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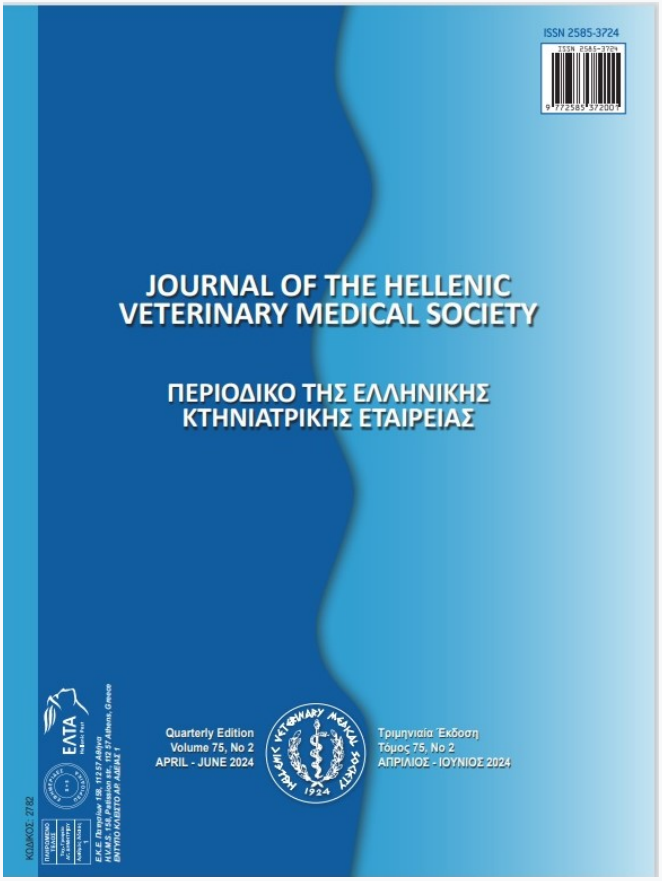

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Comparison of Physiochemical, Biochemical and Antimicrobial Properties of Natural and Artificial *Apis mellifera* L. Beeswax

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ABSTRACT: The interest in bee products is increasing day by day. Beeswax is the honeycomb cells in which bees store their honey. Due to their rich biocompenents, it has both antioxidant and antimicrobial activity. Beeswax could be produced naturally by bees as well as it is commercially available. Commercial beeswax is processed by bees and get ready for honey storage. In this study, the physicochemical properties of commercial and natural beeswax such as water vacuum capacity, oil content, oral secretion and elemental composition were identificated. Then, the beeswax was extracted using different solvents, biochemical and antimicrobial activities of these samples were compared. Natural beeswax was found to have less water absorption capacity, higher oil content (52.79±0.12%) and contained more plant material. Y-acetone extract of beeswax had higher total phenolic content (3.74±0.03 mg GAE/g) and showed a good antioxidant activity (70.23±1.30 µM FeSO4.7H2O/g) than other extracts prepared different solvents. It was clear that both extracts had a good antimicrobial activity. Y-methanol extract was found to be effective on *B. subtilis* and *M. luteus*, D-ethanol on *E. coli*, Y-ethanol on *L. monocytogenes*, and Y-acetone on *P. vulgaris*. It was clear that both artificial and natural beeswax could be used in different applications in a wide range food to medicine.

Keywords: Beeswax; antioxidant activity; antibacterial activity; volatile compounds; water vacuum capacity

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INTRODUCTION

Beeswax is a complicated material utilized to structure honeycombs and is secreted by 12 to 18 days old *Apis mellifera L.* worker bees (Hepburn et al., 1991). It is synthesized from fructose, glucose, and sucrose which are major sugar content of honey. It is secreted in liquid form by four pairs of specialized glands situated on the ventral side of the abdomen. When liquid beeswax aerify directly, it gets dry as slender white particles (Tulloch, 1980; Bogdanov, 2004; Fratini et al., 2016; Svečnjak et al., 2019). Beeswax has an exceptionally, extensive spectrum of useful implementations and occupies a very specific place amongst, plant and animal waxes. It is practically white; merely, after mixing it with honey and pollen, its color changes yellow and over the time it turns brown.

Hydrocarbons, free fatty acids, alcohols, hydroxyl-polyesters and exogenous materials such as propolis residues and pollens are main components of beeswax (Bogdanov, 2009). Nearly 300 different compounds which are fatty acid esters (~67%), hydrocarbons (~14%), and free fatty acids (~13%) predominate were found in natural beeswaxes. Synthetic beeswax were used in industrial production (Tulloch, 1980). It consists chiefly of a mixture of esters of even-numbered, straight chain acids and alcohols containing 16-36 carbon atoms.

The beewax has applications in traditional and complementary medicine to treat skin disorders, infections, burn wounds, eczema and other inflammation (Polat, et al., 2013; Zanoschi, et al.,1991; Sepehr, 2010; Eteraf-Oskouei, et al., 2013; McLoone, et al.,2016). It is also contemplated as a GRAS substance (generally recognized as safe) by the U.S. Food and Drug Administration (Select Committee, 2020). The preponderance of beeswax produced is utilization for technical objectives (candles, modeling, polishes, etc.). Moreover, it could be used in cosmetics, food packaging, processing as coating agent because of its antimicrobial properties (Ka´caniová, et al., 2012). The beeswax has antimicrobial activities and it is instrumented in European and Asian holistic cures for centuries. It was reported that it could affect both Gram-positive and Gram-negative bacteria. The purpose of this study is to evaluate the volatile components, biochemical and physochemical properties and antimicrobial activities of artificial and natural bee waxes extracted in different solvents. The artificial and natural bee waxes were obtained from the Türkiye.

MATERIALS AND METHODS

Natural and Artificial Honey Bee Waxes

One year old combs were used to determine pollen residues. While a small amount of pollen was detected in synthetic beeswax samples, it was determined that the amount of pollen in natural beeswax was quite high. Natural (5 samples) and artificial (5 samples) beeswax samples were harvested from Caykara Halsizen Mountains Trabzon (40° 31′ 59″ North and 40° 22′ 59 East) in the Eastern Black Sea region of Türkiye. Natural beeswax was produced by *Apis mellifera* L. and the artificial bees waxes were purchased and reprocessed by *Apis mellifera* L. After the process, eggs, pupae, and larvae were removed from the bee waxes.

Scanning of Pollens and Determination of The Elemental Composition of Natural and Artificial Wax Honeycombs

The pollen in natural and artificial beeswax samples was detected by using a scanning electron microscope (SEM, Hitachi/ SU1510). For this purpose, samples were coated with 15 nm gold-palladium (SEM coating system, sputtering), and the coated samples were imagined by SEM at 1000x at a voltage of 5-15 kV. The elemental composition of natural and artificial bee waxes was determined using by SEM/ EDX technique.

Determination of Plant Material and Oral Secretion (%)

Dried natural and artificial honey beeswax components were released in 0.5 N KOH solution at 70 °C for two hours. After the treatment, the samples were filtered through weighed filter papers and kept in the laboratory oven until they dried, then the samples were weighed again and the plant material and oral secretion ratio was determined by the formula below and m1 is the weight of the dry sample. Pre-treatment and post-treatment weight of m2 sample: Fiber (cellulose) = (m2/m1) × 100 (Yamane et al. 1999).

Determination of Water Vacuum Capacity (%)

Natural and artificial honey beeswax samples were cut into small pieces and kept in water for one minute. After immersion, the samples were weighed again. The percent absorption capacity was calculated by the following equation, where m1 is the weight of the dried sample before immersion and m2 is the weight

of the sample after immersion: [(m2-m1) / m1] × 100 (Curtis et al. 2005).

Determination of the Amount of Oil Content (%)

To calculate the amount of oil content, known amounts of natural and artificial bee waxes were weighed. After each sample was kept engrossed in the petroleum benzene for one hour, they were weighed again. The oil content was calculated according to the differences between the two weighed (Yamane et al. 1999).

Preparation of Beeswax Extracts

1 g of natural and artificial honeycomb was extracted by 20 mL of solvent (methanol, ethanol, ethyl acetate, acetone, hexane, and ether) separately and was stirred continuously with a shaker at room temperature for 24 hours. Particles were passed through filter paper followed by a syringe filter (0.45 µm). These extracts were stored at -20°C until they were used (Cuce et al., 2020).

Total Phenolic Content of Natural and Artificial Waxes

Total content of the beeswax samples were determined according to Folin-Ciocalteu's method (Slinkard and Singleton, 1977), using gallic acid (GAE) as standard. A standard graph of gallic acid was drawn with the measured absorbance values of gallic acid against methanol solutions at different concentrations (1.0; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/mL). The total phenolic content of the extracts was calculated according to the drawn graph and TPS is expressed as mg GAE/g of beeswax sample using a standard curve. All analyses were performed triplicate.

Ferric Reducing Power (FRAP)

The FRAP method is the most commonly used method for the determination of the antioxidant capacity of natural products, and it is a method based on the reduction of iron (III) ion in the Fe(III)-TPTZ complex of antioxidant substances and hydrogen transfer (Benzie and Strain, 1999). Fe (III) reduced by the antioxidant substances in the solution gives maximum absorbans at 593 nm. Results are expressed in terms of FeSO4.7H2O value. Analyses were performed triplicate.

GC / MS Analysis of Natural and Artificial Beeswaxes

The ethanol extract of beeswaxes was mixed with sodium sulfate (2g) and concentrated to 1 ml by bubbling nitrogen into the solution. The extracted material was taken for GC-MS analysis. Gas chromatography - Mass spectroscopy (Agilent 6890/ Hewlett-Packard 5975) equipped with electron pulse (EI) mode. Helium was used as carrier gas at a flow rate of 1 mL/min. The temperature was programmed at 80 °C for 5 minutes and then it was increased to 300 °C at a rate of 15 °C /min. The temperature of the injector and eI detector (70eV) was 280 °C and 300 °C, respectively. Each extract was manually injected into the GC/MS with a Hamilton syringe (Jiang et al., 2011).

Essential Oil Analysis of Natural and Artificial Beeswax Gas Chromatography/Mass Spectrometry (GC/MS)

It was carried out using a Varian CP 3800 gas chromatograph with a Varian Saturn 2200 MS detector (Walnut Creek, CA, USA). The analyses were performed at Ordu University Central Research Laboratory, Turkey Restek-Rtx-5 was equipped with a column of fused silica capillary tubes (30 m × 0.25 mm × 0.25 µm). The injection volume was 1 µL using the auto sampler at 1 mL/min helium carrier gas (helium) flow with a division ratio of 1:10. Initial oven temperature of 40 °C was maintained for 2 minutes and then increased to 250 °C at a rate of 3 °C/min, then to 250 °C at a rate of 5 °C/min, finally at 15 °C/min. It was raised to 250 °C and held at that temperature for 15 min incubation + 45 min additional. 60 °C. Other settings were interface temperature 300 ◦C, ion source temperature 230 °C, and electron pulse ionization (EI) 70 eV. Mass spectra were analyzed in SCAN mode in the range of 33 to 400 atomic mass units (amu), emission current 34.6 VA electron multiplier voltage 1392V (Jiang et al., 2011).

Bacterial Strains and Growth Conditions

Strains of bacteria and fungi were obtained from American Type Culture Collection (ATCC). The antimicrobial activity of the wax samples was studied using ten bacteria (five gram-positive: *Pseudomonas aeruginosa* ATCC®27853, *Proteus vulgaris* ATCC®7829, *Escherichia coli* ATCC®25922, *Salmonella typhimurium* ATCC®14028, *Staphylococcus aureus* ATCC®25923, *Listeria monocytogenes* ATCC®7677, *Klebsiella pneumoniae* ATCC®13883, *Micrococcus luteus* B1018, *Bacillus subtilis* B209, Mueller Hinton Agar (MHA, Merck) or Mueller Hinton Broth (MHB, Merck) *Candida albicans* ATCC®10231and Sabouraud Dextrose Broth (SDB, Dif-

co) or Sabouraud Dextrose Agar (SDA, Oxoid) were used for growing bacterial and yeast or fungal cells, respectively. For the definition of antibacterial and antifungal efficiency, the diffusion disk plates method was used (Erturk 2017).

Statistical analyses

All measurements were performed in triplicate, the results being expressed as mean plus/minus standard deviation (X ± SD). The non-parametric Mann-Whitney test was used to determine whether differences between the groups were significant ($p < 0.05$).

RESULTS AND DISCUSSION

The ratio of plant material to oral secretion used in the production of natural and artificial wax materials was calculated as a % percentage. The samples for natural and artificial bee wax materials had a similar ratio of plant material to the oral secretion of 50.38 % and 29.33%, respectively. The amount of oral secretion in the nest material of *P. nympha* was calculated as 40.78% - 20.65%, and the water absorption capacity of natural and artificial wax materials was calculated as 29.59% and 39.48%. In addition, the oil percentages of the samples were determined. The results showed that the oil content of natural beeswax (52.79%) was higher than the artificial beeswax (35.58%) (Table 1).

The basic structures of natural and artificial wax surfaces were observed with a stereomicroscope (Figure 1). Natural wax is almost white, however, after contact with honey and pollen, it acquires a variable intense yellowish color and turns brown over time. When it comes into contact with air, it solidifies as flakes and forms a honeycomb structure. The honeycomb gets darkened over time due to the oxidation of the wax cocoons. Larval excrement, pupal skins, and propolis deposition were also observed to change the color of the wax. The nest colors of the artificial wax specimens were dark brown, black stripes, light brown, beige, and a shiny membrane-like structure with white accents. The color of natural wax was white, some parts are brown, orange, dirty white and red due to pollen. It was observed that the natural wax was filled with different colored pollen, and the pollen formed layers of different colors inside the honeycomb. On the other hand, this feature was not observed in artificial wax. In the sections taken from the side faces of the honeycombs, the artificial wax was observed as a very bright membranous structure, while the natural white matte irregular pattern was observed in Figure 2.

Oral secretions of natural and artificial wax were a mixture of saliva, and plant fibers and appeared as a thin layer. The structure of natural beeswax, especially plant material, was seen in SEM micrographs

Table 1. Physicochemical characteristics of natural and artificial honey bee waxes of *AA. mellifera*

	Artificial bee wax	Natural bee wax
Dry weight (mg)	0.224^{a} mg	0.140^{b} mg
Water absorption capacity (%) determination	39.48 ± 1.10^{a}	29.59 ± 1.18^{b}
Plant material and oral secretion (%) determination	$29.33\text{-}20.65^{a}$	$50.38\text{ -}40.78^{b}$
Oil content (%) determination	35.58 ± 0.08^{a}	52.79 ± 0.12^{b}

*Different letters in the same lines show statistically differences between means ($p < 0.05$)

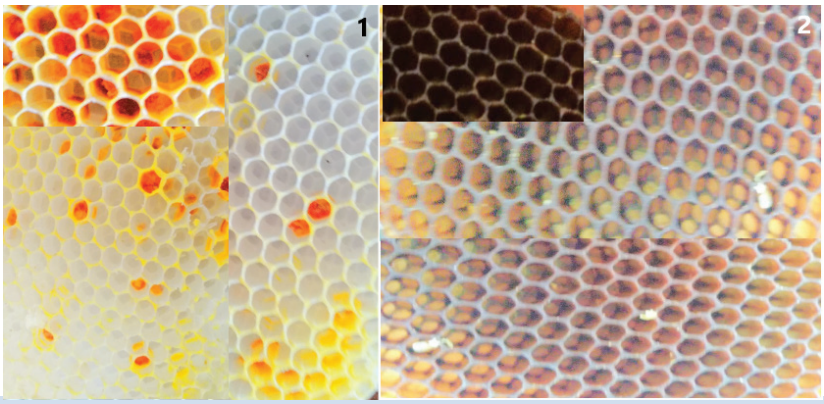

Figure 1. Honeycomb eyes and color images of natural and artificial wax under stereo microscope, 1. natural honeycomb, 2. artificial honeycombs

as fine fibers and sweater knits overlapping each other and in the form of piecemeal flat floors between these fibers (Figure 3 and 4). On the other hand, since the artificial wax was treated a few times, it would seem that most of it was flat and composed of different layers, and occasionally fibers were seen, but very vague, and the ground was mostly bright and in different colors and thicknesses (Figure 3 and 4). As a result, a very different texture pattern and structure difference was detected between the two waxes. The honeycombs formed by the same species from wax in different environments (beehive and rock cavity) had the same structure in appearance, coloration, plant material consumption and pollen accumulation characteristics. Many inorganic particles were seen in SEM micrographs (Figure 5, Table 2). The average fiber thickness of its natural envelope was calculated as 2.01±0.053 µm (min 1.6.04±0.345 µm - max. 2.3.76±0.023 mm) and the artificial honeycomb membrane had no appreciable measurable fiber structure (n = 30 for each well) (Figure 2-5, Table 2).

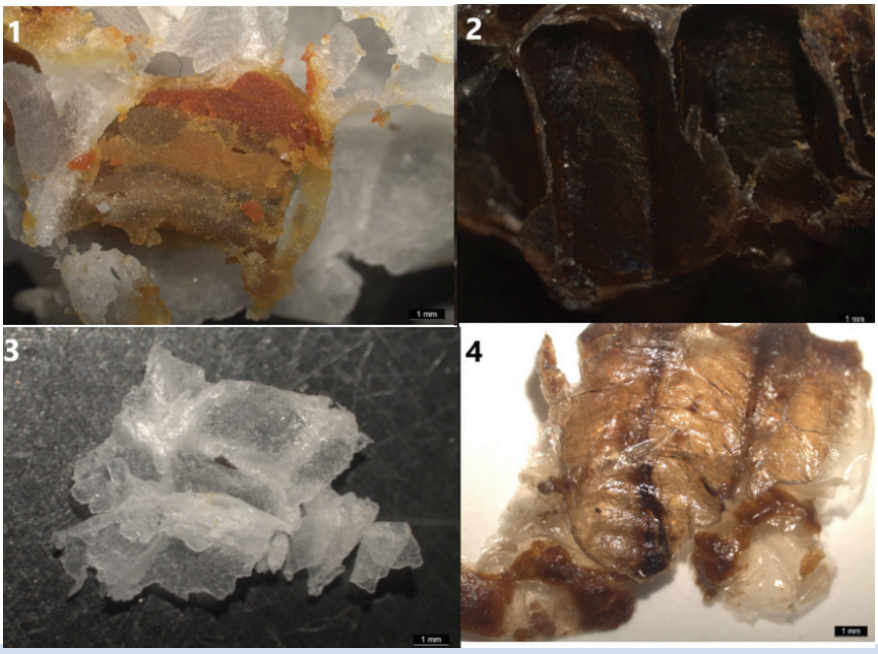

Figure 2. Internal structure view of honeycomb cells of natural and artificial wax and images of pollen layers under stereo microscope, 1-3 natural honeycombs, 2 -4 artificial honeycombs

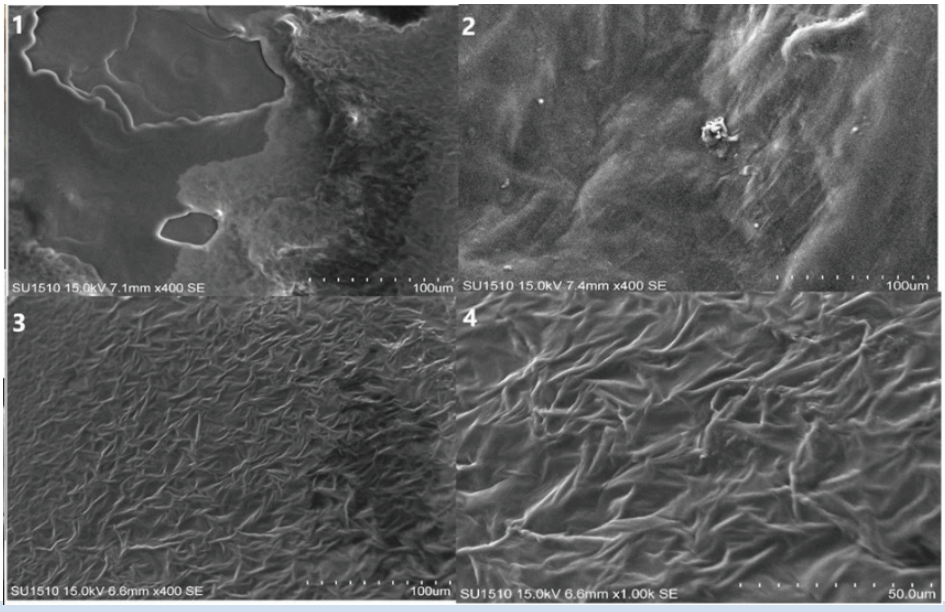

Figure 3. Outer, inner surface and honeycomb coating of the wall of natural and artificial wax in SEM. (1-2) Section of natural wax honeycomb wall; Section of the honeycomb wall of (3-4) artificial wax.

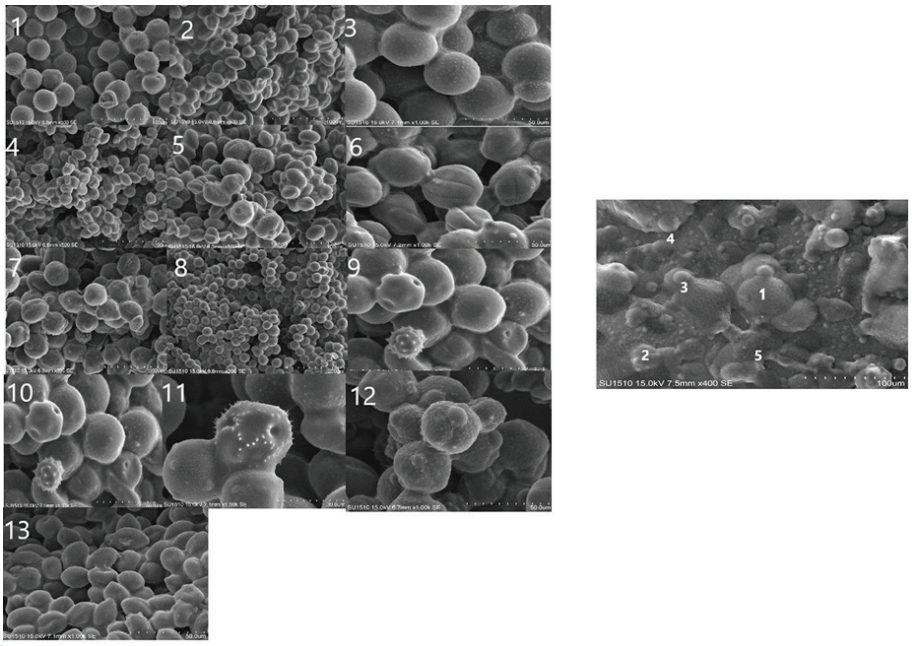

Figure 4. Pollen diversity at the base of the honeycomb knitted from natural and artificial wax, Pollen diversity of natural beeswax (1-13), Pollen diversity of artificial wax (1-5)

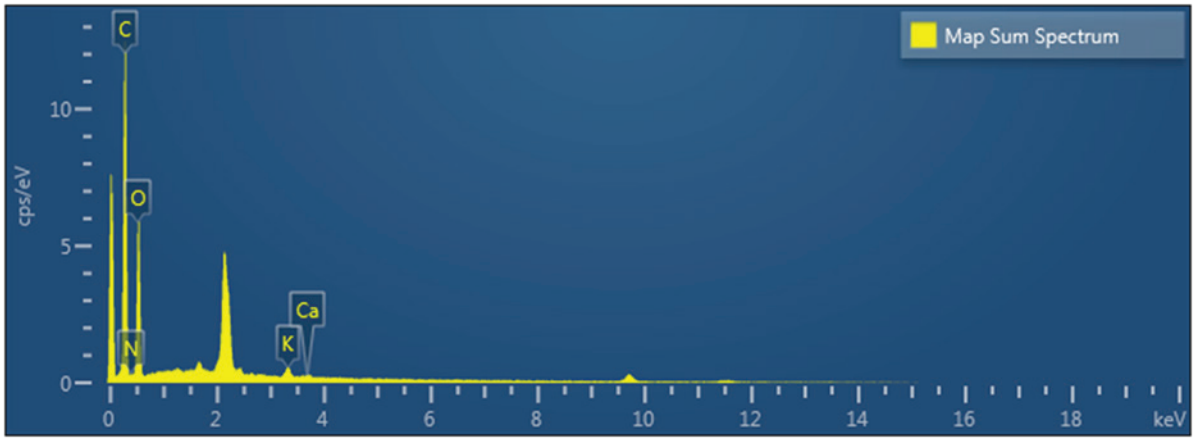

Figure 5. EDX spectrum of elements embedded in the internal structure surface of natural and artificial honey bee waxes of *A.mellifera*

Table 2. Values of parameters natural and artificial honey bee waxes of *A.mellifera* within the area at the final stage of their development

Sample	Max. surface of comb (cm^2)	Dimensions of small cells (mm)	Dimensions of medium cells (mm)	Dimensions of large cells (mm)
Natural bee wax	134.340	40	40	40
Width.		5.26±0.052	6.10±0.045	6.61±0.087
Depth.		8.10±0.865	8.89±1.76	9.45±9.098
Edge length.		5.03±0.47	5.45±0.86	6.03±0.0526
Artificial bee wax	153.860	40	40	40
Width.		5.56±0.543	6.34±0.034	6.75±0.024
Depth.		8.33±0.067	9.80±1.46	10.45±5.945
Edge length.		5.65±0.33	5.78±0.46	6.56±0.334

In this article, the elemental analysis of the extracted bee wax was made with samples of natural material and the wax material used several times. According to the results of the elemental analysis (Table 3), it was seen that it had the maximum amount of carbon, then oxygen, and nitrogen, is that the bee makes its wax naturally and pollen loading before putting honey will increase the quality of the honey. The results have shown that the main elements of the surfaces of natural and artificial beeswax are 100% nitrogen, the element presence of the pollen layers in the honeycombs of the natural beeswax is in the form of increasing values of carbon (C), oxygen (O) and decreasingly nitrogen (N), calcium (Ca) and potassium (K) (Table 3). In artificial wax, no elements were not seen except carbon.

Wax is a product with a very low water solubility due to its hydrophobic structure. For this reason, it must be extracted with a suitable solvent before being used in different applications. In this study, natural and commercial beeswax samples were extracted using twelve different solvents and their total phenolic content and iron reducing capacity (FRAP) were determined. It was determined that the wax samples extracted with Y-acetone had the highest total phenolic substance content and therefore the highest iron reducing capacity. D-hexane was not found to be efficient in the extraction process. It was determined that the total amount of phenolic substance varied between 0.22 and 3.74 mg GAE/g, and the iron reducing capacity ranged between 1.56 and 70.23 µM FeSO4.7H2O/g (Table 4). It was clear that there is less study about biochemical characterization of beeswax and our results were compatible with the literature data (Sawicki et al., 2022; Osae et al., 2022; Kaur et al., 2023).

Volatile compounds of different extracts of bee waxes were detected in high amounts, especially D-Ethanol, D-E. Acetate, Y-Acetone, Y-Methanol, and Y-Ethanol. Volatile compounds were detected in high amounts in all two wax samples tested, and some chemical compounds contained in the alcoholic extract, volatile compounds (Pentane, 2,2-dimethyl-, Hexane, 2,4-dimethyl-, Silver acetate and methylbenzene]bis(. eta.3-2-propenyl)di-) and the aromatic compound Hexacosane (CAS), 1-Heptacosanol (CAS), Hexatriacontane, Heptacosyl heptafluorobutyrate, and -hexamethyl- (CAS) (Table 5 and Table 6). An effective antimicrobial activity could be detect-

Table 3. EDX analyses of the Natural and artificial honey bee waxes of *A.mellifera* Weigth %

Element	Natural Bee Wax	Artificial Bee Wax	1. Layer of Pollen	2. Layer of Pollen	3. Layer of Pollen
C	100.00	100.00	55.30[a]	54.11[a]	57.58[a]
O	0.00	0.00	40.66	41.91	38.99
N	0.00	0.00	0.11	2.42	1.57
Ca	0.00	0.00	0.36	0.33	0.40
K	0.00	0.00	1.25	1.23	1.46
Total	100	100	100	100	100

*Different letters in the same lines show statistically differences between means ($p < 0.05$)

Table 4. Total phenolic content and antioxidant capacity of different extract of beewaxes

Samples	Total Phenolic Content (mg GAE/g)	Antioxidant Capacity (FRAP) (µM FeSO4.7H2O/g)
Y-methanol	2.99±0.04[a]	44.41±0.97
Y-ethyl acetate	0.22±0.01[c]	5.24±0.10
Y-ethanol	2.21±0.02[a]	37.33±0.24
Y-ether	0.41±0.01[c]	9.83±0.26
Y-hexane	0.44±0.03[c]	8.93±0.07
Y-acetone	3.74±0.03[b]	70.23±1.30
D-methanol	1.71±0.01[a]	25.40±0.22
D-ethyl acetate	0.84±0.02[c]	8.31±0.36
D-ethanol	2.11±0.02[a]	31.35±0.16
D-ether	0.40±0.01[c]	6.73±0.01
D-hexane	N.D.	1.56±0.31
D-acetone	0.39±0.01[c]	9.54±0.18

N.D: not determined, different letters in the same columns show statistically differences between means ($p < 0.05$)

Table 5. Chemical composition of artificial and natural beeswax honeycombs by GC/MS

Natural Beeswax			Artificial Beeswax		
Name	**Ret.Time**	**%Area**	**Name**	**Ret.Time**	**%Area**
Methane. nitroso-	1.337	1.51	Carbamic acid. monoammonium salt	1.184	0.54
N.N'-Bis(2-methyl-2-nitrosopentan-4-one)	1.421	0.58	Methane. nitroso-	1.342	0.71
Ammonium acetate	1.78	0.91	Formic acid	1.451	0.99
Pentane. 2.2-dimethyl-	1.851	4.08	Pentane. 2.2-dimethyl-	1.855	8.70
Hexane. 2.4-dimethyl-	2.868	28.67	Silver acetate	1.964	10.88
Molybdenum. di-.mu.-chlorobis[(1.2.3.4.5.6-.eta.)-methylbenzene]bis(.eta.3-2-propenyl)di-	4.132	60.93	1-But aneboronic acid	2.800	0.31
Hexanal	4.925	0.63	Hexane. 2.3-dimethyl-	2.870	21.21
Octanal	11.946	0.81	Molybdenum. di-.mu.-chlorobis[(1.2.3.4.5.6-.eta.)-methylbenzene] bis(.eta.3-2-propenyl)di-	4.133	56.25
Nonanal	15.914	1.15	D-Limonene	12.943	0.21
Decanal	19.797	0.72	2-tert-Butyl-3.4.5.6-tetrahydropyridine	13.008	0.21

ed due to volatile or phenolic compounds of natural products. In studies where some of these compounds were found, E. *coli*, S. *aureus* (Inouye et al., 2001; Nair et al., 2005) and M. *luteus* seem likely to contribute to the increased antibacterial activity. Likewise, it was effective against some gram-negative bacteria, especially E. *coli*. Volatile compounds such as hexacosane and hexatriacontane could be played a role in antibacterial activity. Indeed, in a study, it was reported that hexacosane was effective against the growth of many bacteria, even in small amounts. (Kotan et al., 2010). Based on GC-MS analysis, the most detected compounds of M. *sylvestris* extract were 1-octacosanol (38.4 %), 17- pentatriacontene (19.8%), and 6,9,12,15-docosatetraenoic acid, methyl ester (8.1%). 1-Heptacosanol is a long-chain primary fatty alcohol. As this compound has already been reported to have nematocidal, anticancer, antioxidant, and antimicrobial activities (Al-Abd, et al.,2015; de Oliveira et al.,2012; Everlyne et al.,2015), some of the antimicrobial properties of M. *sylvestris* extract may depend on the presence of 1-octacosanol. The twigs, for instance, contained 8 putative antibacterial compounds (caffeic, p-coumaric, gallic, ferulic chlorogenic acids, adamantyl heterocycle, heptacosanol, and nonadecanol) and they demonstrated moderate antibacterial properties against the majority of the evaluated bacterial strains. In particular, they also exhibited significant antibacterial activities against *Enterococcus faecalis* (Vambe et al., 2020) Given that 1-octacosanol, an antibacterial compound, was the major phytochemical constituent in the hot ethyl acetate fraction, it is logical to suggest that it was probably the one that inhibited the growth of both *Enterococcus faecalis* and *Staphylococcus aureus*.

Antibacterial and antifungal activities of six different solvent extracts of natural and artificial beeswax were tested against 9 bacteria and one fungal species by *in vitro* agar disc diffusion method. The results were summarized in Table 7. The microbial growth inhibition of both samples was extracted in absolute methanol, ethanol, ethyl acetate, acetone, hexane, and ether extracts of the scanned waxes. While D- Methanol extract was effective on E. *coli* with an inhibition zone diameter of 17.5±0.005mm, D- Ether was effective on L. *monocytogenes* with an inhibition zone diameter of 16.0±0.005mm. The most effective solvent of natural beeswax was D- Ethanol. This value was also the highest value with 20±0.005mm for B. *subtilis*, 21±2.005mm for M. *luteus,* and 23.5±0.005mm for E. *coli*, respectively. D- E. Acetate solvent extracted from beeswax sample affected on E. *coli* and S. *aureus* bacteria as 15±0.005mm and 17±0.005mm zone diameters, respectively. No appreciable effect of other solvents on microorganisms was observed. It was determined that the most effective solvent of artificial wax was Y- Ethanol, especially 22±0.005mm for B. *subtilis*, 19.75±0.005mm for M. *luteus*, 22±0.005mm for S. *aureus,* and E. *coli* mm 22±0.005mm. Y- Ethanol solvent extracted from beeswax had shown a 15±0.005mm zone diameter, which is the highest value for C. *albicans* among all solutions. Y-Acetone Y-Methanol extracts also gave similar results and

Table 6. Chemical composition of alcohol extracts of artificial and natural beeswax honeycombs by GC/MS

Natural Beeswax			Artificial Beeswax		
Name	**Ret.Time**	**%Area**	**Name**	**Ret.Time**	**%Area**
Formic acid. 2-propenyl ester (CAS)	3.131	1.59	1-Dodecene (CAS)	4.103	0.10
2-Propanamine. N-methyl-N-nitroso- (CAS)	3.876	0.58	n-Hexadecanoic acid	10.817	1.15
2.3-Dihydro-3.5-dihydroxy-6-methyl-4H-pyran-4-one	3.937	0.51	Heneicosane	12.215	0.58
Guanosine (CAS)	5.562	0.59	9-Octadecenoic acid. (E)-	12.623	0.97
Octadecane (CAS)	10.176	0.76	ETHYL OCTADEC-9-ENOATE	12.911	0.34
FARNESOL 2	10.469	0.28	9.12.15-Octadecatrienoic acid. methyl ester. (Z.Z.Z)- (CAS)	12.945	0.13
n-Hexadecanoic acid	10.817	1.81	1-Docosanol (CAS)	13.873	0.25
Heneicosane	12.216	1.69	Hexacosane (CAS)	14.088	2.89
9.12.15-Octadecatrienoic acid. methyl ester. (Z.Z.Z)- (CAS)	12.298	0.76	1-Heptacosanol (CAS)	15.613	0.80
9.12.15-Octadecatrien-1-ol (CAS)	12.653	0.50	Hexacosane (CAS)	15.801	5.43
1-Heptacosanol (CAS)	13.873	0.36	Hexadecanoic acid. 2-hydroxy-1-(hydroxymethyl)ethyl ester	15.960	0.35
Hexacosane (CAS)	14.089	5.51	Pentadecane. 8-hexyl- (CAS)	16.082	0.72
1-Heptacosanol (CAS)	15.615	2.16	Pentadecane. 8-hexyl- (CAS)	16.607	0.42
1-Heptacosanol (CAS)	15.675	0.37	1-Heptacosanol (CAS)	17.216	0.57
Hexatrıacontane	15.804	8.45	Hexatrıacontane	17.381	11.77
1-Heptacosanol (CAS)	17.219	2.30	Nonadecane (CAS)	17.622	3.31
1-Heptacosanol (Cas)	17.277	0.89	Terephthalic acid. di(2-ethylhexyl) ester	17.842	1.91
Hexatrıacontane	17.382	8.50	Nonadecane (CAS)	18.123	0.41
Octadecanoic acid. 2.3-dihydroxypropyl ester (CAS)	17.611	2.50	2.6.10.14.18.22-Tetracosahexaene. 2.6.10.15.19.23-hexamethyl- (CAS)	18.430	0.35
Terephthalic acid. di(2-ethylhexyl) ester	17.844	2.87	1-Heptacosanol (CAS)	18.730	1.22
2.6.10.14.18.22-Tetracosahexaene. 2.6.10.15.19.23-hexamethyl- (CAS)	18.431	4.26	Hexatrıacontane	18.845	2.78
1-Heptacosanol (CAS)	18.732	2.74	Hentriacontane. 15-methylene-	19.063	1.20
Hexatrıacontane	18.848	1.62	2-Methyl-octadecyne	20.005	0.56
2.6.10.14.18.22-Tetracosahexaene. 2.6.10.15.19.23-hexamethyl- (CAS)	19.751	0.48	1-Heptacosanol (CAS)	20.083	9.40
Heptacosyl heptafluorobutyrate	20.084	8.98	1-Heptacosanol (CAS)	20.130	6.69
Heptacosyl heptafluorobutyrate	20.132	5.03	HEXATRIACONTANE	20.232	1.18
HEXATRIACONTANE	20.232	0.59	Hentriacontane. 15-methylene-	20.450	0.49
Cholesta-5.24-dien-3-ol. (3.beta.)- (CAS)	21.205	0.69	1-Heptacosanol (CAS)	20.844	0.88
9-Octadecen-1-ol. (Z)- (CAS)	21.520	1.13	9-Octadecen-1-ol. (Z)- (CAS)	21.521	2.79
Heptacosyl heptafluorobutyrate	21.668	31.48	Heptacosyl heptafluorobutyrate	21.667	40.36

gave significant zone diameters on microorganisms (Table 7). As a result, extracts of six different natural and artificial beeswax had a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promising extracts open the possibility of evidence of new clinically effective antibacterial and antifungal compounds. Felicioli et al. (2019) showed a high inhibitory activity of methanol and ethanol beeswax extracts with several bacterial strains as well as some yeasts and molds. It was clear that our results are compatible with literature. In addition, ethanol and ethyl acetate extracts of natural beeswax showed the highest antibacterial activity than artificial beeswax. The results concluded that bee waxes have a potential that could be used as an antimicrobial agent in different areas.

Table 7. Measurement results of microbial inhibition growth diameters of natural and artificial wax sample tested according to the agar dilution method (mm)

Microorganism	Y-methanol	Y-ethyl acetate	Y-ethanol	Y-ether	Y-hexane	Y-acetone	D-methanol	D-ethyl acetate	D-ethanol	D-ether	D-hexane	D-acetone	A/N
B. subtilis	20±0.005^{f}	15±0.005^{d}	22±0.005^{j}	13±0.005^{b}	12±0.005^{b}	16.25±0.005^{c}	13±0.005^{c}	11±0.005^{c}	20±0.005^{g}	6.00±0.005^{a}	9±0.005^{c}	9.73±0.003^{d}	42±0.005^{l}
M. luteus	22±0.005^{j}	15±0.005^{d}	19.75*±0.005^{f}	6.00±0.005^{a}	11±0.005^{c}	15±0.005^{d}	13±0.005^{c}	12±0.005^{e}	21±2.005^{g}	6.00±0.005^{a}	9±0.005^{c}	11±0.005^{c}	49.6±0.005^{j}
L. monocytogenes	14±0.005^{a}	9.73±0.003^{d}	17±0.005^{d}	6.00±0.005^{a}	9.73±0.003^{d}	14±0.005^{a}	10.5±0.005^{b}	14±0.005^{a}	11±0.005^{c}	16±0.005^{d}	8.75±0.005^{b}	8.75±0.005^{b}	25.5±0.005^{k}
S. aureus	9.73±0.003^{d}	9.73±0.003^{d}	22±0.005^{j}	11±0.005^{c}	19.75*±0.005^{f}	11±0.005^{c}	10.5±0.005^{b}	17±0.005^{d}	13±0.005^{b}	8.75±0.005^{b}	6.00±0.005^{a}	6.00±0.005^{a}	25.5±0.005^{k}
E. coli	22±0.005^{j}	16.25±0.005^{c}	22±0.005^{j}	11±0.005^{c}	13.75±0.005^{d}	22±0.005^{j}	17.5±0.005^{c}	15±0.005^{d}	23.5±0.005^{j}	6.00±0.005^{a}	13.75±0.005^{d}	13.75±0.005^{d}	42±0.005^{l}
P. vulgaris	6.00±0.005^{a}	12±0.005^{b}	6.00±0.005^{a}	6.00±0.005^{a}	13±0.005^{b}	19.75*±0.005^{f}	11±0.005^{b}	12±0.005^{b}	6.00±0.005^{a}	6.00±0.005^{a}	13.75±0.005^{d}	12±0.005^{e}	43±0.005^{l}
P.aeruginosa	12±0.005^{b}	9.73±0.003^{d}	13±0.005^{b}	11±0.005^{c}	19.75*±0.005^{f}	11±0.005^{c}	8.00±0.005b	14±0.005^{a}	11±0.005^{c}	6.00±0.005^{a}	9±0.005^{c}	8.75±0.005^{b}	25.5±0.005^{k}
S. typhimurium	17±0.005^{d}	11±0.005^{b}	17±0.005^{d}	11±0.005^{c}	9.73±0.003^{d}	16.25±0.005^{c}	9.73±0.003^{d}	11±0.005^{c}	16±0.005^{d}	6.00±0.005^{a}	9±0.005^{c}	9.73±0.003^{d}	25.5±0.005^{k}
K.pneumoniae	6.00±0.005^{a}	9.73±0.003^{d}	13±0.005^{b}	11±0.005^{c}	16.25±0.005^{c}	13.75±0.005^{d}	9.73±0.003^{d}	18.02	13±0.005^{b}	6.00±0.005^{a}	6.00±0.005^{a}	13.75±0.005^{d}	17±0.005^{d}
C. albicans	13.75±0.005^{d}	9.73±0.003^{d}	15±0.005d	6.00±0.005^{a}	13.75±0.005^{d}	13.75±0.005^{d}	10±0.005^{a}	9.73±0.003^{d}	11±0.005^{c}	6.00±0.005^{a}	6.00±0.005^{a}	8.75±0.005^{b}	16±0.005^{e}

No activity (-) *Pseudomonas aeruginosa* ATCC®27853. *Proteus vulgaris* ATCC®7829. . *Escherichia coli* ATCC®25922. *Salmonella typhimurium* ATCC®14028. *Staphylococcus aureus* ATCC®25923. *Listeria monocytogenes* ATCC®7677. *Klebsiella pneumoniae* ATCC®13883. *Micrococcus luteus* B1018. *Bacillus subtilis* B209. . and *Candida albicans* ATCC®10231 Natural beeswax (D). Artificial wax (Y) Nystatine(N). Ampicillin (A)

CONCLUSIONS

Nowadays, the use of natural products in many areas such as cosmetic, food, textile, etc. are popular. Because natural products are biocompatible, non-toxic and had positive effects on human health. Beeswax is a kind of natural product that produced by honey bees. It is commercially available and has biological effects. Thus it could be used in in a wide area from cosmetic to food applications. In this study, natural and artificial beeswax samples were obtained from Türkiye and extracted with different solvents. Physiochemical and biochemical characterizations of both extracts and raw beeswax were performed. The obtained data showed us that both commercial and natural beeswax samples had good antioxidant and antimicrobial effects. In conclusion, it was clear that beeswax have the potential to be used in different fields such as apitherapy, cosmetic technology, medicine, and pharmacy and further studies are needed.

CONFLICT OF INTEREST

None declared

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