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COMPARISON OF ANTIBACTERIAL AND ANTIFUNGAL EFFECTS OF DIFFERENT VARIETIES OF HONEY AND PROPOLIS SAMPLES

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Honey is the most important bee product. There are many secondary metabolites, carbohydrates, enzymes, and vitamins in honey, thus, honey has antimicrobial activity. In this study, in vitro antimicrobial activity of forty-two honey and eight propolis ethanolic extracts (PEE) were investigated against 16 microorganisms. Total phenolic content ranged between 20.00–124.10 mg GAE/100 g and 103–232 mg GAE/g for honey and raw propolis samples, respectively. Pine and oak honeydew honeys had higher antimicrobial activity than four different grades of Manuka Honeys up to 18 mm minimum inhibition zone diameters. The ethanolic propolis extracts showed much higher antimicrobial activity than the honey samples. Fungi species were inhibited by the propolis samples. *Helicobacter pylorii* (*H. pylorii*) was the most sensitive, whereas *Streptococcus agalactiae* was the most resistant bacteria among the studied microorganisms. Brazilian and Zonguldak propolis had the closest antimicrobial activity to ampicillin, streptomycin, and fluconazole. It can be concluded that both honey and propolis could be used in preservative and complementary medicine.

Keywords: pine honey, oak honey, manuka, Brazilian propolis, synthetic antibiotic

Honey and propolis are important apitherapic agents, and they have many different biological activities, such as antimicrobial, antioxidant, anti-inflammatory, immune-modulator, anti-tumor, etc. (Ahuja & Ahuja, 2010; Can et al., 2015; Pobiega et al., 2019). Honey consists of carbohydrates (65–75%), moisture (15–20%), minerals, and various secondary metabolites (1–2%) (Can et al., 2015). The four main reasons explaining why honey is a good antimicrobial agent are: its pH, viscosity, hydrogen peroxide source from glucose oxidase, and secondary metabolites (Kolayli et al., 2016). Except secondary metabolites, the other three substances are common in all honey samples. The amount, variety, and kind of the secondary metabolite differ according to honey types (Ahuja & Ahuja, 2010). Raw propolis is composed mainly of resin (40–50%), wax (25–30%), essential compounds (5–10%), pollens (2–5%), and numerous other organic molecules (polyphenols, vitamins, and sugars) (Keskin & Kolayli, 2018). It was noted that propolis is one of the best pharmaceutical agents, and it contains many different bioactive compounds. The number of flavonoids and its phenyl esters were present in the extracts with antibacterial effects on pathological microorganisms. In this study, antimicrobial and antifungal effects of 42 different honey and eight propolis samples were compared.

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1. Materials and methods

1.1. Samples collections and test microorganisms

In this study, 42 different honey samples were investigated. Honey samples were mostly collected from Turkey in 2016–2017 harvest seasons, and some of them were obtained from different countries as shown in Table 1. Four different grades of Unique Manuka Factor (UMF) certificated Manuka honey samples (UMF-10+, UMF12+, UMF15+, and UMF20+) were purchased from The Real Honey Company, England. Propolis samples were collected from different regions of Turkey. Brazilian Red propolis (raw) was purchased from a Brazilian company, Natura Nectar. All test microorganisms were obtained from the Hıfzıssıhha Institute of Refik Saydam (Ankara, Turkey). Thirteen bacterial strains and 3 fungal strains (Ec*:* *Escherichia coli* ATCC 25922; Yp: *Yersinia pseudotuberculosis* ATCC 911; Kp: *Klebsiella pneumonia* subp. *pneumonia* ATCC18883; Pa: *Pseudomonas aeruginosa* ATCC 27853; Hp: *Helicobacter pyloriii J99;* Sa: *Staphylococcus aureus* ATCC 25923; Ef: *Enterococcus faecalis* ATCC 29212; Sm: *Streptococcus mutans* RSKK07038; Sag: *Streptococcus agalactiae* (clinic strain); Bc: *Bacillus cereus* 702 Roma; La: *Lactobacillus acidophilus* RSKK06029; Lc: *Lactobacillus casei* RSKK591; Ms: *Mycobacterium smegmatis* ATCC607; Ca: *Candida albicans* ATCC 60193; Ct: *Candida tropicalis* ATCC 13803; Sc: *Saccharomyces cerevisiae)* used in the current study were clinical isolates obtained from RTE University's Hospitals, Rize.

1.2. Honey classifications, propolis extraction, and determination of total phenolic content

The honey and propolis samples were obtained from different regions that have different botanical origin (Table 1). The honey samples were classified according to Santiago and co-workers (2018). The propolis extracts were prapered according to Keskin and Kolayli (2018). Total phenolic compounds of the samples were determined using the Folin-Ciocalteu spectrophotometric assay (Singleton et al., 1999).

1.3. Agar well diffusion method

Simple susceptibility screening method was used by employing the agar-well diffusion method (Woods et al., 2003).

1.4. Statistical analysis

The analyses were performed three times, the results were presented as mean values and standard deviations. Regression analysis of the data was performed in Microsoft Office Excel 2013 (Microsoft Corporation, Redmond, WA, USA).

Table 1. Specifications of studied honey and propolis samples

Sample name	Sample code	Sample types	Region	Dominant pollens	Properties
Manuka	H1	Manuka UMF +10	New Zealand	*L. scoparium*	Commercial
	H2	Manuka UMF +12	"	*L. scoparium*	Commercial
	H3	Manuka UMF +15	"	*L. scoparium*	Commercial
	H4	Manuka UMF +20	"	*L. scoparium*	Commercial
Unifloral honeys	H5	Sunflower	Kırklareli/	*Helianthus annuus*	Turkey
	H6	Sunflower	Tekirdag	*Helianthus annuus*	"
	H7	Chestnut	Ordu	*Castanea sativa*	"
	H8	Chestnut	Kure	*Castanea sativa*	"
	H9	Astragalus	Palandöken	*Astragalus microcephalus*	"
	H10	Astragalus	Erzurum	*Astragalus microcephalus*	"
	H11	Thyme	Çanakkale	*Thymus vulgaris*	"
	H12	R.caucasium	Rize	*Rhododendron*	"
	H13	R.ponticum	Trabzon	*Rhododendron*	"
	H14	Pumpkin	Izmir	Pumpkin	"
	H15	Cultivated Thyme	Denizli	*Thymus vulgaris*	"
	H16	Natural Thyme	Denizli	*Thymus vulgaris*	"
	H17	Calltrop	Bursa	*Eryngium campestre*	"
	H18	Thistle	Hatay	*Silybium marianum*	"
	H19	Coriander	Burdur	*Coriandrum sativum*	"
	H20	Harnup	Hatay	*Ceratonia siliqua*	"
	H21	Black Cumin	Adana	*Nigella sativa*	"
	H22	Nettleorurtica	Uskup	*Urtica dioica*	Macadonia
	H23	Heather	Mugla	*Calluna vulgaris*	Turkey
	H24	Heather	Mugla	*Calluna vulgaris*	"
	H25	Buckwheat	Konya	*Fagopyrum esculentum*	"
	H26	Buckwheat	Samsun	*Fagopyrum esculentum*	"
	H27	Gorse	Kırklareli	*Paliurus aculeatus*	"
	H28	Cedar	Hail	*Cedrus* ssp.	Saudi Arabia
	H29	AcaciaThomtree	Taif	*Acacia* ssp.	"
	H30	Talha	Thomtree	Talha tree	"
	H31	Ivy, Hedera	Kırklareli	*Hedera helix*	Turkey
Honey dew	H32	Honeydew	Rize	Forest honey	Turkey
	H33	Honeydew	Gümüşhane	Forest honey	"
	H34	Honeydew	Arsin	Forest honey	"
	H35	Oak	Kırklareli	Oak spp.	"
	H36	Oak	Samsun	Oak spp.	"
	H37	Pine	Muğla	*Pinus* L.	"
	H38	Pine	Izmir	*Pinus* L.	"
Multi-floral	H39	Blossom	Anzer	Plateau honey	"
	H40	Blossom	Gümüşhane	Plateau honey	"
	H41	Blossom	Hakkari	Plateau honey	"
	H42	Blossom	Hakkari	Plateau honey	"
Raw Propolis	P1	Red Brazilian	Brezillia		Brazilia
	P2	Kars	Turkey		Turkey
	P3	Yığılca	Turkey		"
	P4	Zonguldak	Turkey		"
	P5	Ankara	Turkey		"
	P6	Erzurum	Turkey		"
	P7	Konya	Turkey		"
	P8	Artvin	Turkey		"

2. Results and discussion

Total phenolic content of honey and propolis samples depends on geographical origin (Keskin et al., 2020). In a study, it is reported that total amount of phenolic content of Anatolian raw propolis varies between 16.13–178.34 mg GA/g (Keskin & Kolayli, 2018) and total amount of phenolic content of honey samples ranged between 33 mg GA/100 g and 81 mg GA/100 g (Keskin et al., 2020). It is clear from the obtained results that the unifloral and honeydew honey samples had higher phenolic compounds than multifloral honeys (Table 2). Although the honey samples showed different inhibition effects against the 16 microorganisms, the honey samples mostly affected *E. coli*, *Y. pseudotuberculosis*, *K. pneumonia*, *S. aureus*, and *M. smegmatis* (Table 3). *P. aeruginosa*, *S. mutans*, *L. casei*, and yeast like fungus of *C. albicans*, *C. tropicalis*, and *S. cerevisiae* were not affected by any of the honey samples. At the beginning of the study, Manuka honeys were used as positive controls, because numerous investigations in the literature show that these honeys have high antimicrobial activities. Surprisingly, only 4 microorganisms, *Y. pseudotuberculosis*, *K. pneumonia*, *S. aureus*, and *M. smegmatis*, were inhibited by the Manuka honey samples. Although Manuka UMF +10 and +12 samples had moderate antimicrobial effects on *H. pylorii* (8 and 10 mm, respectively), heather honey from Muğla region had better activity against these bacteria (12–15 mm). Moreover, there were no substantial antimicrobial differences among the four Manuka honeys. Among the honey samples, H11-15, H17-19, H21-23, H25-26, H31-32, and H34-36 showed the highest inhibitions against *S. aureus* (Table 3). Although honey samples generally showed inhibition effects against *M. smegmatis*, cedar, acacia, and Talha (H28, H29, and H30) honey samples obtained from Saudi Arabia were the most effective honey samples against this microorganism. The three unifloral honeys of cedar (H28), acacia (H29), and Talha (H41) were found to be very effective especially against *Lactobacillus acidophilus*, *L. casei*, and *M. smegmatis*. Some bacteria (*L. acidophilus*, *L. casei*, and *S. aureus*) are related to dental health and tooth decay (Yadav & Prakash, 2017), and the inhibition of these bacteria by honeys is an important finding. In general, there were no major differences found between the honey samples against the four bacteria (*Y. pseudotuberculosis*, *K. pneumonia*, *S. aureus*, and *M. smegmatis*). Different authenticities of the honeys have also showed dissimilar inhibitions among the 16 microorganisms (Table 3).

For example, only Arabian honeys (H28, H29, and H30) and multifloral honey from Hakkari (H41) showed moderate inhibition against *S. mutans*. In addition, only two buckwheat honeys showed moderate inhibition against *C. albicans* and *C. tropicalis.* At the same time, only the buckwheat honeys and the oak honeys showed moderate inhibition against *S. cerevisiae*. Nearly half of the honey samples showed a weak inhibition against *L. acidophilus*, while the S. Arabic region honeys showed high inhibition effects. Saudi Arabian honeys had the highest phenolic contents (Table 2), and oak, chestnut, heather, buckwheat, and Manuka honeys had higher total phenolic contents than multifloral and blossom honeys. It was reported earlier that oak, chestnut, and heather honeys were dark colored honeys and contained higher phenolic compounds (Can et al., 2015). Cedar, black cumin (*Nigella sativa*), and Manuka honeys showed a good bactericidal-bacteriostatic inhibition effect against only *Staphylococcus aureus* (Almasaudi et al., 2017), and our results supported these findings. Antimicrobial activity of honey samples could be due to the quantity and synergistic effect of key phenolics (Kalogeropoulos et al., 2009). The antimicrobial activities of the propolis extracts are given in Table 3. All propolis samples showed inhibition against the studied microorganisms to diferent extent, but the widest inhibition zone was found againts *H. pylori*,

Table 2. Total phenolic content of honey and propolis samples

Sample	Total phenolic content mg GAE/100 g	Sample	Total phenolic content mg GAE/100 g	Sample	Total phenolic content mg GAE/100 g	Sample	Total phenolic content mg GAE/100 g	Sample	Total phenolic content mg GAE/g
H1	58.11±0.31	H12	35.33±1.55	H23	64.41±2.30	H34	61.60±1.33	P1	232.10± 5.20
H2	56.43±0.46	H13	42.80±2.30	H24	68.05±2.08	H35	74.20±2.10	P2	146.30± 1.20
H3	45.78±0.49	H14	28.60±0.87	H25	52.40±1.04	H36	63.06±1.66	P3	174.50±3.56
H4	49.27±0.31	H15	52.22±2.30	H26	46.32±3.02	H37	48.44±1.40	P4	162.22±2.55
H5	28.20±2.20	H16	60.03±3.70	H27	37.04±1.04	H38	42.20±0.80	P5	106.56±1.15
H6	31.05±1.30	H17	35.63±1.44	H28	105.10±4.20	H39	35.38±0.58	P6	110.45±2.14
H7	58.02±3.20	H18	25.88±0.62	H29	98.20±2.10	H40	28.52±0.60	P7	103.30±0.41
H8	65.20±2.05	H19	47.40±2.01	H30	124.05±2.30	H41	24.20±0.41	P8	132.74 ±0.36
H9	35.40±1.08	H20	20.02±0.35	H31	35.20±1.22	H42	26.39±1.04		
H10	37.10±0.98	H21	56.05±0.74	H32	64.07±3.02				
H11	57.50±2.33	H22	24.32±0.43	H33	53.36±2.00				

which is a fastidious, Gram negative bacterium that grows poorly in broth culture. Our findings showed that propolis extracts have much better inhibition effects than honey samples, which clearly shows that propolis is a much better antimicrobial agent than honey. All samples had the highest antimicrobial activity against *H. pylori*, with Yıgılca (P3) propolis showing the best results. In a previous study, gastric system bacteria were found sensitive to many different Anatolia propolis samples, the inhibition zone diameters ranged from 18 to 22 mm (Velikova et al., 2000). Moreover, in the same study, the anti-urease activity of Anatolia propolis was studied, and the ethanolic extracts showed a good inhibition of the extracellular urease of the bacteria. It was reported that these bee products, either honey or propolis, killed bacteria by inhibition of their urease enzyme (Baltas et al., 2016). It was notably seen that all studied propolis samples showed good antimicrobial activity against Gram negative bacteria. In the previous studies, poplar type propolis samples were found ineffective and Bulgarian type was effective against *E.coli* (Velikova et al., 2000). The good activity found in this study can be due to similar constituents found in Bulgarian and Turkish propolis (Velikova et al., 2000). In this study, the highest total phenolic content in propolis was found in the Brazilian sample, showing a good inhibition against all bacteria and fungi to different extent. Some bacteria are even affected by low doses of propolis, while others need high doses. These findings are also confirmed by other studies (Neto et al., 2017). The propolis samples were also found very effective against oral pathogens such as *Streptococcus mutans*, *Enterococcus faecalis*, and *C. albicans*. Propolis samples have higher antimicrobial activity than honey samples, and the antimicrobial activity of propolis samples depend on their total phenolic content. Therefore, according to typification approach in the standardisation process, similar plant sources should be investigated for Brazilian and Turkish propolis to determine key chemicals providing the antimicrobial effect.

Table 3. Antimicrobial activities of honey samples against a range of microorganisms

Samples	Code	Tested microorganisms and minimum inhibition zone diameters (mm)															
		Gram negative bacteria					Gram positive bacteria							Other	Yeast like fungi		
		Ec	Yp	Kp	Pa	Hp	Sa	Ef	Sm	Sag	Bc	La	Lc	Ms	Ca	Ct	Sc
Manuka	H1	–	8	6	–	8	8	–	–	–	–	–	–	6	–	–	–
	H2	–	6	6	–	10	8	–	–	–	–	–	–	6	–	–	–
	H3	–	6	6	–	–	8	–	–	–	–	–	–	6	–	–	–
	H4	–	8	8	–	–	8	–	–	–	–	–	–	8	–	–	–
Unifloral honeys	H5	–	8	8	–	–	8	–	–	–	6	6	–	8	–	–	–
	H6	–	6	6	–	–	10	–	–	–	6	6	–	8	–	–	–
	H7	8	8	6	–	–	10	–	–	–	–	6	–	10	–	–	–
	H8	8	10	8	–	–	10	–	–	–	–	–	–	10	–	–	–
	H9	6	10	8	–	–	10	6	–	–	–	–	–	6	–	–	–
	H10	6	10	8	–	–	10	6	–	–	–	–	–	6	–	–	–
	H11	–	6	8	–	–	14	–	–	–	–	6	–	6	–	–	–
	H12	6	6	8	–	–	15	–	–	–	–	6	–	–	–	–	–
	H13	8	6	8	–	–	16	6	–	–	–	6	–	–	–	–	–
	H14	–	6	–	–	–	15	–	–	–	–	–	–	6	–	–	–
	H15	–	6	8	–	10	14	–	–	–	10	8	–	6	–	–	–

Table 3. cont.

Samples	Code	Tested microorganisms and minimum inhibition zone diameters (mm)															
		Gram negative bacteria					Gram positive bacteria							Other	Yeast like fungi		
		Ec	Yp	Kp	Pa	Hp	Sa	Ef	Sm	Sag	Bc	La	Lc	Ms	Ca	Ct	Sc
Unifloral honeys	H16	–	6	8	–	8	12	–	–	–	8	6	–	8	–	–	–
	H17	–	6	8	–	–	16	12	–	–	12	12	–	6	–	–	–
	H18	–	6	10	–	–	16	–	–	–	–	6	–	6	–	–	–
	H19	6	6	8	–	–	15	–	–	–	6	8	–	8	–	–	–
	H20	–	6	–	–	–	6	–	–	–	–	–	–	6	–	–	–
	H21	–	8	10	–	–	18	–	–	–	6	6	–	10	–	–	–
	H22	–	6	6	–	–	16	–	–	–	–	–	–	6	–	–	–
	H23	8	6	6	–	15	18	–	–	–	–	–	–	6	–	–	–
	H24	–	10	–		12	10	–	–	–	–	–	–	11	–	–	–
	H25	8	8	10	–	8	20	–	–	–	–	–	–	10	12	10	12
	H26	10	10	10	–	10	16	–			–	–	–	10	10	10	10
	H27	–	6	6	–	–	12	–	–	–	–	8	–	6	–	–	–
	H28	6	10	–	–	8	10	–	8	–	–	15	15	15	–	–	–
	H29	8	12	6	10	10	10	–	8	–	–	20	30	30	–	–	–
	H30	8	10	10	10	10	11	–	8	–	–	20	30	15	–	–	–
	H31	8	12	8	–	–	14	–		–	–	14	10	14	–	–	–
Honeydew	H32	10	10	10	–	–	16	6	–	–	–	10	–	12	–	–	–
	H33	8	8	8	–	8	10	–	–	–	–	10	–	10	–	–	–
	H34	–	6	7	–	10	16	6	–	–	6	10	–	10	–	–	–
	H35	12	16	8	8	10	15	6	–	–	8	10	–	8	–	–	10
	H36	10	14	6	8	8	18	–	–	–	8	–	–	8	–	–	8
	H37	–	12	6	8	–	12	–	–	–	–	–	–	8	–	–	–
	H38	–	10	6	8	–	10	–	–	–	–	–	–	8	–	–	–
Mulifloral	H39	8	8	6	8	–	10	–	–	–	–	–	–	8	–	–	–
	H40	–	–	10	6	10	10	10	–	–	–	–	–	6	–	–	–
	H41	–	6	6	6	8	10	15	8	–	–	8	6	6	6	–	–
	H42	–	–	–	6	8	10	–	–	–	–	–	–	6	–	–	–

Ec*:* *Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Kp: *Klebsiella pneumonia* subsp. *pneumonia* ATCC18883, Pa: *Pseudomonas aeruginosa* ATCC 27853, Hp: *Helicobacter pylorii J99,* Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Sm: *Streptococcus mutans* RSKK07038, Sag: *Streptococcus agalactiae* (clinical strain), Bc: *Bacillus cereus* 702 Roma, La: *Lactobacillus acidophilus* RSKK06029, Lc: *Lactobacillus casei* RSKK591, Ms: *Mycobacterium smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, Ct: *C. tropicalis* ATCC 13803, Sc: *Saccharomyces cerevisiae* RSKK 251, (—): No activity. 6–9 mm; low activity, 9–11 mm; moderate activity, ≥12; good activity

Table 4. Antimicrobial activities of the ethanolic propolis samples against a range of microorganisms

Propolis samples	Tested microorganisms and inhibition zone diameters (mm)															
	Gram negative bacteria					Gram positive bacteria							Other	Yeast like fungi		
	Ec	Yp	Kp	Pa	Hp	Sa	Ef	Sm	Sag	Bc	La	Lc	Ms	Ca	Ct	Sc
P1	8	15	11	12	45	22	20	12	12	18	24	12	20	16	14	20
P2	8	10	8	24	40	18	8	6	6	12	14	6	15	14	6	8
P3	–	10	8	12	50	20	12	10	10	14	25	12	18	12	12	20
P4	12	10	10	18	45	20	15	12	12	12	22	14	25	14	12	–
P5	8	6	14	8	40	10	15	6	6	15	15	6	18	6	6	10
P6	10	10	12	10	45	14	10	6	6	14	18	8	17	15	8	15
P7	12	10	6	10	40	16	15	10	10	14	18	10	15	15	12	14
P8	8	8	6	18	40	16	10	10	8	8	18	10	12	10	8	–
Amp.	10	10	10	18	NT	35	10	NT	NT	15	NT	NT				
Strep.													35			
Flu.														25	25	25

Ec*:* *Escherichia coli* ATCC 25922; Yp: *Yersinia pseudotuberculosis* ATCC 911; Kp: *Klebsiella pneumonia* subsp. *pneumonia* ATCC18883; Pa: *Pseudomonas aeruginosa* ATCC 27853; Hp: *Helicobacter pyloriii J99;* Sa: *Staphylococcus aureus* ATCC 25923; Ef: *Enterococcus faecalis* ATCC 29212; Sm: *Streptococcus mutans* RSKK07038; Sag: *Streptococcus agalactiae* (clinical strain); Bc: *Bacillus cereus* 702 Roma; La: *Lactobacillus acidophilus* RSKK06029; Lc: *Lactobacillus casei* RSKK591; Ms: *Mycobacterium smegmatis* ATCC607; Ca: *Candida albicans* ATCC 60193; Ct: *C. tropicalis* ATCC 13803; Sc: *Saccharomyces cerevisiae* RSKK 251; (—): No activity. 6–9 mm; low activity; 9–11 mm; moderate activity; ≥12; good activity

3. Conclusions

Honey and propolis are substantial antibacterial and antifungal agents, and their antimicrobial effects could result from their floral sources, but antimicrobial activities were found not to be dependent on their total phenolic contents. For this reason, further studies are needed to evaluate those mechanisms. Better antimicrobial effects of propolis implied that wherever they live, bees are created to sense, find, and collect the best chemicals in any environment to protect their hives against microorganisms. Therefore, this natural product could be used in preservative and complementary medicine.

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