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ORIGINAL RESEARCH

Determination and Comparison of Phenolic Compound Content and Antimicrobial Activity of Some Propolis Samples in Türkiye

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Abstract
Objective: Ethanol extraction is the most popular technique for the production of propolis extracts. However, this method may not be suitable for various clinical conditions. Based on it, we composed a trial product with an olive-oil extraction as an alternative method. Furthermore, we crafted combinations to reinforce and synergize the antimicrobial activity of the trial propolis product. Finally, we compared our trial products with the existing marketing products in Türkiye. The present study aimed to determine chemical compounds and the antimicrobial activity of some propolis samples selected from Türkiye and compare the mentioned features with the olive-oil- trial products we composed for the study.
Material-Method: Four different samples, as trial and final products, were crafted for the study. Trail products conducted as sample 1 to 4 (S1, S2, S3 and S4). The trial products were compared with the four other propolis and propolis-containing combined products currently exciting on the market. Four different trademarks were used, and the Trademarks (TM) was called TM1, TM2 TM3, and TM4. Determination of Total Phenolic Compound (TPC) was analyzed according to the Folin-Ciocalteau method. The antimicrobial activity test was determined according to the Kirby-Bauer method.
Results: The highest TPC ratio was detected in the trademark 2 (TM2), and the lowest TPC ratio was determined in the TM4 samples, 19553.12 GAE mg/L and 740.9 GAE mg/L, respectively. The TPC ratio of the final trial product sample 4 (S4) was defined as 6519.3 GAE mg/L. The highest inhibitation zone against *E. coli, K. pneumoniae, S. aureus* strains was observed in S1 (the oleuropein-containing trial product). The highest inhabitation zone against *C. albicans and C. krusei* yeasts was observed in TM1 and S4 (the oleuropein-and boron-containing trial propolis product) samples.
Conclusion: The S4 product, containing boron, oleuropein, and propolis, had a higher inhibitation zone diameters compared to the commercial brands. Furthermore, all the propolis products analyzed in this study had rich phenolic components; the curative and beneficial impacts of phenolic components on health merit further investigations.
Keywords: Propolis, Oleuropein, Boron, Antimicrobial Activity, Phenolic Compounds

INTRODUCTION

Natural living and well-being are a brand trend throughout the world. The steadily rising antibiotic resistance and the increasing healthcare costs may substantially impact.[1]Utilization of a supplementary prepared to treat infectious diseases is widespread as complementary or alternative medicine; propolis is one of the agents commonly preferred beside or alternative to drugs.

Propolis is a natural resinous mixture that has been empirically used for centuries, expecting an immunomodulatory effect.[2]

Propolis is a honeybee (Apis mellifera) product with a broad spectrum of benefits, such as antibacterial, antiviral, antifungal, antioxidant, immunomodulatory, and anti-inflammatory effects.[3] The most-reported biological activity of propolis extracts is its antimicrobial effect; it is widely used to prevent or treat various diseases.[4] The wide use of propolis for different purposes makes it a subject of academic interest.

Propolis is a substance that is difficult to standardize. The bioactive compounds and the chemical structure of propolis vary depending on the endemic vegetation of the region it has been obtained from.[5-7]

Subsequently, the pharmacological effect of propolis differs.[8] The phenolic contents of propolis extracts or a propolis-natural product combination product in the market are always different. The biological activity of extracted propolis is a result of phenolic components. The phenolic compounds play an essential role in human well-being;

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apparently, they have antimicrobial, anti-allergic, anti-inflammatory, and antioxidative effects.[9] The TPC of propolis also differs from the extraction technique and the solvent used.[10]

Because ethanol is the best-known solvent for propolis, ethanol extraction is the most common and effective method to extract propolis.[11] However, it has some disadvantages; it may be not favored in a particular group of the population, it has a strong residual adore, it may be not suitable for the treatment of some ophthalmological cases, pediatric patients, and patients with alcohol intolerance.[12-13] Therefore, in our study, we experimented with olive oil extraction as an alternative method to extract propolis. In addition, numerous studies have reported olive oil polyphenols' antioxidant and antimicrobial activity.[14]

We crafted combined trial propolis samples for the study; with some additional ingredients, we aimed to reinforce and synergize the antimicrobial activity of propolis. We compared the trial samples with market trademark analogs. This study aimed to determine and compare the chemical structure and bioactivity of the olive oil-extracted propolis samples with the existing trademark samples.

MATERIALS AND METHODS

Reagents and chemicals

Refined olive oil was used for the extraction. Other materials used in the product trials (oleic acid, polyethylene glycol 400 (PEG 400), Oleuropein, boron, methanol, Folin-Ciocalteau reagent, Na_2CO_3, gallic acid) were delivered from the Sigma-Aldrich® company.

Propolis extraction

Raw propolis was obtained from a local producer. Different attempts were made for the appropriate combination. To determine the proper dose and the raw material to be used, separate samples were prepared and evaluated in terms of efficacy and phenolic content. The samples prepared are given in Table 1. For propolis extraction, 100 g of raw propolis was frozen at -20 ○C for 24 hours, then passed through a blender and ground into powder.

The final extract was obtained by maceration with olive oil at 25 ○C for 24 hours.

Commercial propolis products

Four different commercial products were selected to compare the effectiveness and TPC of Olive oil-extracted propolis with commercial products in the market. The features of the trademark products are given in Table 2.

Table 1. Trial samples created for the propolis product

Sample	Contents
S1	Oleuropein, Peg 400, Water
S2	Olive oil-extracted propolis
S3	Olive oil-extracted propolis, Oleic acid
S4	Olive oil-extracted propolis, Oleuropein, Boron

Table 2. Commercial propolis products were used in the comparison.

Trademark	Contents
TM1	Water-based propolis extract
TM2	Water-based propolis extract
TM3	Zinc, Vitamin C, Herbal supplement, Water-based propolis extract
TM4	Zinc, Vitamin C, Ethanol based propolis extract

Total phenolic compound determination

The determination of TPC was analyzed according to the Folin-Ciocalteau method. All samples were studied in triplicate. The samples to be tested were diluted with methanol (1:4). 800 µl of 0.5 N Folin-Ciocalteau reagent was mixed with 40 µl of test samples and allowed to react for 5 minutes in the dark at room temperature. Afterward, 800 µl of Na2CO3 (10%) was added, and the volume of the mixture was increased to 3.0 ml with distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured at 760 nm using a spectrophotometer. Gallic acid solution was used as a standard to construct the calibration curve. In the tested samples, TPC was expressed as mg/L gallic acid equivalent (GAE).

Antimicrobial activity

Antimicrobial activity was determined by the disc diffusion (Kirby-Bauer) method (15,16). The microorganism test medium prepared at McFarland 0.5 turbidity was inoculated on Mueller Hinton Agar (MHA, Merck). Test specimens were impregnated with a blank disc (Bioanalyase, blank disc, 6mm). The discs were placed on the agar plate and incubated for 24 hours at 37°C. Gentamicin (Bioanalyse, CN 10µg disc), streptomycin (Bioanalyse, S 10µg disc), and nystatin (Bioanalyase, NY 100U disc) were used as positive controls. Staphylococcus aureus ATCC 25923, Klebsiella pneumoniae ATCC 13883, Escherichia coli ATCC 25922, Candida albicans (Clinical isolate), C. krusei (Clinical isolate) strains were used for the analysis. All experiment performed in triplicate and data are given as mean (± SD).

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RESULTS

Total phenolic compound content

The highest TPC rate was determined in the TM2 sample; 19553.12 (GAE mg/L). The TPC rates obtained from the trial samples were as follow: S1 9446.98 (GAE mg/L), S2 10088.27 (GAE mg/L), S3 5373.03 (GAE mg/L), S4 6519.30 (GAE mg/L). Fig.1 demonstrates the TPC ratio of the study samples.

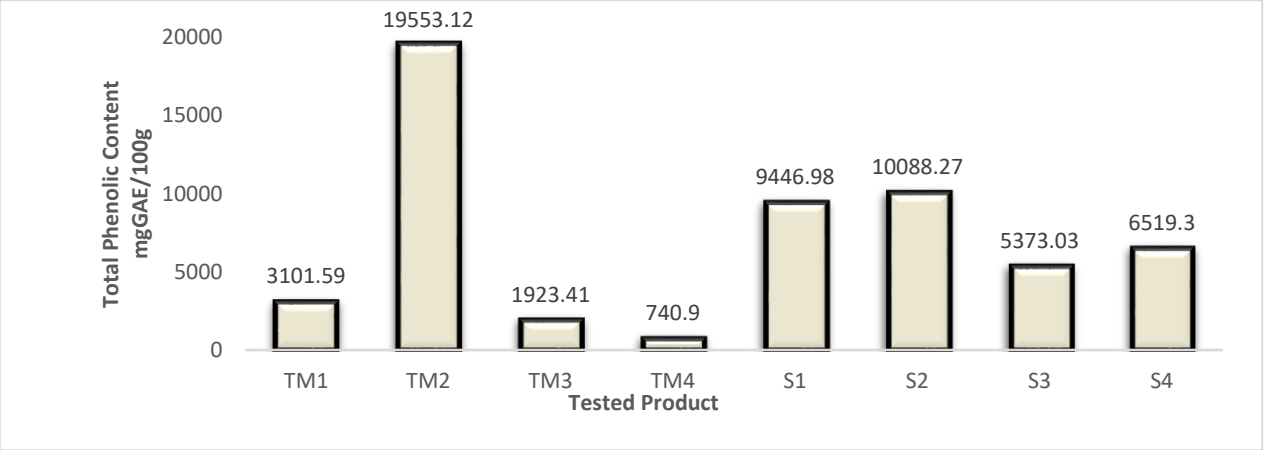

Figure 1. The Phenolic Compound Content of the study samples.

Antimicrobial effect of the products

It has been determined that TM1 has a high effect on Candida strains and no activity against bacteria. The S1 product, containing oleuropein, performed the highest antimicrobial effect against bacteria. TM3 was effective against gram-positive bacteria, and it did not show any activity on gram-negative bacteria. Bacterial strains were generally resistant to market products. TM1 and S4 showed the highest activity against yeasts. Table 3 represents the antimicrobial zone diameters of the products in detail.

Table 3. Zone Diameters of the Test Products

	Zone Diameter (mm) (±SD)				
Product	***E.coli***	***K. pneumoniae***	***S.aureus***	***C.albicans***	***C.krusei***
TM1	R	R	R	15 ±1.15	20 ±0.57
TM2	8 ±0.57	7 ±0.57	10 ±1	11 ±1.52	12 ±0.57
TM3	R	R	10 ±1.15	R	R
TM4	R	R	R	R	R
S1	15 ±2	17 ±0.57	20 ±0.57	R	R
S2	10 ±3	R	R	10 ±1.15	8 ±1.15
S3	11±0.57	10±0.57	11±1.73	R	R
S4	15 ±2.51	16 ±1.15	14 ±2.51	16 ±0.57	20 ±0.57
Gentamicin	25 ±0	22 ±1.15	25 ±0		
Streptomycin	20 ±0	20 ±0	20 ±0		
Nystatin				25 ±0	20 ±0

TM:Trademark, S: Sample, R:Resistant, ±SD:Standart deviation

DISCUSSION

Propolis is a product widely used in folk medicine from ancient times to the present day. Various pharmacological properties have been revealed and reported in the literature.

In their study, Markiewicz et al. have shown a significant reduction in the growth and proliferation of tumor cells with the propolis derivates.[17] Furthermore, in vivo studies represented a positive

effect of propolis on the dysbiosis of the gut microbiota; some studies suggest propolis as a potential agent in the treatment of intestinal diseases such as colitis.[18,19]

The antibacterial effect of propolis has been demonstrated against many gram-positive and gram-negative bacterial strains in vitro analyzes.[20] Propolis has antifungal activity against *Candida* and dermatophyte strains.[21] The antiviral activity against *Herpes simplex* and *Herpes zoster* viruses has been reported.[8,22,23] There are also reports from dentistry investigations; observational studies report successful results in treating dental inflammation, propolis positively contributes to the oral microbiota. A clinical study reports a positive efficacy of propolis-containing mouthwash in reducing plaque index and gingival index.[24]

The phenolic components are responsible for the antimicrobial activity of propolis. Studies show that propolis has more than 300 components. However, the content and variety of phenols vary depending on the solvent or the extraction method used.[25-27]

According to our study, the highest rate of TPC was detected in the TM2 sample (19553.12 GAE mg/L); its antimicrobial activity was not excessive, however. Probably, the reason is not the amount but the variety of phenolic compounds it contains because it is known that not every phenolic component has an antimicrobial property.

The components of a natural product determine the biological action spectrum. For example, phenolic compounds have many pharmacological impacts such as antioxidant, antiproliferative, antiviral, antifungal, antibacterial activities.[28,29]

The TPC ratio in TM3 (1923.41 GAE mg/L) was lower than other products; this may be related to the quantities of the compounds or the extraction method. The TM3 product had no antimicrobial effect against strains except for *S. aureus*, and the TM4 product had no antimicrobial effect against any tested microorganism. We consume that the antimicrobial ineffectiveness of the TM3 and TM4 products may be due to insufficient active substance concentration; because we know that the biological activities of phenolic compounds are dose-dependent.[29,30]

The TM1 and TM2 products had evident inhibition zone against *Candida* strains. The S1 sample had the highest zone diameter activity; the antifungal activity against yeasts. In their study, Kubiliene ve ark. (2015) have reported different antimicrobial activities, and the variation was considered to differ with the phenolic compound.

We prepared the final S4 product based on the S1, S2, S3 samples we crafted previously. As the highest inhibitation zone diameter was detected in the S4 sample, we claim that the boron and oleuropein together with propolis have a synergic antimicrobial effect. Boron is a semi-metal that has antimicrobial effects against many microorganisms. Boron is widely used in oral medical products as it prevents biofilm formation.[31-33] However, MIC (minimal inhibitation concentration) doses should be determined of the products and further studies should be conducted to determine the significance of these zones of inhibition.

CONCLUSION

The sample we developed (S4 product containing boron, oleuropein, and propolis) had higher inhibitation zone diameters against bacteria and yeasts compared to the commercial samples. The TPC ratio in the S4 sample was found twice higher than in TM1 and approximately six times higher than TM3 and TM4. TM2 product contains over three times more phenolic compounds compared to the S4 sample. Therefore, we may claim that olive oil-extracted propolis could be a better alternative method for ethanolic extract. Considering that all propolis extracts analyzed in the present study are rich in phenolic components, we suggest that they benefit well-being. Further researches should be carried to determine the phenolic compound diversity.

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REFERENCES

1. Ağyar Yoldaş P, Yoldaş T, Sipahi N. Cytotoxicity of Some Retail Food Supplements. *International Journal of Traditional and Complementary Medicine Research.* 2020; 1(3): 131-136.
2. Sipahi N. Investigation of Antagonistic Effect of Probiotic Food Supplement against Different Strains of Bacteria.

International Journal of Traditional and Complementary Medicine Research. 2021; 02(02): 95-100.
3. Sipahi N, Göç Rasgele P, Kaya E. 2021. Pharmacological Properties of Apitherapy Products. Atayoğlu AT, editor. Apitherapy, first edition Ankara: Türkiye Klinikleri; p.55-60.
4. Sorucu A. "Bee Product and Apitherapy, *Bulletin of Veterinary Pharmacology and Toxicology Association*. 2019;10(1), 1-15.
5. Seven I, Seven PT, & Silici S. "Effects of dietary Turkish propolis as alternative to antibiotic on growth and laying performances, nutrient digestibility and egg quality in laying hens under heat stress", *Revue de Médecine Vétérinaire*, 2011;162(4), 186-191.
6. Li J, & Kim I H. "Effects of Saccharomyces cerevisiae cell wall extract and poplar propolis ethanol extract supplementation on growth performance, digestibility, blood profile, fecal microbiota and fecal noxious gas emissions in growing pigs", *Animal science journal*, 2014;85(6), 698-705.
7. Wang K, Jin X, Li Q, Sawaya A.C, Leu R.K, Conlon, M. A, Hu F, et al.. "Propolis from Different Geographic Origins Decreases Intestinal Inflammation and Bacteroides spp. Populations in a Model of DSS-Induced Colitis", *Molecular Nutrition & Food Research.* 2018;62(17), 1800080.
8. Yildirim A, Duran G.G, Duran N, Jenedi K, Bolgul B.S, Miraloglu M, Muz, M. Antiviral activity of hatay propolis against replication of herpes simplex virus type 1 and type 2. *Medical science monitor: international medical journal of experimental and clinical research.* 2016;22, 422.
9. Kolaç T, Gürbüz P & Yetiş G. Phenolic Content And Antioxidant Characteristics of Natural Products. *Journal of Vocational School of Health Service.* 2017;5 (1) , 26-42
10. Bakkaloğlu Z. , Arıcı M. Effects of Propolis Extraction with Different Solvents on Total Phenolic Content, Antioxidant Capacity and Antimicrobial Activity. *Academic Food Journal*. 2019; 17(4): 538-545.
11. Dönmez M., Karadeniz Ş, Yoldas T, Aydin G, Karagül P, Aksu O et al. Comparison of Chemical Contents of Extracts in Different Solvents of Propolis Samples Produced in Duzce Province. *International Journal of Traditional and Complementary Medicine Research.* 2020;1(3), 137-146.
12. Mendonça, I. C., Medeiros, M. L., Penteado, R. A., Parolia, A., & Porto, I. C. (2013). An overview of the toxic effects and allergic reactions caused by propolis. drugs, 4, 7.
13. Yıldız, O. Evaluation of Solvents (Menstruums) Used In Consumed Propolis Extracts. *Uludag Bee Journal*. 2020; 20 (1) , 24-37.
14. Malayoğlu, H. B., & Aktaş, B. Antioxidant and Antimicrobial Activities of Olive leaf and Olive Mill Wastewater from Olive Oil Processing By-products. *Journal of Animal Production*. 2011;52(1):49-58, 2011.
15. CLSI. Reference method for antifungal disc diffusion susceptibility testing of yeasts. In Approved Guideline. (M44-A2), 2nd ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2009.
16. CLSI. Performance standards for antimicrobial susceptibility testing. 28th ed. CLSI supplement M100-S28. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2018.
17. Markiewicz Z.R, Moskwa J, Naliwajko S.K, Gromkowska-Kępka K.J, Socha K, Borawska M.H. Isidorov V. Propolis from Poland versus propolis from New Zealand-chemical composition and antiproliferative properties on glioblastoma cell lines 2020.
18. Kačániová M, Rovná K, Arpášová H, Čuboň J, Hleb L, Pochop J, Haščík P. "In vitro and in vivo antimicrobial activity of propolis on the microbiota from gastrointestinal tract of chickens", *Journal of Environmental Science and Health*. 2012;47(11), 1665-1671.
19. Duarte C.R.A, Eyng C, Murakami A.E, Santos T.C. "Intestinal morphology and activity of digestive enzymes in broilers fed crude propolis", *Canadian Journal of Animal Science*. 2014;4(1), 105-114.
20. Robson V, Dodd S, Thomas S. Standardized antibacterial honey (Medihoney) with standard therapy in wound care: randomized clinical trial. *J. Adv. Nurs*. 2009;65(3), 565–575.
21. Petruzzi L, Rosaria Corbo M, Campaniello D, Speranza B, Sinigaglia M, Bevilacqua A. Antifungal and Antibacterial Effect of Propolis: A Comparative Hit for Food-Borne Pseudomonas, Enterobacteriaceae and Fungi. *Foods* 2020; 9(5):559.
22. Bankova V, Galabov A.S, Antonova D, Vilhelmova N, Di Perri B. Chemical composition of Propolis Extract ACF® and activity against herpes simplex virus. *Phytomedicine* 2014;21(11), 1432-1438.
23. Tomanova D, Holcova S, Hladikova M. Clinical study: lotion containing propolis special extract GH 2002 0.5% vs. placebo as on-top treatment of herpes zoster. *Health* 2017;9(10), 1337.
24. Endo M.M, Estrela C.R, Alencar A.H.G, Silva J.A, Decurcio D.A, Estrela C. Antibacterial action of red and green propolis extract in infected root canal. *Revista Odonto Ciência*. 2017;2(2).
25. Marcucci, M. C., Ferreres, F., Garcıa-Viguera, C., Bankova, V. S., De Castro, S. L., Dantas, A. P., ... & Paulino, N. Phenolic compounds from Brazilian propolis with pharmacological activities. *Journal of ethnopharmacology*, 2001;74(2), 105-112.
26. Medana C, Carbone, F, Aigotti R, Appendino G, & Baiocchi C. Selective analysis of phenolic compounds in propolis by

HPLC-MS/MS. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, 2008;19(1), 32-39.

27. Kubiliene L, Laugaliene V, Pavilonis A, Maruska A, Majiene D, Barcauskaite, K., ... & Savickas, A. Alternative preparation of propolis extracts: comparison of their composition and biological activities. *BMC complementary and alternative medicine*, 2015;15(1), 1-7.
28. Bayaz, M. Esansiyel yağlar: antimikrobiyal, antioksidan ve antimutajenik aktiviteleri. Akademik *Gıda*, 2014;12(3), 45-53.
29. Bhuyan, D. J., & Basu, A. Phenolic compounds potential health benefits and toxicity. In Utilisation of bioactive compounds from agricultural and food waste. 2017; pp. 27-59, CRC Press.
30. Ćavar Zeljković, S., Šišková, J., Komzáková, K., De Diego, N., Kaffková, K., & Tarkowski, P. Phenolic Compounds and Biological Activity of Selected Mentha Species. Plants, 2021;10(3), 550.
31. Işık, S. Investigation of Antibacterial Efficiency of Boron Nitride Coated Implants. Hacettepe University Faculty of Medicine Department of Orhtopaedics and Traumatology, Ankara, 2018.
32. Aral K, Çelik Güler Ö, Cooper, P. R., Shoker S, Kuehne S A, & Milward M. R. Antimicrobial Effects of Boric Acid against Periodontal Pathogens. Journal of Ege University School of Dentistry. 2020; 41(1), 20-25.
33. Dede E. Ç, Gizer M, Korkusuz P, & Korkusuz F. Boron containing nano-hydroxyapatite composite applications for implant related osteomyelitis prevention. *TOTBID Journal*. 2020;19:774–779.