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Antibacterial activity of pollen and propolis extracts

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Abstract

The antimicrobial activities of different concentrations of pollen and propolis extracts were determined against nine food-borne pathogens (*Streptococcus salivarius* RSHE 605, *Listeria monocytogenes* NCTC 5348, *Staphylococcus aureus* ATCC 25923, *Salmonella enteritidis* ATCC 13076, *Staphylococcus pneumoniae* ATCC 10015, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* NCTC 5049, *Pseudomonas aeruginosa* ATCC 27853 and *Bacillus anthracis*) in model systems. The extracts and antibiotic discs (ciprofloxacin, cefaperazon sulbaktam, penicilline, amikacine, cefepime, gentamicine, ceftriaxone) exhibited inhibitory effect at the varying levels against tested bacteria. Among the tested bacteria, *L. monocytogenes* and *P. aeruginosa* were the most sensitive ones to 50 ppm concentrations of both extracts. The highest effect concentrations towards *B. anthracis* were 600 ppm dose of pollen and propolis extracts. The 400 and 600 ppm levels of propolis extracts on *L. monocytogenes* NCTC 5348 exhibited similar antimicrobial effects compared with amikacine, gentamicine and ceftriaxone antibiotics. Also, 600 ppm concentration of propolis had higher effect against *B. anthracis* compared with cefepime, gentamicine and ceftriaxone antibiotics. The inhibitory effect of propolis extract was found to be higher than that of pollen against tested bacteria. Propolis especially appears to be promising in this respect.

Key words: Pollen, propolis, extract, antibacterial effect, food-borne pathogens.

Introduction

Pollen is a fine powder-like material produced by flowering plants and gathered by bees. Flower pollens, bees primary food source, contain concentrations of phytochemicals and nutrients and are rich in carotenoids, flavonoids and phytosterols. At the same time, bee pollen has shown to have antimicrobial effects [1-3].

Propolis is a resinous hive product collected from plant buds by bees. Bee products such as honey, pollen and propolis are used for the treatment of several diseases. It is said that propolis was used as a perfect antibiotic agent [4-7]. Propolis is a resin and its color changes from green to dark browning according plant source. Investigations have indicated that propolis contains wax, flavonoids, amino acids, essential oils, pollen, minerals and organic matter [8-10].

Recently, investigations have indicated that interest for natural preservatives had increased. The use of propolis, that is nontoxic, as alternate preservative agent is considered by consumers as safe [11]. In the last thirty years, there has been considerable emphasis on the studies involving propolis [9, 11-16].

The aim of the present study was to determine antibacterial effects of pollen and propolis at different concentrations on some bacteria in microbiological media.

Material and Methods

Material: Both pollen and propolis were collected from Hatay and Taşkent in Turkey, respectively. A 200 g sample of pollen and propolis were each extracted for 8 h in a Soxhlet apparatus with 175 ml methanol at 70°C. The crude extracts were pooled and concentrated in a rotary evaporator and kept in small (20 ml) sterile dark bottles under refrigerated conditions until use.

Assessment of inhibition of bacterial growth: Six Gram (+) bacterial strains (*Streptococcus salivarius* RSHE 605, *Listeria monocytogenes* NCTC 5348, *Staphylococcus aureus* ATCC 25923, *Streptecoccus pneumoniae* ATCC 10015, *Bacillus anthracis* (S.Ü. Vet Fak.) and four Gram (-) strains (*Salmonella enteritidis* ATCC 13076, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* NCTC 5049, *Pseudomonas aeruginosa* ATCC 27853) were used to determine the antimicrobial effect of the pollen and propolis on these bacterial strains, respectively. A disc diffusion method was used to determine the antimicrobial activity [17]. After grown on Brain Heart Infusion broth, these lyophilized strains were inoculated on 5% of blood agar and then incubated for 24 h at 37°C. The pre-cultures of microorganisms were prepared to test their susceptibility against propolis. For this purpose, the bacterial strains were taken by sterile inoculating loop, followed by touching to 4-5 colonies raised from pure microorganism culture. These strains were inoculated with the concentration of $1x10^8$ cfu/ml (to achieve the Mc. Farland No. 0.5 density) and incubated at 37°C for 24 h. Propolis concentrations of 2,500; 5,000; 10,000; 20,000 and 30,000 µg were dissolved in 1 ml of dimethyl sulfoxide (DMSO) solution having no antimicrobial activity to dilute propolis specimens.

All these solutions were prepared to achieve diluted propolis absorption by 6 mm diameter of blank (Oxoid) disks, absorbing capacity of which was two-fold of weight itself, in order that the final concentration of each disc could be 50, 100, 200, 400 and 600 µg, respectively. For the negative control, a second part of the blank disks was provided to absorb only DMSO. For each bacterial strain, ciprofloxacin (CPR), cefaperazon sulbaktam (CES),

ceftriaxone (CRO), gentamicine (GM), penicilline (P), cefepime (FEP) and amikacine (AK) antibiotic discs were used as a positive control. The prepared discs were placed in the inoculated Petri dishes. These Petri dishes were incubated for 24 h at 37°C. The diameters of formed inhibition zones were measured as mm. The results were evaluated according to NCCLS criteria [18]. The present study was conducted in 3 replicates. The obtained results were mean of three measurements.

Statistical analyses: The data were analysed for statistical significance by analysis of variance [19].

Results and Discussion

The antibacterial effect of pollen and propolis extracts on the growth of *Str. salivarius* RSHE605, *L. monocytogenes* NCTC 5348, *Staphylococcus aureus* ATCC 25923, *Salmonella enteritidis* ATCC 13076, *Staphylococcus pneumoniae* ATCC 10015, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* NCTC 5049, *Pseudomonas aeruginosa* ATCC 27853 and *Bacillus anthracis* strains were determined in *in vitro* (Table 1).

Both extracts and antibiotic discs (penicilline, amikacine, cefepime, gentamicine, ceftriaxone, cefaperazon sulbaktam and ciprofloxacin) used as the positive control exerted varying levels of inhibitory effect against tested microorganisms. Among the tested bacteria, *L. monocytogenes* and *P. aeruginosa* were the most sensitive ones to 50 ppm concentrations of both extracts. The highest effective concentrations towards *B.anthracis* were 600 ppm dose of pollen and propolis extracts. Generally, the inhibitory effect of propolis extract was higher than that of pollen against tested bacteria (except for *S. enteritidis*) (Table 1). The 400 and 600 ppm levels of propolis extracts on *L.monocytogenes* NCTC 5348 exhibited similar antimicrobial effects compared with amikacine, gentamicine and ceftriaxone antibiotics. Also, 600 ppm concentration of propolis extract showed similar effect against *S. aureus* ATCC 25923 compared with penicilline. With a few exceptions, the effects of the highest concentrations of both extracts were found to be partly similar. Generally, the inhibition of both extracts increased by the increase of the dosage for all the microorganisms tested (Figs 1 and 2).

This study is a preliminary evaluation of antibacterial activity of pollen and propolis extracts. In previous study, the inhibitory effect of five levels of pollen and propolis extracts on the growth of 13 different species of agricultural bacterial pathogens were investigated *in vitro*. Of the concentrations tested, *Agrobacterium tumefaciens* was the most sensitive one to 1/5 concentration of pollen extract. The last active concentrations towards the tested bacteria were 1/100 of the pollen extract and 1/1000 of the propolis extract [16]. In another study, the antimicrobial activity of pollen and propolis extracts was investigated against 20 species of bacteria. Among the bacteria tested, the most sensitive were *S. aureus* to a 1/5 level of pollen extract and *L. monocytogenes* to a 1/10 level of propolis extract [20].

The variation of the antibacterial activities of the tested extracts may be due to their constituents and the probable presence of nonvolatile compounds of extracts [21, 22]. Bankova *et al.* [23] reported the antibacterial activity of different fractions of Brazilian propolis towards *S.aureus*, and observed that the antibacterial activity is mainly due to polar phenolic compounds. All the propolis samples used in their experiments were active against Gram positive bacteria

Table 1. Inhibitory effect (mm) of pollen and propolis extracts against several food-borne pathogen bacteria.

Microorganism	Concentration (ppm)								Antibiotic disc						
	50		100		200		400		600								
	Pollen	Propolis	Pollen	Propolis	Pollen	Propolis	Pollen	Propolis	Pollen	Propolis	P	AK	FEP	GM	CRO	CES	CPR
Str. salivarius RSHE 605	R	R	R	6±0.7	7±0.1	7±0.1	8±0.2	9±1.1	8±1.3	10±1.2	23	10	R	12	R	16	32
L. monocytogenes NCTC 5348	6±0.1*	7±0.3	6±0.1	7±0.3	10±0.6	9±0.4	11±1.3	11±1.4	12±0.9	13±1.8	R	11	R	10	10	18	24
Staph. aureus ATCC 25923	R	R	6±0.1	7±0.3	7±0.1	8±0.3	8±0.4	10±0.8	9±1.1	12±1.1	12	18	22	18	23	20	18
Salm. enteritidis ATCC 13076	R	R	8±0.3	7±0.5	11±0.5	9±0.8	14±1.7	10±0.8	12±0.7	12±1.3	13	15	16	15	13	30	28
Str. pneumoniae ATCC 10015	R	6±0.4	R	6±0.4	7±0.1	9±0.6	8±0.6	9±0.7	10±0.8	12±1.3	14	22	20	12	26	20	28
E. coli ATCC 25922	R	8±0.7	8±0.2	9±0.6	7±0.3	7±1.1	8±0.5	10±0.8	11±0.5	11±0.8	R	14	23	15	26	28	28
K. pneumoniae NCTC 5049	R	7±0.3	6±0.2	8±0.2	7±0.1	7±0.7	8±0.7	7±0.3	9±0.7	10±0.8	10	20	32	18	34	34	38
P. aeruginosa ATCC 27853	7±0.6	8±0.3	7±0.1	7±0.3	8±1.1	8±0.4	8±0.9	9±0.4	11±1.2	11±0.7	R	18	23	16	13	18	28
B. anthracis (S.Ü. Vet Fak.)	R	6±0.1	7±0.1	6±0.1	10±1.2	11±1.2	11±1.3	11±1.3	14±1.5	23±2.5	34	23	16	22	21	28	30

CPR Ciprofloxacin, CES Cefaperazon sulbaktam, CRO Ceftriaxone, GM Gentamicine, P Penicilline, FEP Cefepime, AK Amikacine.
*mean ± standard deviation.

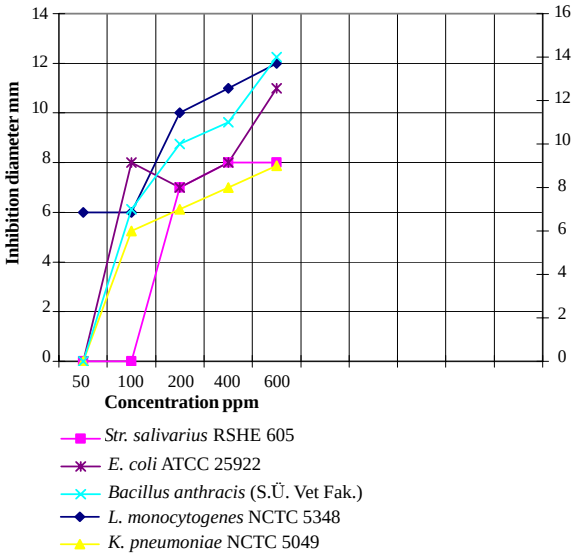

Figure 1. Inhibitory effect of pollen extracts against some bacteria.

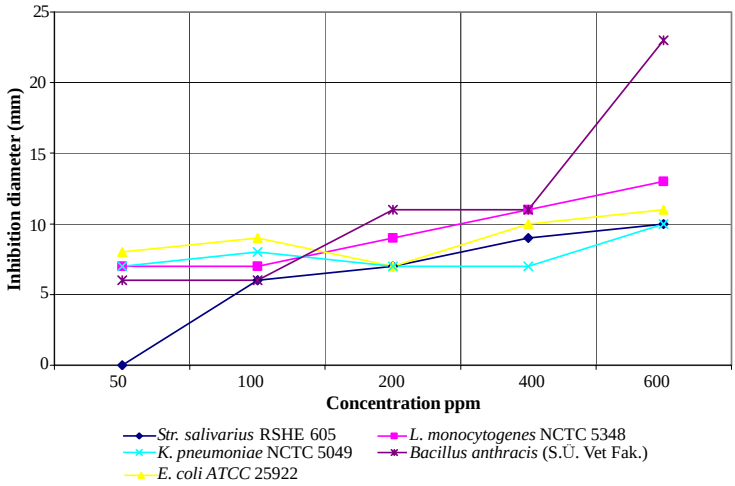

Figure 2. Inhibitory effect of propolis extracts against some bacteria.

and fungal test strains [24]. Propolis samples showed *in vitro* antimicrobial activity mainly against Gram positive bacteria (*Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*) [25].

According to results, the antibacterial activity of propolis extract was found to be higher than that of pollen extract. Propolis and pollen appear to be promising in this respect. The findings of this work further pointed out that propolis has an alternative natural food preservative properties compared with pollen. Further studies on the combined effects of many local bee-product extracts and components in food products are in progress in our laboratory medium.

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References

[1]Balch, J. F. and Balch, P. A. 1990. Prescription for nutritional healing. Avery Publishing Group Inc., New York, pp. 12-39.

[2]Haas, E. M. 1992. Staying healthy with nutrition. Celestial Arts Publish, NewYork, pp. 297-298.

[3]Broadhurts, C. L. 1990. Bee products: Medicine from the hive. Nutrition Science News **4**:366-368.

[4]Grange, J. M. and Darvey, R. W. 1990. Antibacterial properties of propolis (bee glue). J. R. Soc. Med. **83**:159-160.

[5]Cherbuliez, T. 1996. Bee venom therapy: A review. International Conference on Bee Products: Properties, Applications and Apitherapy, May 26-30; Tel-Aviv, Israel, p. 54.

[6]Feraboli, F. 1996. Apitherapy in orthopaedic diseases. International Conference on Bee Products: Properties, Applications and Apitherapy, May 26-30; Tel-Aviv, Israel, p. 55.

[7]Schmidt, L. S. and Schmidt, J. O. 1996. Medical overconcern: What are the real allergic and healty risks from bee product and apitherapy. Int. Confer. on Bee Products: Properties, Applications and Apitherapy, May 26-30; Tel-Aviv, Israel, p. 43.

[8]Walker, P. and Crane, E. 1987. Constituents propolis. Apidologie. **18**:327-334.

[9]Crane, E. 1990. Bees and Beekeeping - Science, Practice and World Resources. Heinemann Newnes, Oxford.

[10]Scheller, S. 1990. Plant origins of propolis: A report of work at Oxford. Bee World**71**(3):107-118.

[11]Ghisalberti, E. L. 1979. Propolis: A review. Bee World **60**:59-84.

[12]Yamauchi, R., Kato, K., Oida, S., Kanaeda, J. and Ueno, Y. 1992. Benzyl caffeate on antioxidative compound isolated from propolis. Bioscience Biotechnology and Biochemistry **56**:1321-1322.

[13]Özcan, M. 2000. Use of propolis extract as a natural antioxidant for plant oils. Grasas y Aceites **51**(4):251-253.

[14]Özcan, M., Ünver, A., Ceylan, D. A. and Yetişir, R. 2004. Inhibitory effect of pollen and propolis extracts. Nahrung/Food **48**:188-194.

[15]Silici, S. and Kutluca, S. 2005. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. Journal of Ethnopharmacology **99**:69-73.

[16]Basim, E., Basim, H. and Özcan, M. 2006. Antibacterial activities of Turkish pollen and propolis extracts against plant bacterial pathogens. Journal of Food Engineering **77**:992-996.

[17]Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M. 1966 Antibiotic suspectibility testing by a standardised single disk method. Am. J. Clinical Pathol. **45**:493-496.

[18]NCCLS 1997. Performance Standards for Antimicrobial Disk Suspectibility Tests. 6th edn. National Committee for Clinical Laboratory Standards. Approved standard M2-A6. Villanova, PA.

[19]Püskülcü, H. and İkiz, F. 1989. Introduction to Statistic. Bilgehan Press, Bornova, İzmir, Turkey (in Turkish), 333 p.

[20]Özcan, M., Sağdıç, O. and Özkan, G. 2004. Antibacterial effects of Tukish pollen and propolis extracts at different concentrations. Archiv für Lebensmittelhygiene **55**:25-48.

[21]Marletto, F. 1984. Particularities of propolis depending on flower source and its utilization. Apiacta **3**:71-74.

[22]Calcagno, C., Evangelisti, F. and Zunin, P. 1989. Chemical/nutritional analysis of pollens obtained from bee-keeping. Ivista della Societa Italiana di Scienza dell'Alimentazione **18**:99-104.

[23]Bankova, V., Christov, R., Kujumgiev, A., Marcucci, M. C. and Popov, S. 1995. Chemical composition and antibacterial activity of Brazilian propolis. Zeit. Naturforschung **50c**:167-172.

[24]Kujumgiev, A., Tsvetkova, I., Serkedjieva, Yu., Bankova, V., Christov, R. and Popov, S. 1999. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. Journal Ethnopharmacology **64**:235-240.

[25]Castaldo, S. and Capasso, F. 2002. Propolis, an old remedy used in modern medicine. Fitoterapia **73**(1):1-6.