Antibacterial Activity and Chemical Composition of Turkish Propolis

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Propolis, Hive Product, Antibacterial Agent

The antibacterial activities of propolis samples have been examined *in vitro,* according to the principles accepted for the determination of a similar activity of antibiotics with the use of solid and liquid media. It has been found that propolis extracts showed antibacterial activity through a range of commonly encountered gram positive cocci (*S. aureus*, beta hem. *Streptococus*), but had weak activity against gram negative bacteria (*E. coli, P. aeruginosa*). GC/MS analysis showed that propolis samples contain a variety of chemical compounds including aromatic compounds, fatty acid esters and sesquiterpenes.

Introduction

Propolis is a hive product, collected by honey bees from the gum of different types of trees (*e.g.*, poplar, birch, willow) and plants containing resins, waxes, flavonoids and some "*impurities*" (*e.g.*, pollen, wood, fragments of bees) (Ghisalberti, 1979; Ackerman, 1991; Bankova *et al.*, 1992). Bees mix this balsam with substances derived from pollen and different types of active enzymes. Propolis is a building and protective material. Bees use propolis to plug the gaps of the hive and more importantly to impede the decomposition of the creatures which have been killed by the bees following an invasion of the hive (Kaal, 1991). Also the high relative humidity, optimum temperature and abundant presence of nutrients in the hives would result in very rapid propagation of moulds and bacteria, without the presence of propolis. Its antimicrobial potency keeps the growth of microbes under control (Szente and Szejtli, 1987; Meresta and Meresta, 1980; Kivalkina, 1976; Bankova *et al.*, 1995).

The use of propolis for treatment of many diseases has been known in folk medicine since the earliest times (Ghisalberti, 1979; Meresta and Meresta, 1980). More detailed studies on the therapeutic properties of propolis were performed in the last thirty five years (Villanueva *et al.*, 1964; Lindenfelser, 1967; Meresta and Meresta, 1983 and 1986; Brumfitt *et al.*, 1990; Grange and Davey, 1990; Zumla and Lulat, 1989; Dobrowolski *et al.*, 1991). Apparently, propolis is very selective in the way it attacks pathogenic bacteria as well as mycoses and virus infections which might be dangerous to human beings. Because of its selectivity normal bacteria such as those of the flora which perform a vital function in our digestive system are not affected. Propolis does not create any harmful side-effects nor do bacteria build up resistance to it. Propolis is now found in tooth pastes, chocolates, shampoos, creams, tablets, etc. and the world production has increased to several thousand kg per year. The composition of propolis may vary depending on its geographical origin (Ackerman, 1991; García-Viguera *et al.*, 1992; García-Viguera *et al.*, 1993; Markham *et al.*, 1996; Borčić *et al.*, 1996; Bankova *et al.*, 1998). We have investigated *in vitro* two different samples of propolis for their potential antimicrobial activities against different bacteria and chemical composition of these samples by GC/MS.

Experimental

Plant material

Two natural native propolis samples collected from two different areas of the Marmara region of Turkey (Balikesir and Istanbul) were used in experiments. Each raw material which consisted of a block of brown waxy material was kept in a

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refrigerator for 48 h then it was ground in a grinder.

Preparation of the extract

Propolis was extracted according to Meresta and Meresta, (1980), with some modification: Powdered propolis was treated with 96% ethyl alcohol and kept at 37 °C for 24 h. The supernatant was decanted, then a mixture of ethyl alcohol and ethyl ether in proportion 1:1, v/v was poured over the rest and again it was kept at 37 °C for 24 h. After the second supernatant was decanted, ether was poured over the rest and it was kept at 3 °C for 24 h. Finally, after the third supernatant was decanted, ethyl acetate was poured over the rest and kept at 37 °C for 24 h. After the last supernatant decantation all the extracts were mixed and filtered through filter paper. Next, waxy substances soluble in the solvents mentioned above were precipitated by centrifugation and the liquid part was again filtered through filter paper. The clear solution of propolis extracts obtained in this way was evaporated at low pressure to eliminate solvent residues.

Simultaneous distillation-extraction (SDE)

After the extracts were evaporated, the dark, sticky, brown substance of propolis was dissolved in 70% ethyl alcohol. Volatiles of the propolis extract were worked up by Likens-Nickerson simultaneous distillation-extraction (SDE) method in pentane (Sandra and Bicchi, 1987).

GC/MS analysis of propolis samples

The propolis volatiles were analyzed by GC/MS using a Hewlett-Packard GC-MSD system. An Innowax FSC column (60 m × 0.25 mm i.d. with 0.25 μm film thickness) was used with helium as the carrier gas. GC oven temperature was kept at 60 °C for 10 min. and programmed to 220 °C at a rate of 4 °C/min., and then kept constant at 220 °C for 10 min. and programmed to 240 °C at a rate of 1 °C/min. Split flow was adjusted to 50 ml/min. The injector temperature was at 250 °C. MS were taken at 70 eV in the EI mode. Mass range was from *m/z* 35 to 425. Library search was carried out using Wiley GC/MS Library and TBAM Library of Essential Oil Constituents (Ardrey *et al.*, 1983; Jennings and Shibamoto, 1980; Masada, 1975; McLafferty and Stauffer, 1988; Swigar and Silverstein, 1981).

Microbial strains

Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and beta-hemolytic *Streptococci* (all of them were clinical isolates) obtained from Hospital of Hacettepe University, Department of Bacteriology were used.

Antibacterial activities of particular propolis extracts were determined by assessment of the minimal inhibitory concentration (MIC) and that of the minimal bactericidal concentration (MBC). The determinations of the MIC were performed in solid medium of pH 6.8 (nutrient agar and blood Agar) while that of the MBC was carried out in liquid medium with pH 6.8/nutrient broth). Propolis extracts were added in arithmetical progression. The concentration of propolis in the media was expressed in μg per ml. The media were inoculated with bacteria and were kept at 37 °C for 18–24 h. Liquid media were inoculated with a 18 h culture. The bacterial density of the culture was determined spectrophotometrically at 490 nm according to Mc Farland's scale using buffered saline solution. Liquid media were inoculated with 104 cells per ml of medium. Solid media were inoculated with an 18 hour liquid culture.

Results and Discussion

To determine antibacterial activities, two different propolis extracts were tested against Gram negative (*E. coli* and *P. aeruginosa*) and Gram positive (*S. aureus* and beta hemolytic *Streptococci*) species. The MIC and MBC values of propolis extracts are shown in Table I.

GC/MS analysis of propolis volatiles are shown in Table II.

For the selected bacteria studied, propolis samples inhibited better the growth of *S. aureus* and beta hemolytic *Streptococci* than gram (–) bacteria (*E.coli* and *P. aeruginosa*). Previous studies also reported that gram negative bacteria were less susceptible showing lower minimal inhibitory concentration (MIC) than gram positive strains (Szente and Szejtli, 1987; Lindenfelser, 1967; Dobrowolski, *et al.*, 1991). The bactericidal dose of the studied extracts was as a rule, approximately twice as large

Table I. The MIC[a] and MBC[b] values of two different samples of propolis.

Region	Amount per ml [mg/ml]	Microorganism tested	MIC [μg/ml]	(MBC) [μg/ml]	Ratio of MIC to MBC
Balikesir	310	*E. coli*	1705	3100	1:1.8
		P. aeruginosa	1705	3100	1:1.87
		S.aureus	175	310	1:1.77
		B. hem. Strep	175	310	1:1.77
Istanbul	160	*E. coli*	88	160	1:1.8
		P. aeruginosa	88	160	1:1.8
		S.aureus	9	16	1:1.7
		B. hem. Strep	88	160	1:1.8

[a] Minimal inhibitory concentration.
[b] Minimal bactericidal concentration.

Table II. Chemical composition of propolis samples.

Compounds	Propolis from Istanbul (%)	Propolis from Balikesir (%)
3-Methyl-2-butenol	16.6	–
Ethyl decanoate	–	1.1
Ethyl benzoate	–	3.0
Diethyl succinate	22.9	–
Ethyl decanoate	–	1.5
Calamenene	–	< 0.1
Ethyl-3-phenyl propionate	2.3	14.6
Phenylethyl alcohol	3.8	–
(*E*)-Ethyl cinnamate	–	1.8
γ-Eudesmol	3.9	10.9
α-Eudesmol	7.1	20.4
β-Eudesmol	11.8	29.6
Ethyl hexadecanoate	14.7	2.6
Decanoic acid	–	1.2
Ethyl oleate	7.1	5.8

as the bacteriostatic dose (Table I) which proves propolis as a highly effective and useful material.

GC/MS analysis showed propolis samples contained a variety of chemical compounds including aromatic components, fatty acid esters, cinnamates and sesquiterpenes (calamenene, eudesmol) (Table II). Many studies have shown that fatty acid esters, phenolic compounds, terpenoid compounds and cinnamic acid are the main propolis constituents and some of them possess antibacterial activity (Bankova *et al.*, 1992; Wollenweber *et al.*, 1987; Greenaway *et al.*, 1988; Kujumgiev *et al.*, 1993) Propolis collected from hives in Balikesir was more chemically diverse (12 compounds) than the material collected from Istanbul (fewer than 10). But it showed a weaker antibacterial activity than the Istanbul extract. The Istanbul sample had three chemical components (3-methyl-2 butenol, diethyl succinate and phenyl-ethyl alcohol) different from the Balikesir sample. The greater activity of the Istanbul sample on bacteria than the Balikesir sample may be attributed to its slightly different chemical composition.

The experiments revealed that there can be minor differences in antibacterial activity of propolis extracts depending on the region from which the stock comes. First of all this is due to nectar flows and the presence of a specific flora within the area of bee circulation.

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