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, peniciline, 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 cepfepine, 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 phytoestrogens. 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 agent4-7. 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 safe11. 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, Strepctococcus 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 and Bacillus anthracis) 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 1x10^6 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 same solutions was provided to absorb only DMSO. For each bacterial strain, ciprofloxacin (CPR), cefaperazon sulbaktam (CES), penicillin, amikacine, cefepime, gentamicine, 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.
Statistical analyses: The data were analysed for statistical significance by analysis of variance (ANOVA).

Results and Discussion

The antibacterial effect of pollen and propolis extracts on the growth of *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 *vitro* (Table 1).

Both extracts and antibiotic discs (penicillin, amikacin, cefepime, gentamicin, ceftriaxone, cepaperazon sulbaktam and ciprofloxacin) were 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 amikacin, gentamicin and ceftriaxone antibiotics. Also, 600 ppm concentration of propolis extract showed similar effect against *S. aureus* ATCC 25923 compared with penicillin. 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 bacterial food-borne 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. 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* ATCC 25923 compared with penicillin. 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).

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.

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. Bankova et al. 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.

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

CPR: Ciprofloxacin, CEF: Cefepime, PEP: Penicilline, AK: Amikacine.
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