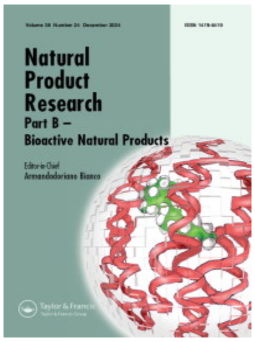

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Chemical profiling and biological activity evaluation of propolis from Çayeli-Rize, Eastern Black Sea Region, Anatolia

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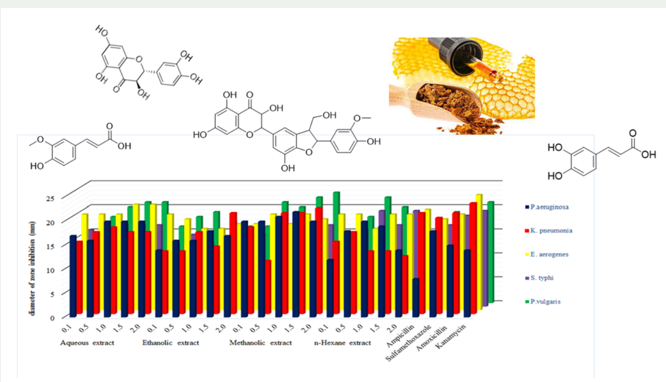
ABSTRACT

The constituents of the aqueous, ethanol, hexane, and methanol extracts of Anatolian propolis collected from the Eastern Black Sea Region (Çayeli-Rize) were investigated by GC–MS, HPLC and AAS. Interestingly, lactulose has been identified. Ten phenolic compounds, namely caffeic acid, ferulic acid, rutin, taxifolin, quercetin, kaempferol, apigenin, silicristin, silibinin and gallic acid were determined. The contents of phenolic acids and flavonoids varied between 17.04–642.59 and 1.18–2749.20 ppm, respectively. Minerals found in propolis were Na, K, Ca, Mg and Zn. The methanol extract had the highest antiproliferative activity against the A549 cell line with an IC_{50} value of 0.1821 µL/mL. The extracts showed higher antibacterial activity against Gram-negative bacteria compared to Gram-positive bacteria.



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1. Introduction

Propolis is a natural substance produced by bees using materials obtained from plants, buds, and leaves of trees. This natural compound is mainly used by bees to seal surfaces, holes, and spaces in the hive. By covering all spaces, bees ensure a sterile environment in the hive. Therefore, propolis can be considered a powerful chemical weapon that protects the bee community against pathogenic microorganisms (Wagh 2013). Propolis also has health-improving properties. It has antiseptic (Castaldo and Capasso 2002; El-Sakhawy et al. 2023), antimicrobial (Freitas et al. 2022), anti-inflammatory (Zamarrenho et al. 2023), antiviral (Ozarowski and Karpinski 2023), antiprotozoal (Alenezi et al. 2022), antiproliferative (Tatlisulu and Ozgur 2023), and antioxidant (Malkoç et al. 2019) properties. For this reason, people have taken advantage of this compound. Propolis is used in food production, food packaging, cosmetics, and over-the-counter preparations (Banskota et al. 2001; Freires et al. 2016). There are some products containing propolis that are approved by the FDA (Fitzmaurice et al. 2011). Propolis owes its valuable properties mainly to the biologically active chemical components it contains. However, the composition of propolis is complex and varies depending on the botanical and phytogeographic origin of the location where it is collected (Shaheen et al. 2011).

Anatolia is a spectacular region that hosts many endemic and non-endemic plant species due to its geographical location, geological structure, and climate diversity (Baser 2002). For example, in Rize, a province located on the eastern coast of the Black Sea, there are 1439 plant taxa, including 225 medicinal and aromatic plants, and 110 endemic plants (Baykal et al. 2011). The diversity of plant species in Rize is much richer than in England and European countries (Baykal and Atamov 2017). However, there have been few reports on Rize propolis (Koru et al. 2007; Gencay and Salih 2009; Erdogan et al. 2011; Temiz et al. 2011; Erturk et al. 2016; Sarikahya et al. 2021; 2022; Guzelmeric et al. 2023). The aim of this study is to investigate the chemical composition, antiproliferative and antimicrobial properties of Çayeli propolis from Rize.

2. Results and discussion

2.1. Chemical composition

In this study, propolis collected from Cayeli in Rize Province, Turkiye was extracted using the Soxhlet method with four different solvents: ethanol, methanol, hexane and water. The chemical compositions of the methanol and ethanol extracts (MEP and EEP, respectively) were identified by GC–MS. Both extracts were found to contain various important chemical constituents such as diterpenoids, fatty acids, organic acids, and high-molecular-weight aliphatic hydrocarbons (Tables S1 and S2). The major constituent in both extracts was benzoic acid, which is found in bark, berries, and fruits as a natural defense against fungi. Benzoic acid was also abundant in French propolis (Hegazi et al. 2000). It is used today as a food-grade FDA-approved preservative, serving as an antimicrobial agent (Del Olmo et al. 2017). According to data, Çayeli propolis was characterised by a rich composition of monosaccharides and disaccharides. An interesting observation was the presence of lactulose (4.29%) in EEP. This unusual reducing sugar is a prebiotic carbohydrate that inhibits the growth of Salmonella. Lactulose also stimulates the growth of bifidobacteria and lactobacilli in the gastrointestinal tract. Therefore, it may be used for the treatment of constipation, Salmonella carriage, and hepatic encephalopathy (Panesar and Kumari 2011). Since lactulose was detected for the first time in propolis, it may serve as an indicator of the authenticity of Çayeli propolis. Additionally, two new compounds, 4-ethyl-3-hexanone and (*2S*)-2-pentyl oxirane, were detected in the analysed propolis sample. The free phenolic acids identified in EEP were *p*-coumaric acid, isoferrulic acid, and caffeic acid, with isoferrulic acid being the most abundant among them (0.39%). Sarikahya et al. (2021) found that caffeic acid was the most abundant phenolic compound in Hemşin-Rize propolis. Conversely, ellagic acid was identified as the major phenolic acid in Çamlıtepe-Rize propolis. They also reported that Rize propolis collected from both Çamlitepe and Hemşin regions contained triterpenes. The main triterpene found in propolis from Çamlıtepe was oleanolic acid, whereas in propolis from Hemşin, pomolic acid was predominant. However, no triterpenes were detected in this study. Çayeli propolis yielded two diterpenoids (dehydroabietic acid and isopimaric acid), a terpene ((*5Z*)-10-methyl-6-(trifluoromethyl)-2-undeca-5,9-dienone), and a terpenoid (cyclofenchene). Isopimaric acid emerged as the predominant compound, comprising 0.26% of the total. Isopimaric and dehydroabietic acids were also found in Brazilian and Canary propolis (Machado et al. 2023; Isidorov et al. 2024). *Isopimaric acid* is a resin acid produced by conifer trees (Keeling and Bohlmann 2006). Dehydroabietic acid is the primary constituent of pine resin obtained from *Pinus* species. It is also present in other conifers such as *Cupressaceae* and *Pinaceae*, as well as various *Angiosperm* species, particularly in the families *Asteraceae*, *Celastraceae*, *Hydrocharitaceae*, and *Lamiaceae* (Feliciano et al. 1993; Helfenstein et al. 2017; Eksi et al. 2020). Çayeli propolis also contained fatty acids such as lauric acid, stearic acid, vaccenic acid, lignoceric acid, and palmitic acid, along with hydrocarbons including eicosane, tetracosane, nonadecane, octadecane, 4-methyl dodecane, 7-propyl tridecane, 2,6,10,15-tetramethyl heptadecane, 1-iodohexadecane, 7,9-dimethyl hexadecane, 3-methyl-5-propyl nonane, and tritetracontane. Additionaly, *muco*-inositol was found in the composition of Çayeli propolis. While *muco*-inositol has been previously reported in edible honeys (Sanz et al. 2004), there is no existing literature data on its presence in propolis. This study marks the first detection of *muco*-inositol in propolis.

The quantitative and qualitative separation and determination of phenolic compounds in the aqueous (AEP), methanol (MEP), ethanol (EEP), and *n*-hexane (HEP) extracts of propolis were investigated using HPLC analysis (Table S3). The sample was found to contain three phenolic acids (gallic, ferulic, and caffeic acids), four flavonols (rutin, taxifolin, quercetin, and kaempferol), two flavonolignans (silicristin and silibinin), and a flavone (apigenin) (Figure S1). The phenolic acids ranged from 642.59 to 17.04 ppm, while the flavonoids varied from 2749.20 to 1.18 ppm. Each extract had a distinct main compound. Rutin was the only glycosylated flavonoid detected. Sarikahya et al. (2021) identified 15 flavonoids in the propolis samples collected from Çamlıtepe and Hemşin, with concentrations ranging from 777.02 to 0.08 µg/g and 1325.52 to 0.01 µg/g, respectively. Diosmetin was found to be the predominant flavonoid in both propolis samples. Çayeli propolis shows significant differences in flavonoid composition compared to propolis from Çamlıtepe and Hemşin. These differences may be due to diverse floral sources within the same geographical region (Baykal et al. 2011), leading a considerable variability in total phenolic compounds.

The mineral composition of propolis preparations was analysed using flame atomic absorption spectrometry (Table S4). Sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), and zinc (Zn) were quantified in the propolis samples, with mineral contents ranging from 1321.63 to 2.64 µg/g. Potassium was identified as the most abundant element in raw propolis. However, the primary element varied among the different extracts. Aksoy et al. (2017) noted that potassium levels were relatively higher in propolis from the Eastern Black Sea region compared to other elements.

2.2. Antiproliferative activity

Four extracts prepared in water, ethanol, methanol, and hexane (AEP, EEP, MEP, and HEP, respectively) were tested on lung cancer cell line (A549) and healthy human embryonic kidney cell line (HEK-293T) using the MTT assay method over a range of concentrations (5, 2.5, 1.25 and 0.625 µL/mL) for 72 h.

The cell viability results, depicted in Figure 1, illustrate that all four extracts demonstrated a dose-dependent decrease in the viability of A549 cancer cells. At the highest concentration (5 µL/mL), the extracts significantly reduced cancer cell viability to below 10%. AEP exhibited a lower toxic effect against healthy HEK-293T cells. In contrast, the other extracts (EEP, MEP and HEP) significantly reduced the viability of healthy cells at the highest concentration, with rates dropping to 10% and below.

AEP demonstrated antiproliferative effects against the lung cancer cell line with an IC_{50} value of 2.5690 µL/mL (Table 1). EEP exhibited strong antiproliferative activity against the A549 cell line with an IC_{50} value of 0.5417 µL/mL; however, it also presented a high toxic effect against non-cancerous HEK-293T cells. In comparison, Algerian propolis ethanol extract (EEP) inhibited A549 cell growth in a dose-dependent manner, with IC_{50} values of 69.94 µg/mL and 14.32 µg/mL at 24 and 72 h of treatment, respectively (Brihoum et al. 2018). This suggests that Algerian propolis is less potent than Çayeli propolis in inhibiting A549 cell proliferation. Additionally, Kouidhi et al. (2010) reported an IC_{50} value of 200 µg/mL for Tunisian propolis ethanol extract against the A549 cell line after 24 h of treatment. Similarly, Sarikahya et al. (2021) reported that Çamlıtepe and Hemşin propolis displayed antiproliferative potency against the A549 cell line, with IC_{50} values of 22.25 and 2.88 µL/mL, respectively, after 72 h of treatment. However, these values are still higher compared to the potent activity observed in Çayeli propolis. This comparative analysis highlights Çayeli propolis as having notably higher antiproliferative potency against A549 cells compared to Algerian and Tunisian propolis extracts, as well as Çamlıtepe and Hemşin propolis samples.

Table 1 indicates that MEP demonstrated the highest activity in inhibiting the growth of cancerous lung cells with an IC_{50} value of 0.1821 µL/mL.HEP showed a more potent antiproliferative effect against A549 cell line than AEP. Conversely, the toxic effect of HEP against healthy cells was found to be higher than AEP but lower than the other two extracts.

In conclusion, all extracts showed an ability to inhibit the growth of lung cancer cells. Research indicates that phenolic acids, flavonoids, and terpenoids are key in

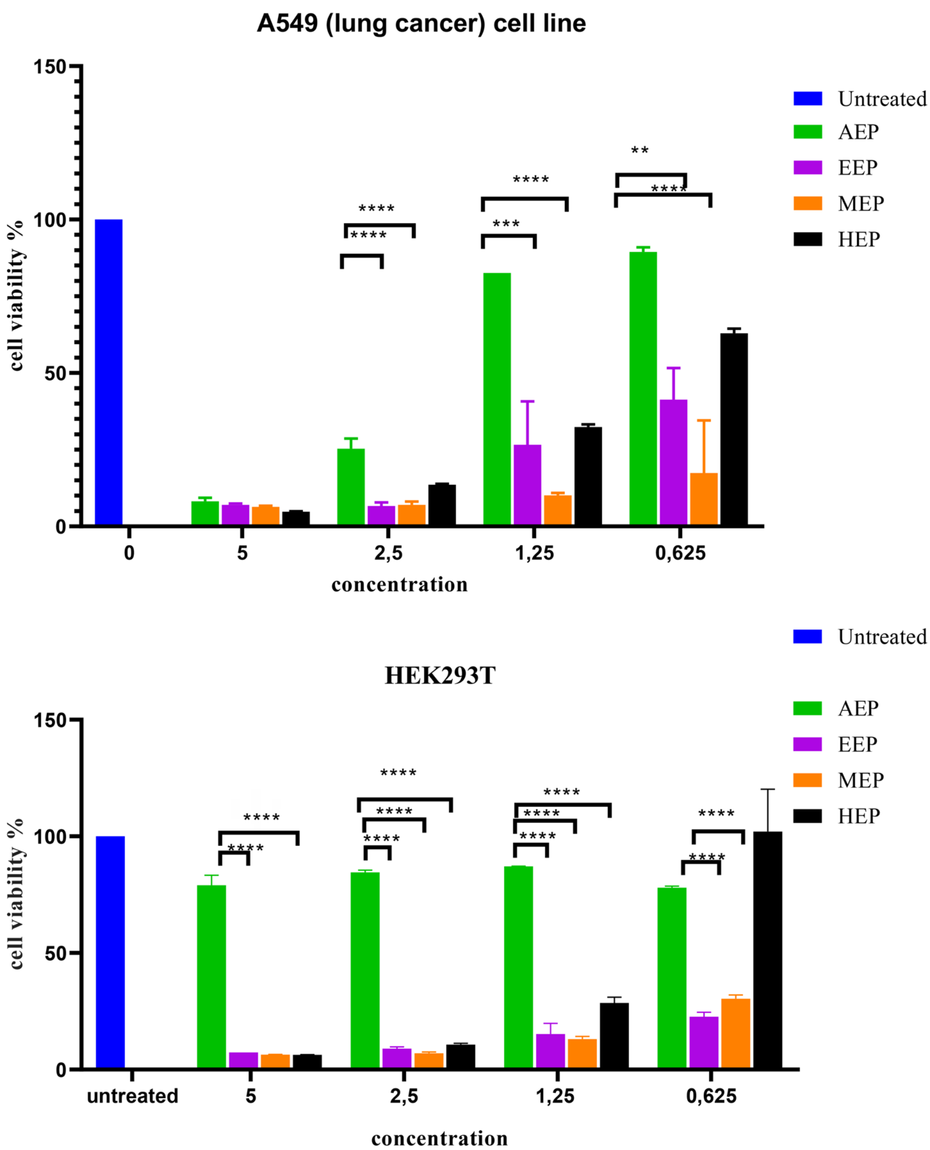

Figure 1. Cell viability rates depending on the concentrations of the extracts. Data were presented as mean ± SD. **$p < 0.001$, ***$p < 0.001$, ****$p < 0.0001$.

Table 1. IC_{50} results of the effect of propolis extracts against human cancer (A549) and healthy (HEK-293T) cell lines.

	IC_{50} (μL/ mL)	
Extracts	A549	HEK-293T
AEP	2.5690	>5
EEP	0.5417	0.3087
MEP	0.1821	0.3624
HEP	0.9298	1.1780

exerting antiproliferative effects (Nan et al. 2023). Compounds such as ferulic acid, *p*-coumaric acid, caffeic acid, taxifolin, quercetin, gallic acid, kaempferol, silibinin, apigenin and isopimaric acid likely contribute to the effects observed in this study. (Das et al. 2021; Sheikh et al. 2021; Kaur and Kaur 2022; Tuli et al. 2022; Goel et al. 2023).

2.3. Antimicrobial activity

The antimicrobial activity of propolis extracts is presented in Table S5. The extracts exhibited a range of activities, primarily varying between moderate and high levels. AEP at the highest concentration exhibited better antibacterial activity against *Staphylococcus epidermidis* compared to sulfamethoxazole, and kanamycin. Pseudomonas is a widely distributed genus that can cause hospital-acquired and opportunistic infections. Çayeli propolis was highly effective against *Pseudomonas aeruginosa*. Even at the lowest concentration of AEP, it was found to exhibit better inhibition activity than the antibiotics ampicillin, amoxicillin, and kanamycin. AEP also showed a better inhibitory effect on the growth of *Enterobacter aerogenes* and *Salmonella typhi*, particularly at higher concentrations. All concentrations of EEP showed higher activity against *S. typhi* compared to ampicillin. Moreover, concentrations of 0.5 ppm and 1 ppm exhibited greater antibacterial efficacy against *Proteus vulgaris* than both ampicillin and sulfamethoxazole. At 5 ppm, EEP demonstrated equivalent potency to ampicillin against *P. vulgaris.* However, *S. epidermidis* exhibited resistance to EEP. Interestingly, MEP at 1 ppm displayed the strongest activity against *Bacillus cereus* (30 mm). MEP showed superior inhibitory activity against *P. vulgaris* at concentrations higher than 0.5 ppm compared to commercial antibiotics. HEP at 1 ppm and 2 ppm exhibited equivalent potency to sulfamethoxazole and kanamycin against *S. epidermis*, and matched the inhibitory action of amoxicillin against *B. cereus*. Moreover, concentrations of 1 ppm (20 mm) and 2 ppm (20 mm) demonstrated higher inhibition against *S. typhi* compared to commercial ampicillin (11 mm), sulfamethoxazole (17 mm), and amoxicillin (19 mm). Additionally, 1 ppm also displayed significant activity against *P. vulgaris* compared to the standard drugs. Overall, *Candida albicans* showed greater sensitivity to HEP and MEP.

An interesting observation in our study is that certain propolis extracts demonstrated higher antibacterial activity at lower concentrations compared to higher ones. This phenomenon may be due to several factors: antimicrobial compounds might exhibit peak efficacy at specific lower concentrations, beyond which increasing the concentration does not proportionally enhance activity and may even reduce it due to saturation effects. At higher concentrations, saturation or non-specific interactions could interfere with the assay, diminishing inhibition despite the increased dosage. Altered bioavailability or interactions at elevated concentrations might also reduce the effectiveness. Additionally, different bacteria might respond variably to concentration changes, with some microorganisms being more sensitive to lower concentrations (Li et al. 2017). Certain propolis extracts, particularly excluding EEP, exhibit high susceptibility against specific Gram-positive bacteria at certain concentrations. However, a broader analysis reveals that propolis extracts provide more consistent and pronounced antibacterial activity against Gram-negative bacteria. For example, the aqueous extract at 2 ppm demonstrated significant inhibition against *S. epidermidis* (26 mm), the methanol extract at 1 ppm showed high activity against *B. cereus* (30 mm), and the hexane extract at 1 ppm exhibited notable activity against *S. epidermidis* (25 mm). Comprehensive data indicates that Çayeli propolis generally exerts more consistent antibacterial effects against Gram-negative bacteria. Previous studies suggest that propolis is more effective against Gram-positive bacteria, as noted by Przybylek and Karpinski (2019) and Erturk et al. (2016). Our findings of enhanced activity against Gram-negative bacteria contrast with these reports, possibly due to differences in bacterial cell wall structures. The additional outer membrane of Gram-negative bacteria may act as a barrier to certain antimicrobial agents, affecting their susceptibility (Afzal et al. 2017).

The antimicrobial activity of Çayeli propolis may also be attributed to its constituents such as flavonoids, fatty acids, phenolic acids, diterpenoids, and benzoic acid. These compounds exert their activity *via* various mechanisms of action. For instance, phenolic acids damage the integrity of bacterial cell walls, flavonoids inhibit essential bacterial processes such as energy metabolism, cytoplasmic membrane function, or nucleic acid synthesis, and fatty acids destabilise bacterial cell membranes (Borges et al. 2013; Casillas-Vargas et al. 2021; Shamsudin et al. 2022). All these findings indicate that the antimicrobial activity of propolis is versatile, influenced by both its specific composition and the structural characteristics of targeted bacterial species. Further research is required to elucidate the precise mechanisms by which Çayeli propolis interacts with different bacterial cell types.

In summary, while literature suggests a general trend towards higher susceptibility of Gram-positive bacteria to propolis, our findings of enhanced activity against Gram-negative bacteria provide new insights. This supports the idea that the antimicrobial activity of propolis is multifaceted and is influenced by bacterial cell wall properties and the specific propolis composition.

4. Conclusion

To conclude, this study investigated the chemical composition and biological activity of Çayeli propolis. The analysis revealed that Çayeli propolis contains a diverse range of chemical constituents, including diterpenoids, fatty acids, organic acids, flavonoids, sugars, high-molecular-weight aliphatic hydrocarbons, and mineral elements. Notably, Çayeli propolis is particularly rich in benzoic acid, phenolic acids, and flavonoids. This richness in bioactive compounds contributes to its notable biological activities. The study demonstrated that all propolis extracts were effective in inhibiting lung cancer cell growth and exhibited moderate to high antibacterial and antifungal properties against both Gram-positive and Gram-negative bacteria, as well as yeast.

These findings suggest that Çayeli propolis has potential as an alternative or complementary resource in combating microbial infections and may offer promising avenues for the development of antimicrobial and anticancer products. Overall, Çayeli propolis shows promise as a valuable natural resource with significant potential for further research and application in these fields.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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