ANTIBACTERIAL EFFECTS OF UNIFLORAL HONEYS AND PROPOLIS IN THE CONTEXT OF THEIR ANTIOXIDANT CAPACITY AND COLOUR

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Abstract

There is a huge potential for honey's therapeutic use, so it becomes increasingly important to examine its antibacterial effects and the factors influencing it. During the work the total polyphenol content and antioxidant capacity of 10 honeys and a propolis sample were examined with respect to their antibacterial effects. Based on this, there is a statistically significant correlation between the total polyphenol content and the antioxidant capacity, and between these properties and antibacterial activity. For the purpose of exploring the connection between the colour of honey and its properties, the colour of 6 honeys and the propolis were examined. Based on the results, dark-coloured honeydew honeys and light-coloured nectar honeys are sharply separated according to their antibacterial and antioxidant capacity.

Introduction

The healing effects of honey have been discovered by ancient cultures. Nowadays the medical community is once again discovering its healing potential, finding that its antibacterial and antioxidant effects can serve human healing in a number of ways [1].

This sweet product can be divided into two main types by its origin: nectar and honeydew. In the latter case, the honey is not harvested from flowers, but from the secretions of evergreens or the excretions of insects that feed on them. The two types show differences in colour, aroma, specific composition and characteristics [2].

Manuka honey comes to the front when it comes to therapeutic use – beside nectar honey. Experiments show that this type has a variety of phytochemical ingredients from several plant sources, and several flavonoids and terpenoids that make it different. In contrast to other honeys which contain hydrogen-peroxide, the activity of Manuka honey was due to the ligth and heat resistant methylglyoxal [3], [4].

Propolis is another important bee product which is a building material and a protective substance for the honeybee colony. Propolis, because of its considerable biological activity, has been used as a remedy in traditional medicine in treating burns, wounds, sore throats, and stomach ulcers for quite some time. Not unlike honey, propolis also has a potential to be used in the field of medicine and the food industry [5].

Numerous degenerative or chronic diseases such as diabetes mellitus, Alzheimer's disease, and heart disease are caused by oxidative stress. Experimental results show that honey due to its bioactive compounds has favourable effects against oxidative damage and degenerative diseases. The phytochemicals such as flavonoids, aromatic acids, phenolic antioxidants contribute the antibacterial properties of honey [5].

Experimental

Materials: 9 honeys were used in this study. Three of them (acacia, buckwheat, forest) were purchased from Golden Nectar Co. Ltd, the three Manuka honeys (Manuka UMF 15+, Manuka UMF 20+, Manuka UMF 22+) were imported from New Zealand. Three of

them (phacelia, pine, rape) were purchased from a local market, these originated from local beekeepers. Another bee product, propolis, (a Propolis Plus EPID Gocce Alcoholfree Propolis Drop) was analysed too. *Escherichia coli; Pseudomonas aeruginosa; Enterococcus faecalis; Listeria monocytogenes; Listeria innocua; Staphylococcus aureus* were used for the microbial analysis.

Preparation of samples for the analytical methods: Honey solutions in 250 mg/ml concentration were used for the analytical methods stored at -32 °C until analysis.

Total phenolic content (TPC): Total phenolic content was determined by the method put forward by Singleton and Rossi (1965) using a spectrophotometer at 765 nm wave length with the chemical of Folin-Ciocalteu. Gallic acid was used as the standard solution, so the results were specified in mg gallic acid equivalent/ 100 g dm. All measurements were performed three times in parallel.

Antioxidant capacity: The antioxidant capacity was determined by FRAP (*Ferric Reducing Ability of Plasma*) method. The reducing ability of ferric tripyridyltriazine complex was used for assessing total antioxidant capacity, which was measured with a spectrophotometer at a 593 wave length. Ferric reducing antioxidant power assay (FRAP) was defined in ascorbic acid equivalent (μg ascorbic acid equivalent/ 100 g dm).

Agar well diffusion method for inhibitory effect measurement: The hundredfold dilution of the 0.5 McFarland density test microorganism suspension was spread onto the TGE agar plate surface. Then, a 7 mm hole was punched aseptically with a sterile cork borer, and 4 drops of preheated sample was pipetted into the well. Plates were incubated for 24 hours at 25 °C. As time progressed the antimicrobial agent diffused in the agar medium and inhibited the growth of the sensitive microbial strain. The inhibition zones were measured by a ruler. The measurements were only repeated in cases were complications arose with the growth of the microorganisms.

Colour parameters: Colour parameters (L*, a*, b*) were established in the CIE system using a Konica Minolta CR-300 Chroma-meter. The L* value represents the brightness, the a* is a red-green coordinate, and the b* is the yellow-blue coordinate. The instrument was calibrated with a white reference tile.

Statistical analysis: Microsoft Excel 2016 was used to calculate averages, deviations and to create the tables and the diagram. The main statistical analysis was performed using IBM Statistics SPSS 22 software. The normal distribution of the measurement data were analysed by Shapiro-Wilk test, and after that the nonparametric Spearman's rank correlation coefficients (r_s) or the Pearson correlation coefficients (r) were calculated in order to determine the correlation between the particular parameters. Significance was set at $p<0.05$.

Results and discussion

High variation was observed for the antioxidant activity among the samples (Table 1.). FRAP was expressed in μg AAE/100 g dm, ranged from 21.454 to 205.451. The FRAP value of the phacelia was the lowest, and the Manuka UMF 15+ was the highest. This value in the case of propolis was extremely high (1002.468 μg AAE/100 g dm) which is almost five-times higher than the highest value of the other honeys. The average deviation in FRAP values was low, 1.91 μg AAE/100 g dm. In the case of total phenolic content, the same pattern was observed. Polyphenols as antioxidant agents can explain the statistically significant correlation between (r_s =0,806) the antioxidant capacity and the total phenolic content. In the case of Manuka honeys and propolis the total phenolic content and the antioxidant capacity were both extremely high. Beside these samples just the buckwheat, forest, and pine honeys have similar results. The results demonstrate that the darkest honeys contain the most phenolic compounds which in turn influence the antioxidant property of honeys. The average deviation in total phenolic content was 11.16 mg GAE/ 100 g dm.

1. Table: Total phenolic content and antioxidant capacity of honey samples and propolis

Honeys	Antioxidant capacity (µg AAE/100g dm)	Total phenolic content (mg GAE/100g dm)
Acacia	41.92	125.72
Buckwheat	151.92	662.78
Forest	179.83	432.72
Manuka UMF 15+	205.45	712.88
Manuka UMF 20+	123.18	551.54
Manuka UMF 22+	169.69	830.52
Phacelia	21.45	179.92
Pine	162.48	238.97
Rape	36.78	185.71
Propolis	1002.47	2984.29

The inhibition zone radii [(the diameter of the zone of inhibition - the diameter of the hole) /2)] around the pure honeys and the propolis sample varied in a wide range of samples (0.0-13.5 mm) (Table 2.). Propolis (6.75-12.25 mm), forest honey (4-12.25 mm), Manuka UMF 20+ (6.5-13 mm) had the highest antibacterial activity. Acacia (0.0-2.5 mm) honey showed to be the least effective against bacteria. The effectiveness of the samples was different for each of the strains. The largest zone was observed against *S. aureus* (2-13 mm) while *E. coli* (0.5-7 mm) turned out to be the most resistant. Different honey properties affected the microorganisms in different ways, but there was a significant correlation between inhibition radii and total phenolic content, and also between the antibacterial effect and antioxidant capacity. This suggests that the components contributing the antioxidant effect of honeys and propolis also have a role in the inhibition of microorganisms.

The antibacterial activity of Manuka honeys is considered to be due to the presence of methylglyoxal. Unique Manuka Factor (UMF) describes the amount of this non-peroxide component and it refers to the measure of antibacterial effect. Manuka honeys with 15+, 20+ and 22+ UMF were tested, but their efficiency were not in direct correlation with their UMF factors. The Manuka UMF 20+ honey had the highest antibacterial activity, but the zones of inhibition of Manuka UMF 22+ were similar to the zones of Manuka UMF 15+ (Table 2.). These samples were effective, but their activities were not remarkable. Only Manuka UMF 20+ honey had a significant effectivity as propolis, against *E. coli*. Antibacterial activity of most honeys is due to the presence of hydrogen peroxide, and while the Manuka honeys do not contain this component they still showed remarkable inhibitory effects. This raises the question that the presence of hydrogen peroxide might not play as an important role as we had thought previously, but this hypothesis needs further studies to prove.

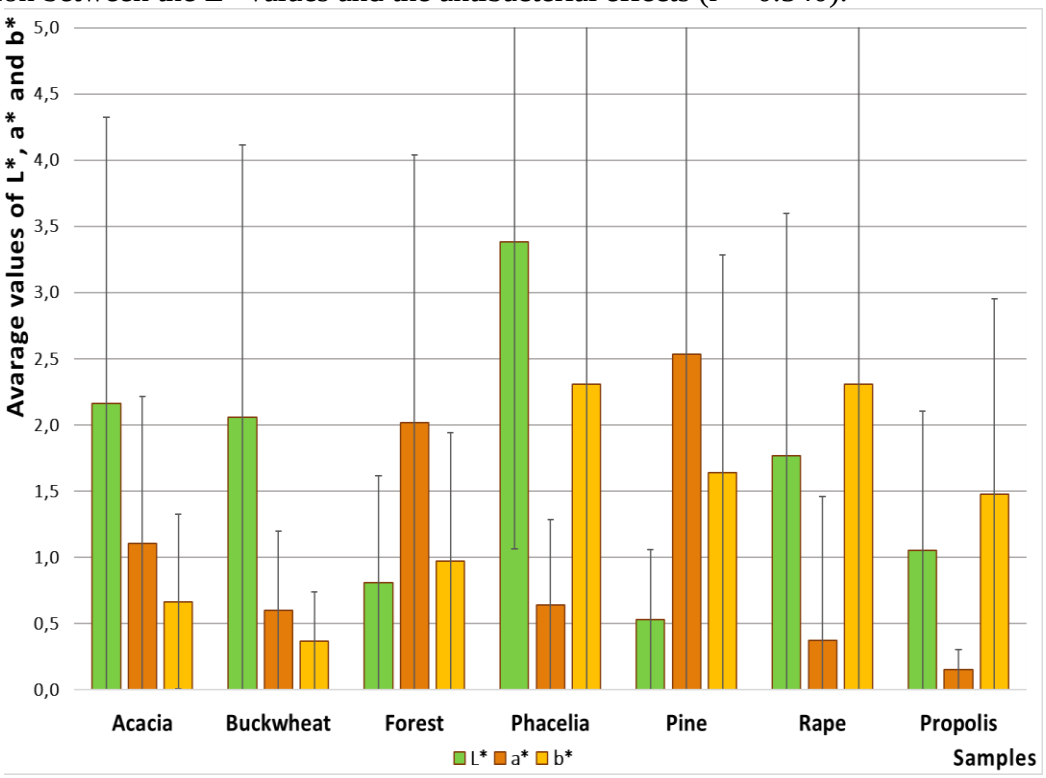
Propolis had a significant antibacterial effect (Table 2) against all strains, but its efficacy was not as pronounced compared to the honey samples, as its total polyphenol content and antioxidant capacity would have suggested. A possible explanation for this phenomenon is that the antibacterial property of propolis is only provoked by its polyphenol content and antioxidant capacity, while in honey there might be another factors that were not analysed in this study.

2. Table: Inhibition zone radii (mm)

Honeys	*E. coli*	*E. faecalis*	*P. aeruginosa*	*S. aureus*	*L. mono-cytogenes*	*L. innocua*
Acacia	0.5	0	2.25	2	2.5	1.5
Buckwheat	4	7.5	8	8.5	10	9.25
Forest	4	8.5	8.5	12.25	8.5	11.5
Manuka UMF 15+	5.5	5	6.5	9	6	7.5
Manuka UMF 20+	7	6.5	8.5	13	7.5	9.5
Manuka UMF 22+	6.5	5.5	7	12	6.5	7.5
Phacelia	2.25	4.5	5.25	5.5	5.5	7
Pine	3	6.5	7.5	7.25	7	9
Rape	2.5	4.75	6.75	6	6.25	6.5
Propolis	6.75	9.75	12.25	10.5	11.5	12

Generally, it can be said that darker coloured, honeydew honeys contain more phenolic compounds with antioxidant properties, and they are more effective against most microorganisms.

The colours of the honey samples (except Manuka honeys), and the propolis sample were analysed to find correlation between colour and other properties. The L* value describes the brightness, and it shows that the honeydew honeys are much darker than the nectar honeys. According to the values of a* and b* there are red compounds in the samples, but yellow pigments are dominant. After propolis and the Manuka honeys, the commercially available darker coloured buckwheat (662.781 mg GAE/100g dm) and forest honey (432.717 mg GAE/100g dm) had the highest polyphenolic content; still no statistically significant correlation could be determined between colour and polyphenol amount. Furthermore, the dark coloured pine honey showed similar results as lighter honeys. There is significant correlation between the L* values and the antibacterial effects (r= -0.540).



1. Figure: The L*, a*, b* values of samples

Conclusion

The propolis sample and the Manuka honeys had a remarkable antioxidant capacity and high total phenolic content. While there is a significant correlation between these properties and antibacterial efficacy, these samples still failed to produce the effectiveness that the results would have suggested. Comparison of nectar and honeydew honeys have shown, that the dark coloured honeydew samples have greater antioxidant capacity and antibacterial effect. Significant correlation was determined between the L* value and the antimicrobial effect.

Acknowledgements

This study would not have been possible without the generous work of the employees from the Department of Microbiology and Biotechnology, the Department of Applied Chemistry, and the Department of Food Preservation in Szent István University.

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