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Full Length Research Paper

Antimicrobial activity and chemical compositions of Turkish propolis from different regions

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The antimicrobial activity of propolis against some gram-positive and gram-negative bacteria and its chemical composition were evaluated by the method of agar-well diffusion and GC-MS, respectively. Some typical compounds samples were identified in the propolis samples. Principal component analysis revealed that the chemical composition of propolis samples was distinct based on the geographical location of the samples. It was found that flavonoids, chrysin, flavanones and cinnamic acid were the predominant compounds in the propolis samples.

Key words: Propolis, chemical composition, antimicrobial activity.

INTRODUCTION

Propolis is a resinous substance collected by honeybees from various plant sources. It is one of the oldest known medicines that are used even today in folk medicine. The medicinal properties of propolis and its use as a therapeutic substance have been known since ancient times (Cardile et al., 2003; Castaldo and Capasso, 2002). It is antibacterial, antifungal, antitrypanosomal and antiviral (Kartal et al., 2003; Prytzyk et al., 2003; Kujumgiev et al., 1999; Güler et al., 2003). Some studies have also shown that propolis has antioxidant, anticancer and anti-inflammatory properties (Wang et al., 2004; Kumazawa et al., 2004; Akao et al., 2003; Blonska et al., 2004). The chemical composition of propolis is quite complicated. Its compounds and biological activities depend on many different factors such as the geographical region, collecting time, and plant source (Bankova et al., 2002; Sforcin et al., 2000).

Although numerous researchers have been reported the biological activities of propolis collected in Europe, information about Turkish propolis are still limited. The aim of this study is to investigate antimicrobial activity of propolis samples from different locations in Turkey and to correlate their antimicrobial activity with their chemical composition.

MATERIALS AND METHODS

Propolis samples and microorganisms

Three samples of propolis were collected from Trabzon, Erzurum and Tekirdağ in 2004. The following eight microorganisms were used in this study: *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 27736, *Pseudomonas aeruginosa* ATCC 27853, *Morganella morganii* (clinical isolate), *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Proteus vulgaris* RSKK 96026 and *Candida albicans* (clinical isolate).

These bacteria were obtained from Pamukkale University, Department of Biology. The cultures of microorganism were sub-cultured on appropriate agar slopes. These stock cultures were stored in the dark at 4°C during the survey.

Preparation of ethanol extracts of propolis (EEP)

Each sample was cut into small pieces after cooling at -20°C, and extracted with 70 and 96% ethanol (1:10, w/v) at 37°C for 5 days. The ethyl alcohol extract was then filtered through a Whatman No. 1 filter paper and evaporated to dryness under vacuum. The dry residual powder was kept at -20°C. About 5 mg of residue were mixed with 75 µl of dry pyridine and 50 µl bis (trimethylsilyl) trifluoroacetamide (BSTFA), heated at 80ºC for 20 min and then the final supernatant was analyzed by GC-MS (Sorkun et al., 2001).

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Table 1. Antimicrobial activities of ethanol extract of Turkish propolis obtained from different regions.

Tested microorganisms	Compound			Standard antibiotic	Zone (mm)
	TE	TR	ER		
Pseudomonas aeruginosa ATCC 27853	++	++	++	Gentamicin (10 µg)	16
Escherichia coli ATCC 35218	+++	+++	+++	Penicillin (10 U)	19
Morganella morganii	+++	θ	θ	Chloramphenicol (30 µg)	15
Klebsiella pneumoniae ATCC 27736	+	θ	θ	Streptomycin (15 µg)	11
Proteus vulgaris RSKK 96026	++	+	++	Ampicillin (10 µg)	10
Staphylococcus aureus ATCC 25923	+	+	+	Ampicillin (10 µg)	9
Bacillus subtilis ATCC 6633	++	++	++	Ampicillin (10 µg)	15
Candida albicans	+++	++	++	Ketoconazole (50 µg)	16

[a]The inhibitory zone was expressed in mm; +: ≤ 8 mm; ++: >8 to < 12 mm; +++: ≥ 12 mm; θ: no inhibition. TE: Tekirdağ, TR: Trabzon; ER: Erzurum.

Table 2. Chemical composition of propolis samples by determined GC/MS

Compound	Trabzon*	Erzurum*	Tekirdağ
Aromatic alcohol	0.15	0.21	0.25
Aromatic acid	0.33	0.31	0.88
Alcoholic terpenes	Very low	0.31	-
Aromatic esters	0.512	0.44	-
Flavanones	4.55	4.72	3.32
Cinnamic acid	1.77	1.09	0.84
Flavonoids	16.45	16.86	4.57
Naringenin	2.76	1.20	8.39
Apigenin	7.89	11.70	14.02
Crysin	13.05	17.89	22.17

* The results of GC/MS were taken from Sorkun et al. (2001).

Antibacterial and antifungal activity tests

Antimicrobial activity of seven propolis samples was determined by the agar-well diffusion method. The antimicrobial activity was performed using nutrient agar for bacteria and YEPD Agar for yeast. The cell culture suspension was adjusted by comparing against 0.4-0.5 McFarland scale standard. These suspensions (100 µl) of target strain were spread on the plates.

For the investigation of the antibacterial and antifungal activity, all extract of propolis were weighed and dissolved with 70 and 96% ethanol to obtain 50 mg/ml and 200 mg/ml extract concentration. These solutions were filtered with a pore size of 0.2 µm. Each sample (100 µl) was filled into the wells of agar plates directly. The diameter of the inhibition zone (mm) was measured after overnight incubation. Commercial discs of gentamicin (10 µg), penicillin (10 U), chloramphenicol (30 µg), streptomycin (15 µg), ampicillin (10 µg) and ketoconazole (50 µg) were used as positive control. All determinations were done duplicate.

Statistical analysis

Data were analyzed and treatments compared using the one-way ANOVA with 95% confidence limits ($p<0.05$) (SPSS 11.0 Version).

RESULTS AND DISCUSSION

In the present study, the antimicrobial activity and chemical compounds of ethanolic extract of propolis collected from different regions of Turkey in 2004 were determined. Antimicrobial activities of propolis gathered from different regions against test organisms are summarized in Table 1. These extracts showed different inhibitory effect against all the tested bacteria.

In generally, ethanol extracts of propolis (EEP) samples did not exhibit inhibitory action on the *K. pneumonia* ATCC 27736 and *M. morganii* (clinical isolate), but thesamples showed a strong inhibitory effect on the growth of *E. coli* ATCC 35218 among gram-negative bacteria.

On the other hand, antifungal activity was showed against *C. albicans* at an amount of 200 mg/ml of propolis samples. The control (96% ethanol, v/v) did not form an inhibitory zone against any of the microorganisms tested. However, the propolis samples due to antibiotic discs no differ significantly in their activity against test microorgan-

isms (F=1.015; df: 12, α=0.05).

Chemical properties are important parameters for propolis characterization and to correlate between antimicrobial activity and chemical composition. Therefore, the three propolis samples were analyzed by GC-MS and determined the chemical patterns. Table 2 shows the major components of propolis samples obtained by ethanol extraction. It was found that flavonoids, apigenin, chrysin, flavonones, and naringenin were the predominant compounds in the samples. Bankova et al. (2002) studied the chemical composition of ten propolis samples from Bulgaria, Italy and Switzerland and concluded that they contain mainly pinocembrin, pinobanksin, chrysin, galangin, prenyl esters of caffeic and ferrulic acids. Numerous researchers have been reported that caffeic acids, flavonoids and phenolic esters are the main biologically active compounds in propolis (Kartal et al., 2003; Kujumgiev et al., 1993; Marcucci et al., 2001; Park et al., 1997). But their biological effects cannot be attributed solely to these components, because, the chemical composition of propolis is complicated. In our opinion, the qualitative and quantitative composition of propolis plays an important role in their biological activity.

In accordance with literature data, the propolis was inactive against the gram negative bacteria. But our samples were found to be active against the gram negative bacteria. This activity can be a synergism between flavonoids, apigenin, crysin, and/or other components in raw propolis samples. Consequently, the purification of these compounds and identification of antimicrobial activity against gram-negative bacteria (especially *E. coli*) and gram-positive bacteria are the next step in our study.

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