

Antimicrobial activity of Iranian propolis and its chemical composition

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ABSTRACT

The objective of this study was to investigate the antimicrobial activity of ethanol extract of Iranian propolis on some microorganisms using disc diffusion method. The chemical composition of the propolis was also investigated using thin layer chromatography and spectrophotometric methods. Ethanol extract of propolis showed activity only against Gram-positives and fungi, whereas no activity was observed against Gram-negatives. Thin layer chromatography screening revealed the presence of pinocembrine, caffeic acid, kaempferol, phenethyl caffeate, chrysin, and galangin in Iranian propolis. The total flavonoid and phenolic contents were 7.3% and 36%, respectively, which suggests that the strong antimicrobial activity of Iranian propolis may be due to high levels of phenolic and flavonoid compounds.

Keywords: Iranian propolis, Antimicrobial activity, Phenolic compounds, Flavonoid compound.

INTRODUCTION

Propolis is a mixture of beeswax and resins collected by the honeybee from plant buds, leaves, and exudates (1). Bees use propolis not only as a building material but also as a means of maintaining low levels of bacterial and fungal concentrations in the hive (2).

Propolis has long been used in oriental folk medicine for curing infections (3) and in European ethno-pharmacology as an antiseptic and anti-inflammatory agent for healing wounds and burns (1).

Many pharmaceutical properties including antibacterial (4), antifungal (5), antiviral (6), anti-protozoan (7), anti-inflammatory (8), antioxidant (9), hepatoprotective (10), immunostimulating (11), antitumor (12), and cytostatic (13) activities have been reported for propolis; hence, its wide recognition as a useful substance in medicine (14).

More than 150 components such as polyphenols, phenolic aldehydes, sesquiterpene quinines, coumarins, amino acids, steroids and inorganic components have been identified in propolis samples (15). The properties and chemical composition of propolis vary with geographical origin (5) and the differences in chemical composition are basically due to differences in the bearing plants (16). Although many active components have been identified in propolis (14, 15) and its antimicrobial activity has been demonstrated for different microorganisms (1),

there is no report that we know on antimicrobial activity of Iranian propolis. The objective of the present study was to investigate the antimicrobial activity of ethanol extract of Iranian propolis and to analyze its chemical compositions.

MATERIALS AND METHODS

Extraction of propolis

Propolis sample was collected from an experimental apiary located in the Lavark area, Isfahan, central Iran. Hand collected propolis was kept in a dry place and stored at 4 °C until its processing. The sample was cut into small pieces, grounded and extracted with 80% ethanol (1:10 w/v) in a shaker (300 rpm) at room temperature for 48 h. The ethanol extract solution was then filtered through a Whatman # 41 filter paper. Based on the dry weight of the solution, the ethanol extract of propolis (EEP) solution was further adjusted with appropriate amounts of 80% ethanol to obtain solutions containing various amounts of EEP.

Microorganisms

The following microorganisms were used in this study to test antimicrobial activity of propolis. *Staphylococcus aureus* PTCC 1189, *Staphylococcus epidermidis* PTCC 1114, *Bacillus subtilis* PTCC 1023, *Bacillus cereus* PTCC 1015, *Bacillus liqueiformis* PTCC 1331, *Candida albicans* PTCC 5027, *Salmonella enteritidis*

PTCC 1091, *Escherichia coli* PTCC 1038, *Klebsiella pneumoniae* PTCC 5027, and *Proteus vulgaris* PTCC 1182. All microorganisms were provided by Biotechnology Institute, Iranian Research Organization for Sciences and Technology, Tehran.

Preparation of inoculums

All bacteria were cultured for 8 h at 37° C in a liquid medium (brain heart infusion) and used as inoculums. The yeast was sub-cultured in yeast glucose broth at 30° C for 8 h and then used for the test. The turbidity of the suspension was adjusted to the McFarland 0.5 turbidity standard.

Antimicrobial activity

Antimicrobial activity of propolis ethanol extract was investigated by the disc diffusion method (17). The antimicrobial screening was performed using brain heart infusion broth for bacteria and sabouraud dextrose agar for yeasts.

Sterile paper discs (Whatman # 4 paper, 6 mm diameter) were loaded with 5 µl of propolis extract dilutions (67, 16.7, 8.3, 4.1, 2.0, 1.0, 0.2, 0.1, 0.06, and 0.01 mg/ml). Discs were dried for 5 hrs at 37 °C in a sterile incubator and then placed on seeded agar plates. Six discs were applied to each petri dish. The 80% ethanol and commercial disc of ampicilline (1µg- oxford) were used as negative and positive controls, respectively. Plates were incubated at 37° C and 30° C for 24 h for bacteria and *C. albicans*, respectively. Inhibitory zone diameters were measured with Scion Image software (Scion Corporation, Frederick, MD). All experiments were performed in triplicates. Values are expressed as means. The data were submitted to analysis of variance using general liner model procedure of statistical analyzer software (18). The chosen level of significance for all statistical tests was $P < 0.01$.

Thin layer chromatography analysis

Thin layer chromatography (TLC) analysis of the propolis ethanol extract was performed on silica gel (Alufolien Kieselgel Merck F₂₅₄) with mobile phase petroleum ether/ethyl acetate 7:3. Spots were visualized by U.V. light (366nm); spraying with 60% sulfuric acid in ethanol and heating at 100 °C. Standard substances were used as described in the literature (2).

Estimation of total flavonoids and phenolics content

Total flavonoid content was determined using a colorimetric method by a previously described method. (19). The Folin-Ciocalteu method was used to determine the quantities of total phenolics (20).

RESULTS AND DISCUSSION

The mean diameters of microbial growth inhibited by different concentrations of EEP are shown in Table 1. Among the bacteria and fungi, *B. cereus* was the most sensitive to the highest concentration of EEP (67 mg/ml). The sensitivity of the tested microorganisms followed the order: *B. cereus* > *B. liqueniformis* > *S. epidermidis* > *S. aureus* and *C. albicans* > *B. subtilis*. The least active concentrations against the tested microorganisms were 4.1 mg/ml for *C. albicans* and *S. epidermidis*, 2.0 mg/ml for *S. aureus* and *B. cereus*, and 8.3 mg/ml for *B. subtilis* and *B. liqueniformis*. The 80% ethanol (negative control) did not show any inhibitory effects on the tested microorganisms. The results also showed that, at a concentration of 67 mg/ml, EEP was more effective than standard ampicilline on *S. aureus*, *S. epidermidis* and *B. cereus* strains, with significant differences ($P < 0.01$), but less active on *B. subtilis*.

Ethanol extract of propolis showed activity only against Gram-positive bacteria and fungi, whereas, no activity was observed against Gram-negative bacteria. Similar results have been reported in other studies (21, 8, 4, 22), which support our findings that propolis is mainly active against Gram-positives. However it has been reported that EEP is effective on Gram-negative bacteria at higher concentrations (23).

The studies carried out on the antimicrobial activity of propolis show conflicting results (14). The variation in the antimicrobial activity of propolis has been attributed to the differences in its chemical components (14). For example, correlation has been shown between the flavonoid content and antimicrobial activity of propolis against *B. Subtilis* (24). It has also been reported that the flavonoid content varies considerably in the 38 samples collected from different parts of Croatia with different climates and vegetation (24). The TLC screening showed eleven spots upon exposure to U.V. light (Figure 1). Compared to standard substances, only six out of eleven spots were recognized, showing the presence of flavonoid aglycones such as pinocembrin, chrysin, galangin, and kaempferol, phenolic acids like caffeic acid and esters such as phenethyl caffeate. It is well documented that among the most potent antimicrobial compounds in propolis are flavonone pinocembrin and flavonol galangin (25). Caffeic acid and its esters, volatile fractions with phenols, terpenoids and chrysin have also exhibited notable antimicrobial activity as well (26). No spot was observed in the TLC pattern sample after obtaining a retention factor (R_f) of 0.64, indicating that the pinostrobin compound ($R_f = 0.82$) either is not present or has

Table 1. The mean of the diameters¹ (mm) of microbial growth inhibited by different concentrations of ethanol extract of Iranian propolis and Ampicilline.

Microorganism	EEP ² concentrations										Ampicilline	S.E.
	1	2	3	4	5	6	7	8	9	10		
<i>S. epidermidis</i>	4.8 ^a	2.6 ^c	1.7 ^d	1.0 ^e	-	-	-	-	-	-	3.0 ^b	0.10
<i>S. aureus</i>	4.1 ^a	2.0 ^b	1.5 ^c	1.0 ^d	1.0 ^d	-	-	-	-	-	2.2 ^b	0.13
<i>B. cereus</i>	6.1 ^a	3.8 ^b	3.1 ^c	2.1 ^d	1.0 ^e	-	-	-	-	-	4.1 ^b	0.20
<i>B. subtilis</i>	2.6 ^b	1.3 ^c	0.5 ^d	-	-	-	-	-	-	-	9.6 ^a	0.20
<i>B. liqueniformis</i>	5.1 ^a	2.1 ^b	1.0 ^c	-	-	-	-	-	-	-	-	0.13
<i>S. enteritidis</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>P. vulgaris</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>K. pneumoniae</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>C. albicans</i>	4.1 ^a	2.6 ^b	1.7 ^c	1.0 ^d	-	-	-	-	-	-	-	0.22

¹Values expressed are averages of three replicates. Values within each row followed by different letters are significantly different at P<0.01.

²1: 67mg/ml, 2: 16.7 mg/ml, 3: 8.3 µg/ml, 4: 4.1 mg/ml, 5: 2.0 mg/ml, 6: 1.0 mg/ml, 7: 0.2 mg/ml, 8: 0.1 mg/ml, 9: 0.06 mg/ml, 10: 0.01 mg/ml.

a too low concentration to be detected. Furthermore, the sample used was free from benzyl ferulate ($R_f = 0.57$).

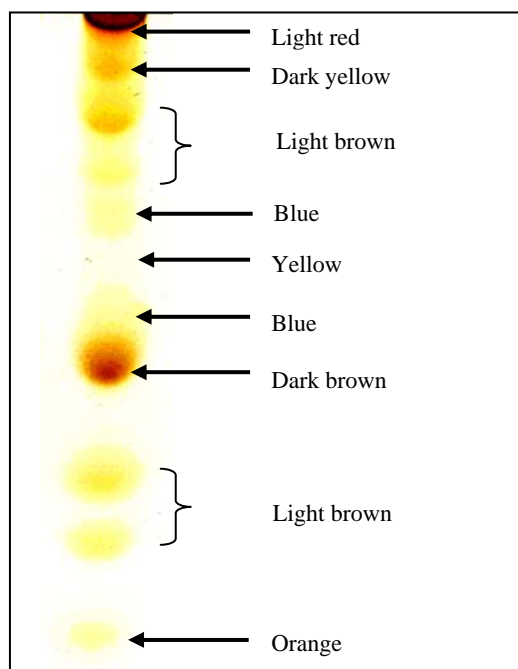


Figure 1. Charting Thin layer chromatography (visible at daylight) of ethanol extract of Iranian propolis. Colors are reported as seen at U.V. light (366 nm).

The sample was also analyzed for total flavonoid and total phenolic contents, using spectrophotometric procedures. The total flavonoid and phenolic components were 7.3% and 36%, respectively. The concentrations of these compounds compared to Turkish propolis (2) were considerably higher.

CONCLUSION

The results of this study indicate the antimicrobial activity of ethanolic extract of Iranian propolis. From these results it may be concluded that Gram-positives bacteria are more susceptible to EEP antibacterial activity than Gram-negatives bacteria. The results of TLC analysis confirmed the presence of pinocembrin, caffeic acid, kaempferol, phenethyl caffeate, chrysin, and galangin in Iranian propolis. The strong antimicrobial activity of Iranian propolis may be due to high total phenolic and flavonoid contents. There are numerous questions yet to be answered concerning chemical compositions and antibacterial properties of Iranian propolis and further research is required for clarification.

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