

RESEARCH OF ANTIMICROBIAL EFFECTS OF PROPOLIS FROM PROVINCE OF ORDU

Gurkan DEMIRKOL

Ordu University Faculty of Agriculture Department of Field Crops, 52200, Ordu, Turkey

Corresponding author email: demirkol_52@hotmail.com

Abstract

Propolis is a resinous product collected by bees used in their hives to have a safe place. Bees provide it from various plants. It is rich in terms of phenolic compounds so it is very important for the role in contributing to human health. In this study, antimicrobial effects of ethanol extract of propolis were determined against Escherichia coli, Streptococcus mutans, Pseudomonas aeruginosa, Listeria monocytogenes, Candida albicans and Aspergillus niger using disc-diffusion and agar dilution method. According to the results, propolis showed antimicrobial activity against Pseudomonas aeruginosa, Streptococcus mutans, Listeria monocytogenes and Candida albicans. The most sensitive microorganism was Pseudomonas aeruginosa to propolis. This study offers that propolis may provide an alternative to chemical preservatives against several diseases.

Key words: Antimicrobial, propolis, Ordu.

INTRODUCTION

Propolis is a strongly adhesive, resinous substance collected, transformed and used by bees to seal holes in their honeycombs. It smooths out the internal walls and protects the entrance against intruders. Honeybees (*Apis mellifera* L.) collect the resin from the cracks in the bark of trees and leaf buds. This resin is masticated, salivary enzymes added and the partially digested material is mixed with beeswax and used in the hive (Ghisalberti, 1979; Marcucci et al., 1996; Burdock, 1998).

Propolis is extensively used in folk medicine and a number of investigations have shown that propolis have antimicrobial and antiviral properties (Mirzoeva et al., 1997; Park et al., 1998; Kujumgiev et al., 1999; Hegazi and El Hady, 2001; Ota et al., 2001; Kartal et al., 2003; Güler et al., 2003; Prytyk et al., 2003). The resin contains most of the compounds found in alcohol extracts consumed by people from many countries as food complements or alternative medicine (Gabrys et al., 1986; Marcucci et al., 1996).

It has been shown that there were variations in the antimicrobial activity according to the propolis origin (Hegazi and El Hady, 2001; Stepanovic, 2003). The constituents of propolis vary widely due to climate, season, location and year. Its chemical formula is not stable

(Ghisalberti, 1979; Cheng and Wong, 1996). The most important pharmacologically active constituents in propolis are? avonoids (? avones,? avonols,? avonones), phenolics, and aromatics. Flavonoids are thought to account for much of the biologic activity in propolis (Uzel et al., 2005).

A great enthusiasm characterizes present-day propolis research, driven by positive results in pharmacological tests, dealing not only with antimicrobial activity, the first (Lavie, 1960) and as yet the most investigated effect in propolis research, but also with a wide diversity of effects, including immune activation and cytotoxicity (Banskota et al., 2001).

Turkey, which is the fourth largest honey producing country in the world, has a rare mix of suitable conditions for beekeeping. Turkey has both European and Asian flora characteristics, enriching the bee products, such as honey, pollen and propolis (Aliyazicioglu et al., 2013).

The present study was designed to determine the antimicrobial activities of propolis gathered from Ordu province of Turkey.

MATERIALS AND METHODS

Propolis Sample and Preparation of Extract
Propolis sample in the form of hard lumps were collected from Ordu province of Turkey during

October and November 2012. The crude sample was stored in air-tight glass container in dark at -20°C until used. Propolis extract was prepared by stirring 30 g samples in 150 ml of 95% ethanol at room temperature and the extract was kept at 4°C for a week. The extract was filtered through 45 µm membrane filter and then the solution was dried with an evaporator. The crude extract was stored at -20°C until used.

Test Strains and Culture Media

Strains of bacteria and fungi were obtained from ATCC (American Type Culture Collection, Rockville, USA). Antimicrobial activities of propolis extract sample was assayed against *Escherichia coli* ATCC 25922, *Streptococcus mutans* ATCC 25175, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 7677, *Candida albicans* ATCC 25922 and *Aspergillus niger* ATCC 9642. The species of bacteria were grown in Mueller Hinton Agar (Oxoid Ltd., Basingstoke, Hampshire, UK) and Mueller Hinton Broth (Merck Co., Darmstadt, Germany). The species of fungi were grown in Sabouraud Dextrose Agar (Oxoid Ltd., Basingstoke, Hampshire, UK) and Sabouraud Dextrose Broth (Difco Laboratories, Detroit, MI, USA). The concentrations of bacterial suspensions were adjusted to 10⁸ cells/ml, while those of fungal suspensions to 10⁷ cells/ml.

Antibacterial and Antifungal Assay

Antibacterial and antifungal activity were measured using methods of diffusion disc plates on agar (Ronald, 1990). In order to test antibacterial and antifungal activity, the fractions of propolis sample was dissolved in ethanol. Mueller Hinton Agar medium (20 ml) for bacteria and Sabouraud Dextrose Agar (20ml) for fungus were poured into a 15 cm petri dish. All bacterial strains were grown in Mueller Hinton Broth medium for 24 h, at 37°C and the fungal strains were grown in Sabouraud Dextrose Broth at 27°C for 48 h. Growth was adjusted to 600 nm of 0.1 by dilution with Mueller Hinton Broth medium for bacteria and Sabouraud Dextrose Broth for fungi. Suspension (100 µl) with approximately 10⁸ microorganisms per milliliter was placed in petri dishes. Then, sterile paper discs (6 mm in diameter) were placed on the agar to load 15 µl

of the sample (20 mg/ml). One hundred units of nystatin for fungus, ampicillin and cephalosporin for bacteria, all obtained from a local pharmacy, were used as a positive control and ethanol as a negative control. Inhibition zones were determined after incubation at 37°C for 24 h for bacterial tests and 27°C for 48 h for fungal tests. All tests were made in triplicates (Aliyazicioglu et al., 2013).

Minimum Inhibition Concentration

The agar dilution method was used for the antimicrobial screening with slight modifications (Vanden Berghe and Vlietinck, 1991). Instead of 96 well microtiter plates 24 well tissue culture (Corning Costar Co., Corning, NY, USA) plates were used. The crude propolis extract was dissolved in ethanol and physiological tris buffer (1:4) and mixed with an equal amount of 3% agar solution at 45°C to a final concentration of 10, 5, 2.5 and 1.25 mg of extract/ml. An amount of 400 µl from the solution was transferred into each well of the tissue culture (Corning) plates. After solidification, each well was inoculated with 10 µl of freshly prepared bacterial suspension of 10⁸ bacterial/ml and incubated at 37°C for 24 h. Ampicillin and cephalosporin for bacteria and nystatin for fungi, were used at (1.25-10 mg/ml) as positive controls. The microbial growth was assessed by a stereo microscope after the incubation period. All tests were made in triplicates (Aliyazicioglu et al., 2013).

RESULTS AND DISCUSSIONS

In the present study, the antimicrobial activity of ethanol propolis extract from Ordu province, was investigated. The antimicrobial activity of propolis extract was initially evaluated by the disc diffusion method using two gram-positive (*S. mutans*, *L. monocytogenes*), two gram-negative bacteria (*P. aeruginosa*, *E. coli*) and two fungi (*C. albicans*, *A. niger*). The results obtained in the disc diffusion assay regarding the growth inhibition zones of the tested microorganisms are shown in Table 1. Propolis showed the highest antibacterial activity against *P. aeruginosa* (25 mm). Some researches reported that ethanolic propolis extracts inhibited *P. aeruginosa* (Uzel et al., 2005; Aliyazicioglu et al., 2013). The antifungal activity was highly showed against *C. albicans*

(18 mm). Also, propolis showed strong inhibitory action against *S. mutans* (19 mm) and *L. monocytogenes* (17 mm), which correlates well with the literature data (Lepekhn and Leonova, 1970; Gebara et al., 1996; Koo et al, 2000; Ophori et al., 2010; Aliyazicioglu et al., 2013). Propolis extract did not show antimicrobial activity against *E. coli* and *A. niger*. However, in the studies of Aliyazicioglu et al. (2013) and Rahman et al. (2010), propolis showed antimicrobial activity against *E. coli*. It is well known that the type of propolis sample may vary highly according to regional and environmental vegetation. Evaluation of minimum inhibitory concentration of extract by means of agar dilution experiment method is reported in Table 2. The extract of propolis sample required minimum inhibitory concentration of =1.25 mg/ml for *P. aeruginosa* and > 1.25 mg/ml for *S. mutans*.

The most sensitive microorganism to propolis was *P. aeruginosa* in the gram negative group and *S. mutans* in the gram positive group. According to the results, it may not be concluded that, in general which gram group is more susceptible to propolis sample about antimicrobial action. In the study, the least sensitive microorganism was *A. niger*. A control test run with standard antibiotics revealed that propolis sample have a similar or greater inhibitory effect on *C. albicans* and *P. aeruginosa*.

Table 1. Results of antimicrobial screening of the ethanolic propolis extract determined by the disc diffusion method (inhibition zone in mm).

Samples	Microorganisms					
	<i>E.c.</i>	<i>S.m.</i>	<i>P.a.</i>	<i>L.m.</i>	<i>C.a.</i>	<i>A.n.</i>
Propolis	10	19	25	17	18	8
AMP	15	25	28	23	NT	NT
CEP	15	30	24	33	NT	NT
NYS	NT	NT	NT	NT	15	15
Ethanol	-	-	-	-	-	-

-: no inhibition, NT: Not tested, *E.c.*: *E. coli*, *S.m.*: *S. mutans*, *P.a.*: *P. aeruginosa*, *L.m.*: *L. monocytogenes*, *C.a.*: *C. albicans*, *A.n.*: *A. niger*, Control: AMP: Ampicillin 10 µg. CEP: Cephazolin 30 µg. NYS: Nystatin 100 Units.

Table 2. Results of antimicrobial screening of the ethanolic propolis extract determined by the agar dilution method (minimum inhibitory concentration, in mg/mL).

Sample	Microorganisms					
	<i>E.c.</i>	<i>S.m.</i>	<i>P.a.</i>	<i>L.m.</i>	<i>C.a.</i>	<i>A.n.</i>
Propolis	> 10	> 1.25	=1.25	> 2.5	> 2.5	NT

NT: Not tested, *E.c.*: *E. coli*, *S.m.*: *S. mutans*, *P.a.*: *P. aeruginosa*, *L.m.*: *L. monocytogenes*, *C.a.*: *C. albicans*, *A.n.*: *A. niger*.

CONCLUSIONS

Antibacterial activity of propolis depends on chemical composition and concentration of the active components and compounds. It has distinctive features that may be beneficial to our health as an antimicrobial since it has important chemical contents such as flavonoids, phenolics and aromatics. The studies show that propolis which is the polyphenolic-rich natural product may provide an alternative to chemical preservatives and it may be used as a source of natural antimicrobial.

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