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ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF SOME PLANT EXTRACTS WITH PROPOLIS

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ABSTRACT

For many years, plants have been utilized in food, healing materials, and curing for many illnesses. Lately, improvements in biological searches have displayed the notable potential of natural compounds.
Objective: In this study, biological activities of extracts of herbal mixtures with propolis were investigated.
Materials and Methodology: Ethanol and hexane extracts of propolis-*Syzygium aromaticum* mixture, propolis-*Papaver somniferum* mixture, propolis-*Foeniculum* sp. mixture were used in the assays.
Results: Ethanol extracts exhibited higher antibacterial activity compared to hexane extracts. While ethanol extracts inhibited bacterial growth ranges from 7±1.41 mm to 19.5±2.12 mm, hexane extracts showed inhibition zones ranges from 7±0.00 mm to 14±1.41 mm. The maximum and the minimum total phenolic contents were detected in propolis-S. *aromaticum* ethanol extracts as 389.81±0,001 µg GAE/mL and in propolis-*Foeniculum* sp. as 100.57±0.012 µg GAE/mL, respectively.
Conclusion: Studied plant extracts with propolis might be an option to synthetic antioxidant and antibacterial compounds.

Keywords: Antibiotic, Antioxidant, Free Radical

1. INTRODUCTION

Plant are valuable sources that can be utilized in many industries. Products based on natural substances attract attention in many industries, such as cosmetics and pharmacy in recent years Stuper-Szablewska et al. (2022).

Antimicrobial agents have important roles against pathogens. On the other hand, using excessive antimicrobial in the world cause drug resistance and undesirable effects. Many bacteria gained resistant to current antibiotics. The increase in untreatable infections require the discovery and development of brand antibacterials.

Plants have been utilized to cure bacterial infections thousands of years ago. Many people in developing countries utilize herbal drugs for antibacterial illnesses Liang et al. (2022).

Oxidative stress may lead Alzheimer's, cancer, and cardiovascular illnesses. Free radicals can be harmful proteins, lipids, nucleic acids and carbohydrates. The utilization of antioxidants can delay or retard the oxidation of biomolecules. Medicinal plants have been utilized as natural antioxidant agents for centuries by people Guchu et al. (2020).

Synthetic antioxidants create negative health effects. Hence, studies about natural antioxidants is valuable. Polyphenolic compounds can behave as antioxidants and free radical scavengers. Studies about polyphenols from plants has now achieve considerable attention Shahinuzzaman et al. (2020).

Papaver somniferum is an annual plant which belons to the family Papaveraceae. P. somniferum use as a foodstuff in manufacturing of bakery products. Moreover, it's oil has various medicinally important metabolites Chmelová et al. (2018).

Syzygium aromaticum possess various medical features like antimutagenic, antibacterial, anti-inflammatory and radical scavenging activity Faujdar et al. (2020). Moreover, It has antioxidant action so it utilizes in preventing some degenerative diseases. *Syzygium aromaticum* bud oil heals wounds and burns Alanazi et al. (2022).

Foeniculum sp. belongs to family Apiaceae and cultivated in India, China and Egypt. The volatile oil of fennel possess anti-allergic, diuretic, anti-spasmodic, antimicrobial and antioxidant potencies Eliuz et al. (2016).

Bees gather resins from plants, stir them with their own salivary enzymes and beeswax which composes propolis. Propolis possess many important effects such as antibacterial, radical scavenging, antiparasitic, antifungal and antiproliferative Przybylek & Karpinski (2019).

This investigation aims the compare biological features of extracts of propolis-*Syzygium aromaticum* mixture, propolis-*Papaver somniferum* mixture, propolis-*Foeniculum sp.* mixture.

2. MATERIALS AND METHODS

2.1. SUPPLYING OF PLANTS USED IN THE STUDY

Propolis, *Syzygium aromaticum, Papaver somniferum and Foeniculum* sp. were brought from a herbal shop in Giresun, Turkey.

2.2. TEST BACTERIA

Listeria monocytogenes ATCC 7644, *Salmonella enterica serovar typhimirium* ATCC 14028, *Staphylococcus aureus subsp. aureus* ATCC 25923, *Bacillus cereus* 702 ROMA, *Yersinia pseudotuberculosis* ATCC 911, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* IMG 22, *Enterobacter aerogenes* CCM 2531, *Gordonia rubripertincta* (lab isolate) and *Proteus vulgaris* (lab isolate) were utilized in antimicrobial activity tests.

2.3. PREPARATION OF THE EXTRACTS

15 g of propolis-15 g of *Syzygium aromaticum*, 15 g of propolis-15 g of P*apaver somniferum* and 15 g of propolis-15 g of *Foeniculum* sp. were extracted in a shaker

for 24 h utilizing 300 mL ethanol and hexane, separetely. The extracts were filtered through Whatman filter paper No. 1 and residues were evaporated (40 °C) with rotary evaporator Murugan & Parimelazhagan (2014).

2.4. ANTIBACTERIAL ACTIVITY

The discs (6 mm diameter) on the petri were impregnated with 20 μL of extracts, separetely. Gentamycine was used as standard antimicrobial agent. DMSO was used as negative control. Plates were incubated for 24 h at 37°C. Diameter of zones were measured with a ruler Murray et al. (1995), Šariš et al. (2009). The tests were carried out twice.

2.4.1. THE DETERMINATION OF MINIMUM INHIBITION CONCENTRATION (MIC)

Minimum inhibition concentration of extracts which created ≥10 mm inhibition zones were determined. Method of Yiğit et al. (2009) were used to reveal MIC values of the tested extracts Yiğit et al. (2009).

2.5. ANTIOXIDANT ACTIVITY

All antioxidant tests were carried out three times. Results are expressed as the mean ± standard deviation (S.D.) of each triplicate test.

2.5.1. TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND TOTAL ANTIOXIDANT CAPACITY

The total phenolic, flavonoid content and total antioxidant capacity were expressed as μg of gallic acid equivalent (GAE)/mL Slinkard & Singleton (1977), μg of cateschin equivalent (CE)/mL Zhishen et al. (1999) and μg of ascorbic acid equivalent (AAE)/mL Prieto et al. (1999), separetely.

2.5.2. CUPRAC ACTIVITY

CUPRAC activity of the extracts were studied according to the method of Özyürek et al. (2009). Butylated hydroxytoluene (BHT) was used as a standard antioxidant agent.

2.5.3. DPPH RADICAL SCAVENGING ACTIVITY

Extracts were prepared at 250-1000 μL/mL concentrations by the method of Blois (1958). BHT and Rutin were used as standards.

The DPPH radical scavenging activity was calculated using the following equation:

$$DPPH\ Radical\ Scavenging\ Activity\ (\%) = \frac{A0-A1}{A0} X100$$

A0: Absorbance of control

A1: Absorbance of sample or standard

3. RESULTS AND DISCUSSION

In the current study it was tested antibacterial efficiencies of ethanol and hexane extracts of propolis-*Syzygium aromaticum*, propolis-*Papaver somniferum* and propolis-*Foeniculum* sp. against test bacteria. Table 1 demonstrates inhibition zones.

Ethanol extracts exhibited higher activity than hexane extracts. Antibacterial activities of mixtures were increased in the following order: propolis-*Syzygium aromaticum*> propolis-*Papaver somniferum*> propolis-*Foeniculum* sp. While inhibition zones was created by ethanol extracts ranges from 7±1.41 mm to 19.5±2.12 mm, inhibition zones was created by hexane extracts ranges from 7±0.00 mm to 14±1.41 mm. Gentamycine showed higher activity when compared with tested extracts. DMSO showed no activity against test bacteria.

Table 1

Table 1 Inhibition Zones, Which Was Created by Extracts, DMSO and Gentamycine (mm)

Bacteria	PSE	PFE	PPE	PSH	PFH	PPH	CN	DMSO
B. cereus 702 ROMA	8.5±0.70	NA	7.5±0.70	11±1.41	7±0.00	9±0.00	18±1.41	NA
K. pneumoniae (lab isolate)	11±0.00	11±1.41	15.5±0.70	7.5±0.70	7.5±0.70	11±1.41	21±1.41	NA
E. aerogenes CCM 2531	12±1.41	NA	11±0.00	7±1.41	7±1.41	NA	20±1.41	NA
G. rubripertincta (lab isolate)	NA	NA	NA	14±1.41	8±0.00	15±0.00	19±1.41	NA
E. faecalis ATCC 29212	14.5±0.70	10.5±0.70	17±1.41	12.5±0.70	10.5±0.70	13±2.82	20.5±2.12	NA
P. vulgaris (lab isolate)	18.5±2.12	10.5±0.70	12±1.41	9.5±2.12	8.5±0.70	8.5±2.12	21.5±2.12	NA
S. enterica serovar typhimirium ATCC 14028	11.5±0.70	9±0.00	10±0.00	9±0.00	8.5±0.70	7.5±0.70	22±2.82	NA
L. monocytogenes ATCC 7644	16±1.41	8±0.00	11.5±0.70	7.5±0.70	7±0.00	NA	19.5±0.0	NA
B. subtilis IMG 22	7.5±0.70	7.5±0.70	7±1.41	8±0.00	7±1.41	7.5±0.70	16.5±2.12	NA
S. aureus subsp. *aureus* ATCC 25923	19.5±2.12	8±0.00	15.5±2.12	9.5±0.70	7.5±0.70	11±1.41	18.5±2.12	NA
Y. pseudotuberculosis ATCC 911	12±0.00	12.5±0.70	12.5±2.12	9±0.00	8±0.00	10.5±0.70	21.5±2.12	NA

PSE: Ethanol extract of propolis-*Syzygium aromaticum*; PFE: Ethanol extract of propolis-*Foeniculum* sp.; PPE: Ethanol extract of propolis-*Papaver somniferum*; PSH: Hexane extract of propolis-*Syzygium aromaticum*; PFH: Hexane extract of propolis-*Foeniculum* sp.; PPH: Hexane extract of propolis-Papaver *somniferum*; CN: Gentamycine 10 µg/mL, NA: No Activity

MIC values of the extracts were given in Table 2. MIC describes as the minimum antimicrobial agent that inhibits the growth of microorganisms. Low MIC values means higher antibacterial effect. While the lowest MIC value was found in PSE as 0.0003 mg/mL against S. *aureus*, the highest MIC value was found in PSH as 1.5 mg/mL against B. *cereus* and in PPH as 1.5 mg/mL against Y. *pseudotuberculosis.*

Table 2

Table 2 MIC values of the extracts (mg/mL)

Bacteria	PSE	PFE	PPE	PSH	PFH	PPH
B. cereus 702 ROMA	-	-	-	1.5	-	-
K. pneumoniae (lab isolate)	0.0468	0.0468	0.1875	-	-	0.75
E. aerogenes CCM 2531	0.0468	-	0.0058	-	-	-

G. rubripertincta (lab isolate)	-	-	-	0.1875	-	0.1875
E. faecalis ATCC 29212	0.0117	0.0117	0.1875	0.1875	0.75	0.1875
P. vulgaris (lab isolate)	0.375	0.1875	0.1875	-	-	-
S. enterica serovar typhimirium ATCC 14028	0.1875	-	0.375	-	-	-
L. monocytogenes ATCC 7644	0.1875	-	0.1875	-	-	-
B. subtilis IMG 22	-	-	-	-	-	-
S. aureus subsp. *aureus* ATCC 25923	0.0003	-	0.1875	-	-	0.0234
Y. pseudotuberculosis ATCC 911	0.1875	0.1875	0.1875	-	-	1.5

- **Antioxidant activity**

Table 3 summarizes total phenolic content of the extracts. When total phenolic content was compared between extracts, it was found that ethanol extracts have higher phenolic content than hexane extracts. The highest and the lowest phenolic contents were found as 389.81±0.001 µg GAE/mL and 100.57±0.012 µg GAE/mL in PSE and PFH, respectively.

Table 3

Table 3 Total Phenolic Contents of the Extracts (µg GAE/mL)

Extract	Total Phenolic Content (µg GAE/mL)
PSE	389.81±0.001
PFE	169.3±0.001
PPE	286.93±0.003
PSH	205.03±0.003
PFH	100.57±0.012
PPH	142.15±0.004

Total flavonoid content of the extracts were presented in Table 4. Ethanol extracts possess higher flavonoid contents than hexane extracts. Total flavonoid contents of the extracts as follows: PSE>PPE> PSH>PFE>PFH>PPH.

Table 4

Table 4 Total Flavonoid Content of the Extracts (µg QE/mL)

Extract	Total Flavonoid Content (µg QE/mL)
PSE	91.25±0.024
PFE	22.31±0.022
PPE	68.43±0.016
PSH	57.5±0.013

PFH	13.64±0.010
PPH	1.7±0.002

Total antioxidant capacity of the extracts were given in Table 5. The highest and the lowest values were found as 190.54±0.031 µg AAE/mL in PSH and 46.03±0.018 µg AAE/mL in PFH.

Table 5

Table 5 Total Antioxidant Capacity of the Extracts (µg AAE/mL)

Extract	Total antioxidant capacity (µg AAE/mL)
PSE	119.33±0.021
PFE	65.44±0.012
PPE	147.11±0.009
PSH	190.54±0.031
PFH	46.03±0.018
PPH	65.85±0.005

Table 6 shows DPPH radical scavenging activity of the extracts. It was found no activity in PFH and PPH. PSE exhibits higher activity than Rutin and BHT which were used as standard antioxidant agents.

Table 6

Table 6 DPPH Radical Scavenging Activity of the Extracts and Standards

Extract	Concentration (µg/mL)	DPPH Radical Scavenging Activity (% inhibition)
PSE	250	91.08±0.002
	500	91.87±0.001
	750	93.14±0.001
	1000	94.42±0.003
PFE	250	86.62±0.002
	500	87.26±0.001
	750	89.35±0.005
	1000	89.72±0.004
PPE	250	87.03±0.003
	500	87.8±0.003
	750	89.49±0.002
	1000	89.95±0.004
PSH	250	85.01±0.007
	500	86.94±0.004
	750	88.74±0.002
	1000	89.41±0.005

PFH	250	NA
	500	NA
	750	NA
	1000	NA
PPH	250	NA
	500	NA
	750	NA
	1000	NA
BHT	250	88.85±0.012
	500	89.55±0.005
	750	90.27±0.011
	1000	91.55±0.008
Rutin	250	86.80±0.008
	500	87.91±0.003
	750	90.6±0.004
	1000	91.89±0.011

NA: No Activity

CUPRAC activity of the extracts were presented in Table 7. The highest activity was determined in PSE. All extracts were showed higher activity than BHT except for PPH and PFH.

Table 7

Table 7 CUPRAC Activity of the Extracts and Standard

Extract	Concentration (µg/mL)	CUPRAC Activity (nm)
PSE	250	2.022±0.018
	500	2.073±0.010
	750	2.104±0.008
	1000	2.143±0.003
PFE	250	2.007±0.003
	500	2.029±0.004
	750	2.066±0.005
	1000	2.081±0.056
PPE	250	1.902±0.006
	500	1.988±0.008
	750	2.004±0.003

	1000	2.015±0.003
PSH	250	2.009±0.008
	500	2.062±0.009
	750	2.091±0.005
	1000	2.136±0.004
PFH	250	0.569±0.038
	500	0.473±0.019
	750	0.650±0.016
	1000	0.840±0.003
PPH	250	0.352±0.027
	500	0.427±0.035
	750	0.620±0.008
	1000	0.812±0.012
BHT	250	0.6945±0.023
	500	0.7519±0.020
	750	0.8509±0.029
	1000	1.0567±0.012

There is no study about antibacterial activity of propolis-*Syzygium aromaticum*, *propolis-Papaver somniferum and propolis-Foeniculum* sp. *On the other hand, there are many studies about propolis, Syzygium aromaticum, Papaver somniferum and Foeniculum sp.*

One of the most common and known most studied properties of propolis is its antimicrobial activity. Numerous studies have been conducted on its effects on fungi and viruses Albayrak & Albayrak (2008).

Veiga et al. (2017) reported that propolis inhibited gram posivite bacteria and gram negative bacteria such as methicilline resistant *Staphylococcus aureus*. Yildirim et al. (2016) investigated effect of propolis against tuberculosis and they found that propolis is efficient against many mycobacteria species.

It was recorded that propolis has antibacterial activity against many aerobic bacteria such as *Bacillus cereus, Bacillus subtilis, Enterococcus faecalis, Micrococcus luteus, Nocardia asteroids, Staphylococcus auricularis, Staphylococcus epidermidis, Staphylococcus capitis, Staphylococcus haemolyticus and Staphylococcus warnerius* Fokt et al. (2010).

In a study which was carried out by Nzeako et al. (2006) water extract and essential oil of *Syzygium aromaticum* showed antibacterial activity against *Streptococcus pyogenes, Corynebacterium spp.*. *Salmonella spp.* and *Bacteroides fragilis.*

Gupta & Prakash (2021) used extracts and essential oil of *Syzygium aromaticum against Halobacteria sp., Lactobacillus sp., Pseudomonas sp., Micrococcus sp. and Streptococcus mutans* which cause dental plaques and cavities. It was concluded that essential oil of *Syzygium aromaticum* had better activity than extracts of *Syzygium aromaticum*.

Masood et al. (2008) found aqueous infusions of *Papaver somniferum* seeds had no activity and aqueous decoction had very weak activity against 188 bacterial isolate.

Mishra and Pathak (2021) reported methanol and water extracts of *Papaver somniferum* seeds had activity against *Salmonella* and *Escherichia coli* but they were no activity against *Pseudomonas*.

Eliuz et al. (2016) revealed essential oil of Foeniculum sp.had antibacterial effect against *E. coli, Klebsiella pneumoniae, Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus and Enterococcus faecalis*.

Yıldırım et al. (2010) carried out a study about antibacterial activity of *Foeniculum sp*. It was reported that ethanol and aqueous extracts of Foeniculum sp. didn't inhibit *E. coli, S. aureus, B. cereus, K. pneumoniae and Enterobacter sp.* but methanol extract of *Foeniculum sp. inhibited S. aureus and Enterobacter sp.*

Like antibacterial studies, there is no study about antioxidant activity of propolis-*Syzygium aromaticum,* propolis-*Papaver somniferum* and propolis-*Foeniculum* sp. On the other hand, there are many studies about propolis, *Syzygium aromaticum, Papaver somniferum and Foeniculum sp.*

Kocot et al. (2018) found that extracts of propolis had better DPPH and ABTS radicals scavenging activities than standard antioxidant agents such as BHT and ascorbic acid.

Can et al. (2016) investigated total phenolic content and antioxidant activity of propolis samples from Azerbaijan. It was stated that total phenolic contents of samples ranges from 10.94 to 79.23 mg GAE/g. Propolis samples which was obtained from Ismayilli. Zerdap and Qax had higher antioxidant activity when compared with other districts.

Muhson & Al-Mashkar (2015) investigated total phenolic content, total flavonoid content and DPPH radical scavenging activity and acetone extracts of stem and fruit parts of *Syzygium aromaticum*. DPPH radical scavenging activity was found %87.50 and %79.41 in fruit and stem, respectively.

Elaleem et al. (2017). studied DPPH radical scavenging activity of methanol, petroleum ether and chloroform extracts of seeds of *Papaver somniferum*. It was found that only methanol extract had activity.

4. CONCLUSION

The results revealed that these plant-propolis mixtures could play as a promising antibacterial and antioxidant agents, because of their high activities. Moreover, the current study suggests that these mixtures might be developed as pharmaceutical products. Hence, studies on the isolation and identification of substances responsible for biological activities in these mixtures should be expanded.

CONFLICT OF INTERESTS

None.

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