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ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF WATER SOLUBLE FRACTION OF PROPOLIS AND ITS ANTIBACTERIAL POTENTIAL ON BACTERIA CAUSING CONJUNCTIVITIS

Propolisten Suda Çözünür Bir Fraksiyonun İzolasyonu, Karakterizasyonu ve Bakteriyel Konjonktivite Karşı Potansiyel Etkinliğinin Belirlenmesi

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ABSTRACT

Propolis is a bee product with a variety of biological activities. Although chemical composition of propolis differs by the location but all propolis types possesses antimicrobial activity. The usage of propolis for apitherapeutic purposes has increased recently. But its ethanol solubility limits its usage in certain areas like ophthalmology. Main objective of this study is to isolate water soluble components of propolis and determination of its antimicrobial activity against two bacteria causing conjunctivitis namely *Neisseria gonorrhoeae* and *Haemophilus influenzae*. Isolation of water soluble fraction of propolis was carried out in two steps by using pectin-propolis micro beads. Isolated water soluble fraction and crude extract was examined by thin layer chromatography and HPLC analyses. Three main spots were screened on TLC plate after isolation. These spots could be explained by the presence of different class of compounds in the isolate. HPLC analyses showed that water soluble fraction contained phenolic acids, their esters and flavonoids like p-OH benzoic acid, *t*-cinnamic acid, pinocembrin and caffeic acid phenethyl ester. Low antimicrobial activity was achieved against tested microorganisms for the fraction. It can be concluded that isolation of water soluble fraction of the propolis extract could be a solution for its usage in restricted areas.

Keywords: Propolis, Water soluble fraction, Conjunctivitis, Antimicrobial activity

ÖZ

Propolis farklı biyolojik aktivitelere sahip doğal bir arı ürünüdür. Propolisin kimyasal bileşimi lokasyona göre farklılık gösterse de tüm propolis türleri antimikrobiyal aktiviteye sahiptir. Propolisin apiterapötik amaçlarla kullanımı son zamanlarda artmıştır. Ancak propolisin etanolde çözünürlüğü, oftalmoloji gibi bazı alanlarda kullanımını sınırlamaktadır. Bu çalışmanın temel amacı propolisin suda çözünen bileşenlerini izole etmek ve izolatların konjonktivite neden olan iki bakteriye karşı

antimikrobiyal aktivitesini tespit etmektir. Propolisin suda çözünür fraksiyonunun izolasyonu pektin-propolis mikro boncukları kullanılarak iki aşamada gerçekleştirildi. İzole edilmiş suda çözünür fraksiyon, ince tabaka kromatografisi ile incelendi ve ham ekstraktla karşılaştırıldı. İzolasyondan sonra TLC plakasında üç ana nokta tarandı. Bu lekeler izolatta fenolik asitler, flavonoidler ve kafeik asit esterleri gibi farklı sınıftaki bileşiklerin varlığıyla açıklanabilir. Suda çözünebilen fraksiyonun fenolik asitler, bunların esterleri ve p-OH benzoik asit, t-sinnamik asit, pinosembrin ve kafeik asit fenetil ester gibi flavonoidleri içerdiği belirlendi. Fraksiyon için test edilen mikroorganizmalara karşı düşük antimikrobiyal aktivite elde edildi. Propolis ekstraktının suda çözünen kısmının izolasyonunun kısıtlı alanlarda kullanımı için bir çözüm olabileceği sonucuna varılabilir.

Anahtar Kelimeler: Propolis, Suda çözünen fraksiyon, Konjonktivit, Antimikrobiyal aktivite

GENİŞLETİLMİŞ ÖZET

Amaç: Propolis arılar tarafından bitkilerin tomurcuk, yaprak ve benzeri farklı kısımlarından toplanan reçinemsi, rengi sarıdan koyu kahverengiye kadar değişen doğal bir arı ürünüdür. Propolis antimikrobiyal, anti-inflamatuvar, antikanser, antioksidan gibi farklı biyolojik aktivitelere sahip doğal bir arı ürünü olmakla birlikte propolisin kimyasal bileşimi ve biyolojik aktivitesi toplandığı bölgeye göre değişiklik göstermektedir. Propolisin kimyasal bileşimi toplandığı bölgeye göre farklılık gösterse de tüm propolis türleri antioksidan ve antimikrobiyal aktiviteye sahiptir. Apiterapi; arı zehri, bal, polen, arı sütü ve propolis gibi arı ürünlerinin hastalıkların tedavi edilmesi veya önlenmesi amacıyla kullanılması olarak tanımlanmaktadır. Propolisin apiterapötik amaçlarla kullanımı son zamanlarda artmış olmasına karşın yapışkan yapısından dolayı doğrudan kullanılması veya tüketilmesi mümkün değildir. Etanol, methanol, zeytinyağı gibi farklı çözücüler kullanılarak ekstrakte edilen propolis için en iyi çözücü %70'lik etanol çözeltisidir. Bu durum propolisin oftalmoloji gibi bazı alanlarda kullanımını sınırlamaktadır. Bakteriyel konjonktivit, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Neisseria gonorrhoeae* ve *Haemophilus influenzae* gibi bakterilerin neden olduğu en sık karşılaşılan göz hastalıklarından biridir. Bakteriyel konjonktivit, gözün mukoza zarının enfeksiyonudur ve antibiyotik içeren göz damlaları kullanılarak tedavi edilir. Bu çalışmanın temel amacı, propolisin suda çözünebilen bileşenlerini ve konjonktivite neden olan gram negatif bakterilerden olan *Neisseria gonorrhoeae* and *Haemophilus influenzae*'ye karşı antimikrobiyal aktivitesini tespit etmek ve göz hastalıklarının tedavisinde ya da önlenmesinde kullanılabilecek yeni bir ürün geliştirilme potansiyelinin tespit edilmesidir.

Gereç ve yöntem: Propolisin suda çözünür fraksiyonunun izolasyonu, pektin-propolis mikro boncukları kullanılarak iki aşamada gerçekleştirildi. İlk olarak pektin-propolis boncuklar 1:100 oranında su içerisinde homojenize edilerek gece boyunca karıştırıldı. Elde edilen homojenat 4000 rpm'de 5 dakika santrifüj edildi ve süpernatan toplandı. İkinci olarak aynı hacimde etanol eklenerek süpernatanttan çözünmüş pektin çökeltildi ve karışım süzüldü. Daha sonra propolisin suda çözünebilen bileşenlerini içeren süzüntü evaporatörde kurutuldu. İzole edilmiş suda çözünür fraksiyon, ince tabaka kromatografisi ile incelendi ve ham ekstraktla karşılaştırıldı. Hem suda çözünür fraksiyonun hem de ham ekstraktın kimyasal bileşimi RP-HPLC-UV analizi ile belirlendi.

Bulgular: İzolasyondan sonra TLC plakasında üç ana nokta tarandı. Bu bantlar izolatta fenolik asitler, flavonoidler ve kafeik asit esterleri gibi farklı sınıftaki bileşiklerin varlığıyla açıklanabilir. Suda çözünen fraksiyonun fenolik asitler, bunların esterleri ve p-OH benzoik asit, siringik asit, t-sinnamik asit, hesperidin, pinocembrin ve kafeik asit fenetil ester (CAPE) gibi flavonoidleri içerdiği belirlendi. Genel olarak propolisler gram negatif bakterilerdense gram pozitif bakterilere karşı daha güçlü antimikrobiyal aktivite göstermektedirler. Bu çalışmada da gram negatif bakteriler olan *Neisseria gonorrhoeae* ve *Haemophilus influenzae* karşı düşük antimikrobiyal aktivite göstermiştir. Ancak bu her iki bakteri de nazlı üreme özelliği olan bakteriler olup etkinliğin yüksek olması beklenmekteydi. Bu sonuçun propolis bileşenlerinin düşük konsantrasyonundan dolayı fraksiyon için test edilen mikroorganizmalara karşı düşük antimikrobiyal aktivite elde edildiği düşünülmektedir. Fraksiyonda daha yüksek bileşenler elde edildiği takdirde etkinliğin yüksek olacağı düşünülmektedir. Bu konuda daha fazla çalışmaların gerektiği düşünülmektedir.

Sonuç: Propolis ekstraktının suda çözünen kısmının izolasyonunun kısıtlı alanlarda kullanımı için bir çözüm olabileceği sonucuna varılabilir. Bu ön çalışma niteliğinde olan bu çalışma geliştirilerek propolisli göz damlası veya göz temizleme solüsyonlarının hazırlanmasına temel oluşturabilir.

INTRODUCTION

Propolis is an important bee product with a variety of biological activities (Kolaylı and Keskin 2020). It is collected from different parts of the plants by honey bees. Biological activity with relation to chemical composition of a propolis depends on the flora of collection site (Keskin and Kolaylı 2018). Even though chemical composition of propolis samples differs by the location but all propolis samples around the world possess antimicrobial activity (Bouchelaghem 2022, Nichitoi et al. 2021, Stepanović et al. 2003,). Apitherapy is defined as the usage of bee products in the treatment either for curative or preventive purposes (Kolaylı and Keskin 2020). The usage of propolis for apitherapeutic purposes has increased recently. Many biological activities of propolis has been reported up to now (Bouchelaghem 2022, Keskin and Kolaylı 2018, Kolaylı and Keskin 2020, Nichitoi et al. 2021, Stepanović et al. 2003, Weis et al. 2022). But the usage of propolis in the treatment of eye diseases is not possible because of its resinous nature and its alcohol solubility.

Propolis contains many active substances and some of them are soluble in water but it is not possible to extract these substances from the raw propolis directly (Chua et. al. 2023). It is clear that new methods are required for this purpose. Although it is clearly stated in the literature that the best solvent for propolis extraction is ethanol but it is also a limiting factor for the usage of propolis in certain areas. When achieved to separate them, water soluble components of propolis have good potential to be used in restricted areas like bacterial conjunctivitis.

Bacterial conjunctivitis is an infectious inflammation of the conjunctiva which is a flexible transparent mucous membrane covering the *Bulbus oculi* (Azari and Arabi 2020). Due to direct exposure of the conjunctiva to the environmental factors, inflammation of the conjunctiva, either infectious or non-infectious, is seen commonly and one of the common reasons for visiting eye care clinics (Pisano et al. 2023). Viral and allergic reasons are the other common causes of conjunctivitis and usually cause mild inflammation and ocular discomfort that healed spontaneously (Chan et al. 2022). However, bacterial conjunctivitis has a relatively more severe course, and topical antibiotics, either drop or ointment, are generally required in the treatment (Mohammed et al. 2020, Banks et al. 2020, Stiles 2021). The high prevalence and frequent occurrence of this disease and the need for isolation of cases from school and work cause a huge economic impact on governments. In the United States, the estimated cost of bacterial conjunctivitis per year was $377 million to $857 million (Azari and Arabi 2020).

Topical antibiotic drugs are commonly used in ophthalmology clinics for various indications in addition to conjunctivitis (Aramă 2020). They are suggested for almost after any surgical intervention through the ocular surface. Increasing administrations of intravitreal anti-VEGF drugs for Age Related Macular Degeneration (AMD), diabetic macular edema and other retinopathies caused a serious increase in antibacterial drug use in the last decades (Xu et al. 2023). In addition to the cost of these drugs, the common use of topical antibacterial drugs may cause toxic and allergic reactions to the ocular surface and lead to an increase in antibiotic resistance (Aramă 2020). Therefore, various natural and herbal products are investigated for a possible antimicrobial activity to use in infectious inflammation instead of antibiotics.

Main objective of this study is to isolate water soluble components of propolis and identification of isolated compounds by HPLC analyses. Antimicrobial activity of the isolated compounds against two common pathogens of bacterial conjunctivitis (Devipriya 2020) namely *Neisseria gonorrhoeae* and *Haemophilus influenza* was also carried out.

MATERIAL AND METHODS

Bacterial strains were supplied from Refik Saydam Institutes (Ankara). Propolis was obtained by a local beekeeper in Bilecik city. Propolis was collected by using traps in the summer season of 2020. Pectin, ethanol, Na_2CO_3, and HCl were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, catechin, syringic acid, *p*-coumaric acid, epicatechin, rutin, *t*-cinnamic acid, luteolin and ferulic acid were obtained from Sigma Aldrich

Chemie GmbH (Munich, Germany) and used as HPLC standards. Other chemicals used in the study were off analytical grade.

Preparation and Encapsulation of Propolis Extract

The propolis sample was extracted with 70% ethanol/water (v/v) in the ratio of 1:10 (g/v) (Keskin and Kolaylı 2018). Extraction was carried out for 48 h on a magnetic stirrer under constant stirring at 150 rpm. Finally, the obtained mixture was filtered and labeled as ethanol propolis extract (EPE).

Microencapsulation of propolis extract with pectin was carried out by using both ionic gelation and solvent exchanging methods (Keskin et al. 2019). 5% pectin solution was prepared by dissolving 5 g pectin in 100 mL water. In another beaker 1.72 g $CaCl_2$ was dissolved in 100 mL ethanol propolis extract. Pectin solution was added into ethanol propolis extract drop by drop under constant stirring. Finally obtained mixture was dried in a vacuum oven at 50 °C and obtained beads were grounded in a fine powder (Keskin et al. 2019).

Isolation of Water Soluble Fraction of Propolis

Isolation of water soluble fraction of propolis was carried out in two steps. Firstly, pectin micro-beads were homogenized in water at 1:100 ratios (1 g pectin beads/100 mL water). Obtained mixture was centrifuged at 4000 rpm for 5 min and supernatant was collected, precipitate was named as residue. Secondly dissolved pectin was precipitated from the supernatant by adding the same volume of ethanol. Then the mixture was filtered and the filtrate was collected and dried in a rotary evaporator (IKA-Werke, Staufen, Germany).

Examination of Water Soluble Fraction/ Thin Layer Chromatography

Isolated water soluble fraction was examined by thin layer chromatography. Commercially obtained silica gel was used. Mobile phase was composed of ethanol/chloroform in a 9.5/0.5 (v/v) ratio.

Determination of Chemical Composition by HPLC Analyses

Propolis samples, both water soluble fraction and ethanol propolis extract, were examined by using HPLC analyses. For this, the solvent of the samples was removed. Obtained residues were dissolved in acidified (pH 2) distilled water separately. 5 mL diethyl ether after then 5 mL ethyl acetate was used for re-extraction for 3 times for each. Obtained phases were separately combined and the solvents were evaporated in a rotary evaporator at 45 °C. The residues were re-dissolved in 2 mL of methanol and filtered with 0.45 µm filter. The filtrates were injected to HPLC. Nineteen phenolic standard compounds were analyzed using HPLC (Elite LaChrome; Hitachi, Tokyo, Japan) equipped with a reversed phase Fortis C18 column (Chromex Scientific, 150 mm* 4.6 mm, 5 µm). Mobile phase was composed of acetonitrile/water (7/3 ratio) and acetic acid 2%. A programmed gradient was applied. Reservoir A contained 2% acetic acid and reservoir B contained 7/3 ratio of acetonitrile/water. Elution of samples began with the eluent composed of 95% of reservoir A and 5% of reservoir B for 3 min. Then, the ratio of reservoir A was decreased gradually to 20% at the end of 30 min. Later, gradient program was shifted to starting point of 95% reservoir A for 20 min more. 20 µL of samples was injected individually at room temperature and flow rate was set as 0.75 mL/min (Can et al. 2015).

Determination of Antimicrobial Activity

Antimicrobial activity of the sample was determined by using agar well diffusion method. Chocolate and Blood agar was used for *Neisseria gonorrhoeae* and *Haemophilus influenza* respectively. Hemin and Vitamin K were added to both media at a rate of (%1) and used. Dried propolis samples were dissolved in ethanol at 100 mg/mL concentration as stock solution. Working solution of samples was prepared by tenfold dilution of the stock solution. A serial dilution of the propolis samples was achieved by diluting the working solution in the range of ½ to 1/16. After microorganisms were homogeneously seeded into the media with the help of swab sticks, wells (5 mm) were opened. Each dilution was tested three repetitions and in 50 microliter quantities. Petri dishes were placed in an incubator at 37 °C for 48-72 hours with microaerophilic conditions. Finally, zone diameter was measured as mm (Kolaylı et al. 2020). The analyses were performed three times, the results were presented as mean values and standard deviations.

RESULTS

Main objective of this study is to isolate water soluble compounds of propolis and to determine its potential as an antimicrobial agent. As mentioned above it is not possible to extract them from the raw propolis

directly. It is suggested that micro encapsulation of ethanol extract of propolis and then homogenization of the obtained beads in water may be applicable for this purposes.

Isolation of water soluble fraction was carried out in two steps by starting the homogenization of 10 g of pectin-propolis beads in 1L of water. Then the homogenate was centrifuged at 4000 rpm for 5 min and the supernatant (800 mL) was collected in a clean beaker. In the second step, the same volume of ethanol was added into the supernatant and dissolved pectin was precipitated. Obtained mixture was filtered to separate the pectin from the propolis active compounds. Finally, solvent of the filtrate was evaporated to dryness in a rotary evaporator and 2.16 g dry residue (water soluble fraction) was achieved. Obtained residue was examined by thin layer chromatography. The spots obtained were colorless in day light and they could be visualized under UV light (Figure 1). Identification of chemical compounds in ethanol propolis extract, water soluble fraction and residue was carried out by HPLC analyses and obtained results were summarized in Table 1. It was determined that water soluble fraction contained phenolic acids, their esters and flavonoids like p-OH benzoic acid, syringic Acid, t-cinnamic acid, hesperidin, pinocembrin and caffeic acid phenethyl ester (CAPE). Ethanol propolis extract, remaining residue and water soluble fraction were tested as an antimicrobial agent against two bacteria causing bacterial conjunctivitis. Antimicrobial activity was determined as the zone diameter (Figure 2) and results were summarized in Table 2. Antimicrobial activity of samples was defined as high (zone diameter > 15mm), moderate (zone diameter between 10-15 mm) and low (zone diameter < 10 mm).

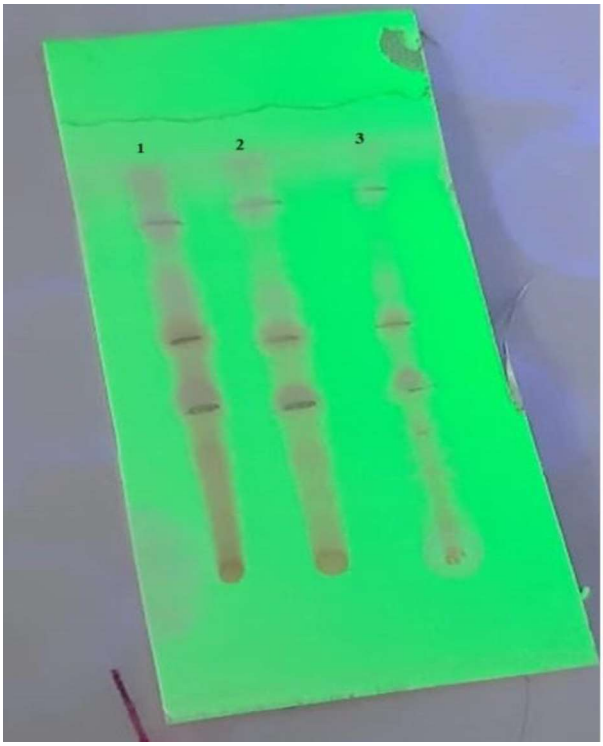

Figure 1. TLC image of samples.
Lines as 1,2,3, Represents ethanol propolis extract, Residue and Water Soluble Fraction respectively.

Figure 2. Antimicrobial activity of samples

Figure 2. Antimicrobial activity of samples

Table 1. Phenolic composition of samples

	Ethanol Extract	Residue	Water Soluble Fraction
Gallic acid	-	-	-
Protocatequic acid	-	-	-
p-OH benzoic acid	91.45[a]	33.05[b]	7.52[c]
Catechin	-	-	-
Caffeic Acid	-	-	-
Syringic Acid	240.61[a]	35.16[b]	17.42[c]
Epicatechin	-	-	-
p-coumaric acid	-	-	-
Ferulic acid	-	-	-
Rutin	778.27[a]	287.88[b]	142.85[c]
Myricetin	489.39[a]	75.55[b]	49.79[c]
Resveratrol	-	-	-
Daidzein	-	-	-
Luteolin	-	-	-
t-cinnamic acid	157.09[a]	46.67[b]	30.08[c]
Hesperetin	64.30[a]	16.29[b]	24.29[c]
Chyrisin	-	-	-
Pinocembrin	1581.93[a]	601.35[b]	663.94[c]
CAPE	112.53[a]	21.38[b]	34.02[c]

Results were expressed as µg/ g sample. – means not detected.

Different letters represent signifcant diferences at $p < 0.05$ probability level

Table 2. Antimicrobial Activity of Samples

	Dilution	Concentration (mg/mL)	Zone Diameter (mm) *Neisseria gonorrhoeae*	*Haemophilus influenzae*
Water Soluble Fraction	1/10	20 mg/mL	8±0.2	8±0.2
	1/2	10 mg/mL	6±0.1	6±0.1
	1/4	5 mg/mL	nd	nd
Residue	1/10	20 mg/mL	12±0.3	8±0.2
	1/2	10 mg/mL	10±0.3	6±0.1
	1/4	5 mg/mL	8±0.2	nd
Ethanol Extract	1/10	20 mg/mL	12±0.3	20±0.5
	1/2	10 mg/mL	10±0.3	18±0.5
	1/4	5 mg/mL	8±0.2	10±0.3

* nd: Not detected

DISCUSSION

This is the first study reporting the fractionation of propolis extract after encapsulation. Propolis is highly resinous substances and not readily soluble in water. Many biological activities of ethanol-propolis extract have been reported up to now but there is a limit for the usage of this extract as a treatment agent especially in ophthalmology (Kubiliene et al. 2015). Main reason is its water insolubility. The application

of water based products for treatment is easier than other solvent based products. That is why isolation of water soluble components of propolis is still an attractive research area. Many attempts have been made for improving the water solubility of propolis up to now. In an early attempt, Ivanovska et. al. (1995) described the production of water soluble derivative of ethanol propolis extract by using L-lysine. Some phenolic compounds were purified from Mexican propolis by using chromatographic methods (Guzmán-Gutiérrez et al. 2018). It is a well-known fact that chromatographic methods require many experimental steps and usage of huge amount of organic solvents for purification (Hostettmann et al. 1986). The method we described in this paper is new and relatively easier for application and does not require the usage of such amount of organic solvents. This method is also easy to scale up for commercial purposes.

Water soluble fraction and propolis ethanol extract were analyzed by thin layer chromatography. Three main spots were screened on TLC plate after isolation. These spots could be explained by the presence of different class of compounds in the isolate like phenolic acids, flavonoids and caffeic acid esters. A chemo metric fingerprinting of Chinese propolis was developed by using TLC and it was reported that five main spots were obtained for Chinese propolis. R_f value for four of these spots were reported as the same with quercetin, kaempferol, chrysin and galangin (Tang et al. 2014). In a study it was reported that standardized TLC method could be developed for investigation of phenolic acids and flavonoids in propolis. The researchers reported that many spots were visualized for propolis samples where some of them representing the phenolic acids and flavonoids (Medić-Šarić et al. 2004).

Chemical structure of water soluble fraction, propolis ethanol extract and the residue was also identified by using HPLC analyses. Findings of this analyses support the findings of thin layer chromatography analyses. It is clearly seen from the Table 1 that water soluble fraction is rich in some phenolic acids, esters and flavonoids. Propolis sample used in this study was obtained from Bilecik province. When compared with the literature, it is clear that similar findings were also reported earlier (Keskin et al. 2019). It is also mentioned in literature that Anatolian propolis is rich in phenolic compounds (Özkök et al. 2021).

The findings of the present study showed that it is an easy and cheap way of fractionation of propolis extract. Propolis is a resin like substance and not readily soluble in water. It is stated in literature that water extract of propolis contains lesser compounds of propolis (Wieczorek et al. 2022). The resinous nature of propolis makes it unusable in the treatment of certain diseases like ophthalmology. Isolation of water soluble fraction of the propolis extract could be a solution.

It is clear from the results that the water soluble fraction showed lower antimicrobial activity compared to propolis ethanol extract. In general, propolis shows stronger antimicrobial activity against gram-positive bacteria than gram-negative bacteria (Kolayli et. al. 2020). In this study, low antimicrobial activity against the gram-negative bacteria *Neisseria gonorrhoeae* and *Haemophilus influenza* was achieved for the water soluble fraction. However, both of these bacteria have fastidious growth characteristics and the activity was expected to be high. This result is thought to be due to the low concentration of propolis components in the fraction resulting in low antimicrobial activity against the microorganisms tested. It is thought that the effectiveness will be higher if higher components are obtained in the fraction.

Our findings also showed that antimicrobial activity of residue was moderate, higher than water soluble fraction and lower than propolis ethanol extract. This result could be explained by the synergy between phenolic and non-phenolic compounds of propolis. It was stated in literature that propolis is composed of plant resins containing phenolic and non-phenolic compounds like plant waxes, hydrocarbons, alcohols and esters (Hossain et al. 2022). Although biological activities like antioxidant and antimicrobial activity of propolis extract have been associated with its phenolic content (Silva et al. 2012), our finding somehow is opposing this consideration. When the phenolic compounds were separated from the ethanol extract, decreased antimicrobial activity was achieved.

This is the first study reporting the separation of active compounds from the propolis ethanol extract by combining the microencapsulation and homogenization techniques. For improving the yield, the effect of pH and ionic strength of water should be studied. Also the type of encapsulant used for microencapsulation might help the yield of

separation. It is clear that more study is required for improving the yield.

Conclusion: Propolis is a highly resinous material composed of plant resins and bees wax. Its highly apolar nature makes it not readily soluble in pure water. Although propolis contains some water soluble components nevertheless extraction of these components from the raw propolis by using pure water is not possible because of the chemical structure of raw propolis. In this study water soluble components of propolis were separated in two steps. Obtained fraction is completely soluble in pure water. Antimicrobial activity of ethanol extract, water soluble fraction and the residue left was tested against two bacterial strains responsible for conjunctivitis. It could be concluded that despite of the low antimicrobial activity of water soluble fraction, it might be a step in the production of eye drop for the treatment of bacterial conjunctivitis.

Author contributions: ÖEM and ŞK:Conceptualization, Project administration, Formal analysis, Data curation, Writing - original draft; YK: Formal analysis; ŞAK: and MK: Formal analysis, Data curation and Writing - review & editing.

Conflict of interest: There is no conflict of interest between the authors.

Data availability: The data used in the present study could be obtained from the corresponding author upon request.

Ethics: No animal or human experiments were performed during the conduct of the study.

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