inhibition percentages (%) were used to express the results.

FRAP (Ferric Reducing Antioxidant Power) assay was made after (Benzie and Strain, 1999) and (Aljadi and Kamaruddin, 2004). The samples' absorbance was measured at a wavelength of 593 nm with a multichannel spectrophotometer (Microplate reader BioTek Synergy HT). The results were expressed as FRAP value in mM Fe^{2+}/100 g sample.

Statistical analysis

The data obtained were statistically analysed using two programs, GraphPad Prism 8.0.1 and Microsoft Excel 2016. Data were analysed in triplicate and the following statistical parameters were calculated: minimum, maximum, mean, standard deviation (SD), and standard error of the mean (SEM). To reveal a direct correlation

between the total polyphenol content and the total antioxidant capacity of the studied samples, a linear regression test was performed to determine the equations and the R^2 coefficient. The results were plotted using Microsoft Excel. The principal component analysis (PCA) was performed using the Paleontological Statistics (PAST) software version 4.12b (Hammer et al., 2001).

RESULTS AND DISCUSSIONS

Quality parameters of the honeydew honey sample

Melissopalynological analysis.

The pollen analysis of honey is frequently used to confirm the honey's botanical and geographical origin (Margaoan et al., 2023; Mureşan et al., 2022; Louveaux et al., 1978). Honeydew honey is distinguished by the presence of honeydew elements such as microalgae, fungal mycelia, or spores (Louveaux et al., 1978; Pita-Calvo and Vázquez, 2017). The microscopic slides' examination using palynology atlases indicated the existence of particular honeydew elements, such as those in Figure 1.

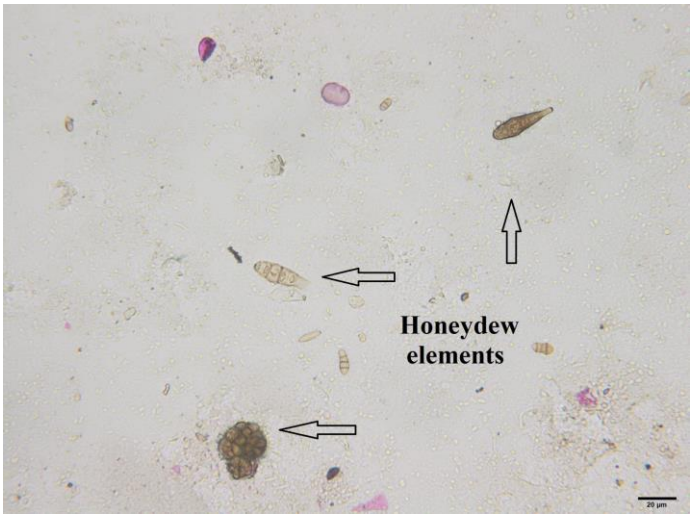

Figure 1. Fungal spores present in honeydew honey.

Electrical conductivity.

Compounds like organic acids and minerals that are found in honey have the ability to split up into ions and conduct electricity in an aqueous solution (Makhloufi et al., 2021). This indicator may be greatly influenced by a range of parameters, including the floral source, the quantity of organic acids, minerals and proteins, the environmental conditions, and the length of storage (Karabagias et al., 2014). International law mandates that honeydew, chestnut, and combinations of these with various floral honeys have electrical conductivity values of at least 800 µS cm^{-1} (Council Directive 2001/110/EC; Pita-Calvo and Vázquez, 2017). The honey sample under investigation had an average value of 940 µS cm^{-1} (Table 1).

Water content

Water content is particularly crucial for determining the "shelf life" of any honey variety. The findings showed that the analyzed honey sample had an average water level of 16%, which is in accordance with legal regulations (Table 1). The water content of bee honey must be less than 20% following the mandatory framework (Codex Alimentarius, 2001) (Council Directive 2001/110/EC). The influence of multiple elements, notably environmental, geo-climatic, and botanical factors, the honey's level of maturation, extraction and processing methods, and storage conditions may contribute to variations in water content (da Silva et al., 2016). Additionally, this investigation assesses how stable honey is and how well it can withstand the fermentation-based deterioration process brought on by a variety of pathogens. In this case, honey with a high water content is more prone to deterioration through fermentation (Sakač et al., 2019).

Deterination of pH, free acidity, lactones and total acidity

Depending on the amount of organic acids present, bee honey has a pH range of 3.5 to 5.5 (Pita-Calvo and Vázquez, 2017). The stability and validity of honey are also thought to be highly connected to the pH value (Terrab et al., 2004). The average pH of the honey sample under investigation was 3.84, which is within the standard range

(Table 1). The presence of organic acids and inorganic ions is linked to the acidity of honey (Silva et al., 2017). The natural acidity of honey can increase with its storage and maturation, as well as during the fermentation process. Moreover, honey adulterated with inverted sugar exhibits a pronounced level of acidity (Yadata, 2014). The studied honey sample had an average free acidity value of 10.20 meq NaOH/kg honey, which is within the permissible range of 50 meq NaOH/kg honey as requested by Council Directive 2001/110/EC. According to the current investigation, the average lactone acidity was 22.94 meq NaOH/kg of honey and the average total acidity was 33.14 meq NaOH/kg of honey (Table 1).

Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural (HMF) is a valid indicator of the freshness of honey. In this regard, the HMF level of honey may rise as a result of extreme or prolonged heat treatment, poor storage conditions, or even both (Fallico et al., 2004). As a result, aged honey has a much higher HMF content than fresh honey. The maximum limits for honey are set at 40 mg/kg in EU legislation ("Council Directive 2001/110/EC Relating to Honey). The analyzed honey sample revealed a very low quantity of HMF compared to EU regulations, with an average value of 0.27 mg/kg (Table 1).

Diastase activity

Diastase activity is provided by the honey's enzyme content. Because their activity diminishes in old or heated honey, diastase and invertase activities are widely used in Europe for assessing the freshness of honey. (Pita-Calvo and Vázquez, 2017). European law (Council Directive 2001/110/EC) sets a minimum value of 8 on the Schade scale while imposing a minimum value of 3 for honey with low natural enzyme content, such as citrus honey. A value of 37.12 DN was the average for the honey sample that has been evaluated in the present study (Table 1).

Table 1. The honeydew honey sample's physico-chemical profile (expressed as fw)

No. Crt.	Electrical conductivity (μS cm⁻¹)	Water (%)	pH	Free acidity (meq NaOH/kg)	Lactones acidity (meq NaOH/kg)	Total acidity (meq NaOH/kg)	HMF (mg/kg)	Diastase (DN)
No. values	3	3	3	3	3	3	3	3
Minimum	934	15.8	3.56	10.1	22.54	32.96	0.19	36.68
Maximum	950	16.4	4.02	10.5	23.16	33.25	0.31	37.61
Mean	**940**	**16.03**	**3.843**	**10.23**	**22.94**	**33.14**	**0.27**	**37.12**
SD	8.963	0.322	0.248	0.231	0.347	0.157	0.069	0.467
SEM	5.175	0.186	0.143	0.133	0.200	0.091	0.04	0.269

Sugars content

Honey primarily consists of monosaccharides, among which fructose and glucose make up the majority (approx. 65%). Minor elements found in honey include proteins, flavour and aroma, phenolic compounds, free amino acids, organic acids, vitamins, and minerals (Silva et al., 2009, González-Miret et al., 2005). The main disaccharides found in floral honey are sucrose, maltose, trehalose, and turanose (Pita-Calvo and Vázquez, 2017). In comparison to floral honey type, honeydew honey has a larger concentration of oligosaccharides, particularly the trisaccharides, melezitose and raffinose, which are both lacking from floral honey (Bogdanov et al., 2004, Pita-Calvo and Vázquez, 2017). One aspect that is thought to help differentiate honeydew honey from other types is the predominance of melezitose (Doner, 1977, Vasić et al., 2020).

The honey sample under investigation contained two monosaccharides (fructose and glucose), four disaccharides (sucrose, turanose, maltose, and trehalose), and two trisaccharides (erlose and melezitose) (Table 2).

Table 2. The free sugars detected in the honeydew honey under study.

No. Crt	Fructose %	Glucose %	Sucrose %	Turanose %	Maltose %	Trehalose %	Erlose %	Melezitose %
No. of values	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Minimum	34.26	34.54	0.08	1.98	2.12	0.07	0.28	0.78
Maximum	36.15	36.82	0.22	2.99	2.49	1.42	0.41	1.02
Mean	**35.27**	**36.06**	**0.14**	**2.593**	**2.353**	**0.727**	**0.357**	**0.86**
SD	0.952	1.316	0.072	0.539	0.203	0.676	0.068	0.139
SEM	0.550	0.76	0.042	0.311	0.117	0.390	0.039	0.08

The distinction between floral honey and honeydew honey can also be influenced by the sum of glucose and fructose. Current regulations state that floral honey must have a fructose plus glucose percentage ≥ 60, whereas honeydew honey and honeydew honey-floral honey combinations must have a fructose plus glucose value ≥ 45% (Council Directive 2001/110/EC). The honey sample under investigation had an average glucose plus fructose level of 71.33%.

Total lipids

This class of compounds is much reduced or even absent in honey, which means that the latter cannot be regarded as a source of fat (Tan et al., 1988). The honeydew honey subjected to the study indicated very low lipid content, recording an average value of 0.025% (Table 3).

Total proteins

The Kjeldahl method measures the total nitrogen concentration; this value is multiplied by 6.25, which is the factor used to convert nitrogen into protein equivalents (Tewari and Irudayaraj, 2004). Proteins frequently appear in honey in small amounts (0.1%–0.5%) (Pereira et al., 2022). Several studies have revealed low protein levels in honeydew honey, including those from Brazil, Slovakia (average values of 0.04%), Lebanon (0.08%-0.14%), and Croatia (0.03%-0.10%) (Azevedo et al., 2017, Horniackova et al., 2017, Jaafar et al., 2017, Flanjak et al., 2016). The honeydew honey sample used in the present research exhibited an average value of 0.56% of the total nitrogen content, referred to as total proteins (Table 3).

Table 3. The honeydew honey sample's total proteins and lipids.

No. Crt.	Total Lipids (%)	Total Proteins (%)
No. values	3	3
Minimum	0.012	0.54
Maximum	0.039	0.593
Mean	**0.025**	**0.561**
SD	0.014	0.028
SEM	0.008	0.016

Tetracyclines

This class of drugs is frequently used in beekeeping to treat several bacterial bee brood disorders, such as American foulbrood, which is caused by *Paenibacillus larvae,* and European foulbrood, which is caused by *Melissococcus plutonius* (Martel et al., 2006). Consumers might experience adverse effects due to the antibiotics in honey, including possible allergic responses, liver damage, and gastrointestinal issues (Thanasarakhan et al., 2011). The maximum limits of 20 g/kg for the screening of tetracycline residues in honey were set by the community reference laboratories in 2007, and Romania approved these values as the highest level that is permissible for tetracycline residues in honey. The tested sample was devoid of any tetracycline or oxytetracycline.

Overall, the honeydew honey sample that served as the main component for the two elaborated products met all quality requirements and was following all applicable laws.

Compositional analysis of sea buckthorn oil

Total carotenoid content

Total carotenoid content is one of the main attributes of sea buckthorn oil used in the industry (Ciesarová et al., 2020). Depending on the source of the oil, carotenoids can range from 0.5 to 21.4 g/kg in oil (Li and Beveridge, 2003). The profile of carotenoids detected in the oil sample under study is comparable to the profile outlined in the literature for different sea buckthorn varieties, where zeaxanthin, as an esterified form, is one of the major carotenoids reported (Pop et al., 2014; Tudor et al., 2019). The individual carotenoids detected in the investigated sample are listed in Table 4. The sea buckthorn oil under study included substantial amounts of zeaxanthin, lutein, and β-cryptoxanthin and a lesser amount of β-carotene (cis and trans isomers). The high concentration of β-carotene and zeaxanthin, which both improve eye health, is particularly helpful for enhancing dietary intake (Ciesarová et al., 2020).

Table 4. The carotenoids' content of the studied sea buckthorn oil

Peak	Individual Carotenoids	Mean ± Sd (mg/100 g)
1	Lutein	14.94 ± 0.747
2	Zeaxanthin	96.84 ± 4.842
3	*Cis-β*-carotene	2.89 ± 0.145
4	*Cis-β*-carotene	4.17 ± 0.209
5	*β*-cryptoxanthin	11.25 ± 0.563
6	*Cis-β*-carotene	1.64 ± 0.082
7	*Cis-β*-carotene	2.88 ± 0.144
8	*Trans β*-carotene	5.27 ± 0.264
9	*Cis-β*-carotene	2.45 ± 0.123
	Total carotenoids	**142.33**

Fatty acids

Comparing sea buckthorn oil with different vegetable-based oils, it has a distinctive fatty acid content (Bialek et al., 2016). The fatty acid composition of the sample under analysis was typical for oil derived from the pulp and peel of sea buckthorn fruits of *Hippophae rhamnoides*. As a result, 8 fatty acids, both saturated and unsaturated, were detected in the investigated sea buckthorn oil sample (Table 5). Monounsaturated fatty acids made up an average of 53.50% of the examined sample, with palmitoleic acid serving as the predominant constituent as well as oleic and vaccenic acids. Given its rarity amongst vegetable oils, palmitoleic acid is regarded as a taxonomic marker of sea buckthorn oil (Ranjith et al., 2006). The average amount of palmitoleic acid found in the examined sample (35.12%) is comparable to previous sea buckthorn oil samples from Romania. In this regard, (Tudor et al., 2019) found that 28% of palmitoleic acid was present, and (Vescan et al., 2010) showed that this proportion varied from 13.35% to 36.68%. According to (Madawala et al., 2018), the results varied between 31.9% and 43.3% in Sweden and India. The polyunsaturated fatty acids identified in the studied oil sample were linoleic (3.63%) and linolenic (1.57%). These findings were similar to those reported by Tudor et al. (2019), who also revealed the presence of linoleic acid (4.13%) and linolenic acid (1.27%).

The three saturated fatty acids found in the oil sample under study were palmitic acid, stearic acid, and myristic acid, with an average amount of 41.30% (Table 5). The most prevalent of them all was palmitic acid (41.03%) (Table 5). This value is comparable to that obtained by Tudor et al. (2019), where a sample of sea buckthorn oil contained 49.42% palmitic acid. Regarding the UFA/SFA ratio, the reported value (1.42) is close to that reported in other studies (Suryakumar and Gupta, 2011).

Table 5. The fatty acid composition of the sea buckthorn oil under investigation

PEAK	Fatty acids	Analyzed Sample (Mean ± SD) (% of total fatty acids)
1	Myristic acid 14:0	0.07 ± 0.004
2	Palmitic acid 16:0	41.03 ± 2.052
3	**Palmitoleic acid 16:1n-7**	35.12 ± 1.756
4	Stearic acid 18:0	0.20 ± 0.01
5	**Oleic acid 18:1n-9**	13.74 ± 0.687
6	**Vaccenic acid 18:1n-7**	4.64 ± 0.232
7	**Linoleic acid 18:2n-6**	3.63 ± 0.182
8	**Linolenic acid 18:3n-3**	1.57 ± 0.079
ΣSFA		41.30
ΣUFA		58.70
UFA/SFA ratio		1.42

Tocopherols

Three different types of tocopherols were found in the sea buckthorn oil that was analyzed (Table 6). The most prevalent of them, with average values of 128 mg/100 mL oil, was α-tocopherol. (Zadernowski et al., 2003) showed that α-tocopherol made up 62–68% of tocopherols in an oil sample derived from whole fruits of *H. rhamnoides* varieties. Additionally, we found low concentrations of β-tocopherols and traces of δ -tocopherols in our oil sample. Another study also emphasized low levels of β-tocopherols (Kallio et al., 2002b). The method of extraction, the portions of the sea buckthorn berries, genetic variation, environmental conditions, growing factors, seasonal fluctuations, degree of maturity, time and manner of harvesting, and the conditions of storage are few examples of

the several elements that might affect the amount of tocopherols (Ranjith et al., 2006, Cenkowski et al., 2006, Zadernowski et al., 2003).

Sterols

Sea buckthorn is an excellent additive for preventing heart conditions caused by hypercholesterolemia (Ciesarová et al., 2020). Dietary phytosterols may also help lower the risk of developing different types of cancer (Saleem, 2009). The primary sterol present in sea buckthorn oil is β-sitosterol (Yang et al., 2001). Stigmasterol, campesterol, citrostadienol, avenasterol, cycloartenol, and obtusifoliol are other sterols detected in sea buckthorn oil (Yang and Kallio, 2002a). Both β-sitosterol and stigmasterol were found in the sample under investigation (Table 6).

Table 6. Tocopherols and sterols' content in the studied sea buckthorn oil

Tocopherols		
No.	**Compound**	**Mean ± Sd** (mg/100 mL)
1	α- Tocopherol	128 ± 6.4
2	β- Tocopherol	6.24 ± 0.312
3	δ - Tocopherol	0.65 ± 0.033
Sterols		
No.	**Compound**	**Mean ± Sd** (mg/100 mL)
1	β-Sitosterol	3000 ± 150
2	Stigmasterol	32 ± 1.6

Compositional analysis of thyme essential oil

Organic compounds

21 chemical substances have been identified by the GC-MS analysis (Table 7). Thymol was the main compound (52.15%), while *p*-cymene (23.55%) and γ-terpinene (5.31%) also appeared in considerable quantities. These results indicated that the examined sample corresponds to the thymol chemotype and are comparable than those reported by other studies (Grigore, 2010, Borugă et al., 2014). On the other hand, some investigations have revealed greater amounts of linalool (De Lisi et al., 2011) or camphor in thyme oil (Imelouane et al., 2009). The existence of a wide variety of chemotypes can be associated with these variations (Rota et al., 2008, De Lisi et al., 2011). The composition of *T. vulgaris* essential oil can also be affected by environmental variables, genetics, the time of harvest, and its geographical origin (Imelouane et al., 2009).

Table 7. The organic compounds of thyme essential oil

Retention Time	**Compound**	**Mean ± Sd (%)**
6.45	α- Thujene	0.89 ± 0.01
6.60	α- Pinene	0.95 ± 0.02
6.91	Camphene	0.93 ± 0.02
7.40	3- Octanone	0.17 ± 0.01
7.52	α- Phellandrene	1.02 ± 0.01
7.88	3- Carene	0.34 ± 0.01
8.04	α- Terpinene	1.01 ± 0.01
8.21	*p*- Cymene	23.55 ± 0.07
8.32	cis-β- Ocimene	0.53 ± 0.02
8.72	γ- Terpinene	5.31 ± 0.02
9.38	Linalool	1.65 ± 0.03
10.32	Camphor	0.55 ± 0.01
10.68	Terpene-4-ol	1.71 ± 0.01
10.76	Cryptone	0.47 ± 0.01
11.01	α- Terpineol	0.07 ± 0.01
12.38	Thymol	52.15 ± 0.05
12.49	Carvacrol	2.08 ± 0.01
13.02	Neryl acetate	0.03 ± 0.01
14.10	Caryophyllene	3.81 ± 0.08
14.59	Germacrene D	0.05 ± 0.01
16.25	Caryophyllene Oxide	0.14 ± 0.01

The content of the biologically active compounds of the honeydew honey and products

Total polyphenol content

The regression equation of the gallic acid calibration curve, y=5.3634x + 0.0812, R^2=0.9991, was used to calculate the total amount of polyphenols. The average results are given in Table 8 for all the investigated samples.

Table 8. Total polyphenols content

Parameter	Total Polyphenols (mg GAE/100 g)		
	Honeydew honey (1%)	Product I (1%)	Product II (1%)
No. of values	3	3	3
Minimum	75	947	988
Maximum	94	1023	1013
Mean	**86.67**	**973.3**	**997.3**
SD	10.21	43.04	13.65
SEM	5.897	24.85	7.881

When compared to the honeydew honey sample that served as their baseline, the two products showed greater values. These characteristics can be attributed to the additional components, which include soft propolis extract and thyme essential oil for product II, and sea buckthorn oil for product I, respectively sea buckthorn oil, thyme essential oil, and propolis extract, all comprising polyphenols, as demonstrated by different studies (Zielińska and Nowak, 2017, Mancini et al., 2015, Galeotti et al., 2018). The evaluated honeydew honey sample, on the other hand, exhibited a high amount of total polyphenols, which was comparable to prior reported findings (Bobis et al., 2008, Stanciu et al., 2008).

Flavones-flavonols content

Based on the regression equation of the calibration curve with quercetin (y=10.73x + 0.049, R^2=0.9994), flavones-flavonols were quantified. The acquired results showed that the two products' values were almost similar. The honeydew honey sample contained lower flavones-flavonols than the other two samples (Table 9), demonstrating yet another time the higher quality of the two products due to their additional ingredients. Flavones and flavonols are among the most significant subclasses of flavonoids, which are linked to a variety of health benefits and are a vital element of many nutraceutical, pharmacological, medical, and beauty products. These features are a consequence of their anti-oxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic effects, as well as their capacity to modulate important cellular enzyme processes (Walker et al., 2000).

Table 9. Flavones-flavonols content of the studied samples

Parameter	Flavones-Flavonols (mg QE/100g)		
	Honeydew honey (1%)	Product I (1%)	Product II (1%)
No. of values	3	3	3
Minimum	18	27	34
Maximum	22	41	42
Mean	**20.33**	**33.33**	**37.33**
SD	2.082	7.095	4.163
SEM	1.202	4.096	2.404

The antioxidant activity of the honeydew honey and products

DPPH free radical method

The antiradical activity has been determined using the following equation and reported as a percentage of DPPH radical inhibition:

% Inhibition = [(DPPH Absorbance – Sample Absorbance)/DPPH Absorbance] x 100.

According to the antioxidant content, different values were obtained (Table 10). The honeydew honey was found to have the lowest percentage of inhibition, whereas the other two products had substantially greater values. Product I, which comprises honeydew honey and sea buckthorn oil, showed a percentage of inhibition of 41.75%, but product II, which contains honeydew honey, thyme essential oil, and soft propolis extract, acquired the greatest percentage of inhibition (84.29%). Investigations on six samples of honeydew honey (concentration 10%) from various Transylvanian regions revealed that the percentage of inhibition varied between 47.84 and 62.99% (Bobis

et al., 2008). However, the botanical source, season and external factors, along with the circumstances during processing, all have major effects on the antioxidant capacity of honey (Kıvrak and Kıvrak, 2017).

Table 10. Free radical scavenging capacity of the studied samples (DPPH method)

Parameter	Antioxidant Capacity (Inhibition %)		
	Honeydew honey (1%)	Product I (1%)	Product II (1%)
No. of values	3	3	3
Minimum	20.02	39	81.86
Maximum	21.58	45.36	85.71
Mean	**21.03**	**41.75**	**84.29**
SD	0.873	3.267	2.114
SEM	0.504	1.886	1.221

FRAP assay

The regression equation y=0.6379X + 0.2464, R^2=0.9955 was used to calculate the total antioxidant activity. The results were given in FRAP value as mM Fe^{2+}/100 g sample. The product II showed the strongest antioxidant activity (2734 mM Fe^{2+}/100 g product). The lowest antioxidant activity was found in honeydew honey (29.68 mM Fe^{2+}/100 g honey) (Table 11).

Table 11. Antioxidant activity of the studied samples (FRAP method).

Parameter	ANTIOXIDANT ACTIVITY FRAP value (mM Fe^{2+}/100 g sample)		
	Honeydew honey (1%)	Product I (1%)	Product II (1%)
No. of values	3	3	3
Minimum	15.05	527.7	2716
Maximum	44.83	549.6	2757
Mean	**29.68**	**541.3**	**2734**
SD	14.9	11.87	20.88
SEM	8.601	6.854	12.05

The product II that contains honeydew honey, thyme essential oil, and soft propolis extract could potentially be seen as the finest in terms of antioxidant effectiveness based on the data collected both by the DPPH and FRAP methods. Thyme essential oil and soft propolis extract are additional ingredients known in literature for their exceptional antioxidant effects, along with the honeydew honey included in this formula (Castaldo and Capasso, 2002, Viuda-Martos et al., 2008, Al-Hariri, 2011, Martinotti, 2014, El-Guendouz et al., 2017, Machado et al., 2017).

Although antioxidant activity of product I made from sea buckthorn oil and honeydew honey displayed lower values than product II, the results were still considerably greater than those recorded by the honeydew honey sample. In this sense, sea buckthorn oil's contribution is noteworthy because research suggests that it has a potent antioxidant effect (Gęgotek et al., 2018). The total amount of polyphenols and the antioxidant activity were also found to be directly correlated (Figure 2). Such characteristics concerning different honey types were likewise found by other researchers (Hołderna-Kędzia and Kędzia, 2006, Alves et al., 2013, da Silva et al., 2013, Alvarez-Suarez et al., 2010, Silici et al., 2010).

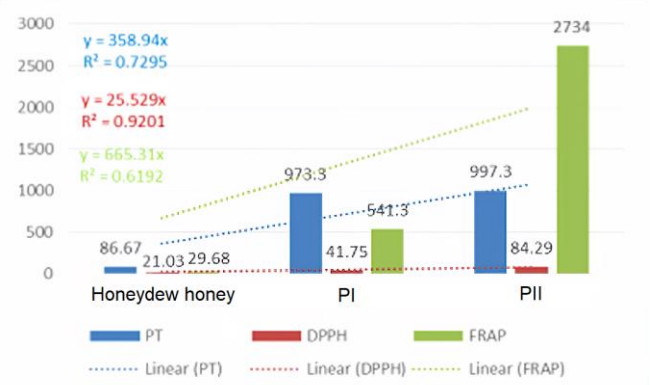

Figure 2. The average values of the antioxidant potential of the honeydew honey and the two products.

Based on the PCA biplot (Figure 3), the flavones-flavonols content was strongly correlated with Product 1 as seen by the closely related values between the investigated samples. This may be due to the high content of zeaxanthin and fatty acids, particularly palmitic and palmitoleic acids present in sea buckthorn oil. Conversely, in the 2nd quadrant, Product II proved to have the highest concentrations in terms of both polyphenols and antioxidant activities. This may be due to the presence of thymol and *p*-cymene in the thyme oil. Lastly, the honeydew honey sample presented relatively low antioxidant capacities, but closely related flavones-flavonols content compared to the other investigated samples.

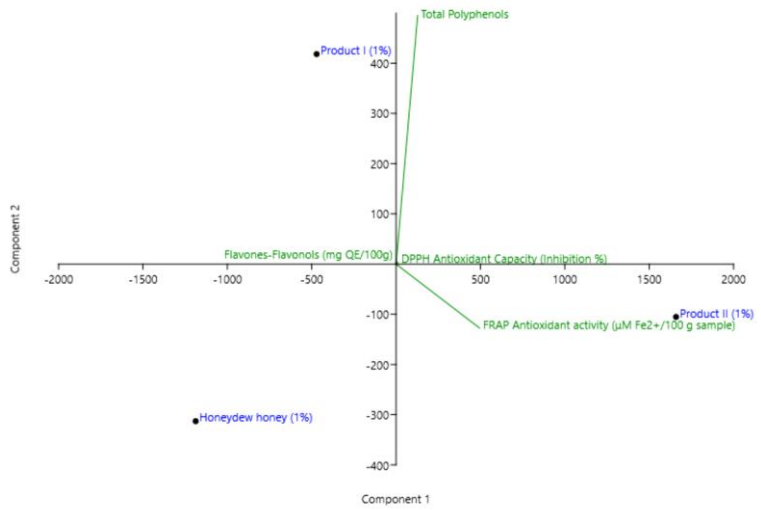

Figure 3. Principal component analysis (PCA) biplot for the investigated samples. The first two components explained 93% of the data variance.

CONCLUSIONS

The overall analyses have demonstrated the authenticity and quality of the examined honeydew honey sample. The information acquired for the examined sea buckthorn and thyme oils also showed an array of biologically active substances. Additionally, the two products' high polyphenol content and strong antioxidant potential imply major health benefits, reinforcing the importance and significance of further studies. The current documentation might also encourage the creation and evaluation of as many honey-derived and natural oil-based formulas as possible, followed by their eventual usage within medical care.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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