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Brief Original Article

Inhibitory effects of propolis and essential oils on oral bacteria

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Abstract

Introduction: Propolis is a natural composite balsam. In the past decade, propolis has been extensively investigated as an adjuvant for the treatment of periodontitis. This study aimed to investigate antimicrobial activities of propolis solutions and plant essential oils against some oral cariogenic (*Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguis, Lactobacillus acidophilus*) and periodontopathic bacteria (*Actinomyces odontolyticus, Eikenella corrodens*, *Fusobacterium nucleatum*).

Methodology: Determination of the minimum inhibitory concentration (MIC): The antimicrobial activity of propolis and essential oils was investigated by the agar dilution method. Serial dilutions of essential oils were prepared in plates, and the assay plates were estimated to contain 100, 50, 25 and 12.5 µg/mL of active essential oils. Dilutions for propolis were 50, 25, 12.5 and 6.3 µg/mL of active propolis solutions.

Results: Propolis solutions dissolved in benzene, diethyl ether and methyl chloride, demonstrated equal effectiveness against all investigated oral bacteria (MIC=12.5 µg/mL). Propolis solution dissolved in acetone displayed MIC of 6.3 µg/mL only for *Lactobacillus acidophilus*. At the MIC of 12.5 µg/mL, essential oils of *Salvia officinalis* and *Satureja kitaibelii* were effective against *Streptococcus mutans* and *Porphyromonas gingivalis*, respectively. For the latter, the MIC value of *Salvia officinalis* was twice higher.

Conclusions: The results indicate that propolis and plant essential oils appear to be a promising source of antimicrobial agents that may prevent dental caries and other oral infectious diseases.

Key words: propolis; essential oils; oral bacteria; antibacterial activity.

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Introduction

Extracellular polysaccharides, mainly glucans, which are synthesized from dietary sucrose by streptococcal glucosyltransferases (GTFs), play a key role in the pathogenesis of dental caries and in plaque formation and accumulation as well [1]. During the last several decades, a remarkable increase has been observed in the use of natural products, especially propolis and essential oils [2,3]. The two mechanisms by which propolis exerts anti-caries properties, such as antimicrobial activity against cariogenic bacteria and inhibition of glucosyltransferase enzymes (GTFs) activity, have been described in earlier studies [4,5]. Among many active compounds present in propolis, flavonoids and terpens display distinct biological properties as effective GTF inhibitors and antibacterial agents, respectively [6]. The main components of the *Salvia officinalis* and *Satureja kitaibelii* essential oils are ketones and monoterpene hydrocarbons [7,8]. The products of these plant are lipophilic and capable of penetrating through the cell wall and cellular membranes. They can also increase cell permeability, affect the proton-pump mechanism and deactivate cellular enzymes after denaturing the plasma proteins to cause cellular death [9].

Since propolis and plant essential oils appear to be a promising source of antimicrobial agents, the aim of this work was to evaluate *in vitro* antibacterial effects of these natural products on the development of caries and strains that cause periodontopathy.

The minimal inhibitory concentrations (MIC) of essential oils (*Salvia officinalis* and *Satureja kitabelii*) and of propolis solutions (dissolved in benzene, diethyl ether, acetone and methyl chloride) have been determined for the following strains: *Streptococcus mutans* (*S. mutans), Streptococcus mitis* (*S. mitis*), *Streptococcus sanguis* (*S. sanguis*), *Lactobacillus acidophilus* (*L. acidophilus*), *Actinomyces*

odontolyticus (*A. odontolyticus*)*, Eikenella corrodens* (*E. corrodens*), *Fusobacterium nucleatum* (*F. nucleatum)*, and *Porphyromonas gingivalis* (*P. gingivalis)*.

This study aimed to investigate the antimicrobial activities of propolis solutions and plant essential oils against some oral cariogenic (*Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguis, Lactobacillus acidophilus*) and periodontopathic bacteria (*Actinomyces odontolyticus, Eikenella corrodens*, *Fusobacterium nucleatum*).

Methodology

Study design

This prospective study was conducted at the Faculty of Stomatology, Pančevo, from 2010 to 2014.

Collection of the plant material

Salvia officinalis and *Satureja kitaibelii*, two free-growing and also cultivated medicinal plant species, were collected in northern Serbia for the purpose of the study.

Bacterial strains

The investigated bacterial strains were*: A. odontolyticus* ATCC 17929, *S. mitis* ATCC 6249, *S. sanguis* ATCC 10556, *E. corrodens* ATCC 23834, *F. nucleatum* ATCC 25586, *L. acidophilus* ATCC 4356, *S. mutans* ATCC 25175 and *P. gingivalis* ATCC 33277 (Microbiologic).

Extraction of essential oils

The essential oils of *Salvia officinalis* and *Satureja kitaibelii* were obtained by distillation in a Clevenger-type apparatus. With respect to the preparation of propolis solutions in the study, propolis of the same species and origin was used – from one apiary near the mountain of Kopaonik (southern part of Serbia), and collected during only one time section (autumn), to ensure the highest homogeneity of the basic raw material. Extraction, as a chemical method, was performed as follows, regardless of the type of solvent. A mixture of solvent and water in a volume ratio of 60:40 to 96:4 was placed in a double-pot mixer (for necessary cooling). Four non-polar solvents (ether, acetone, methyl chloride and benzene) were used to dissolve propolis as well as ethanol, the solvent most commonly used for these purposes. When non-polar solvents, such as ether, acetone, methyl chloride and benzene were used as solvents during the extraction process, 500 mL of each solvent was added to 150 g of propolis. The extraction process took 48 hours, after which 360 mL, 450 mL, 486 mL and 500 mL of filtrate was obtained, respectively. The weight of the propolis extract was 80 g, 80 g, 40 g and 150 g, respectively. The containers with propolis extracts were cooled from 5 °C to 15 °C for thirty minutes and the contents were stirred at a rate of 20 m/s. After that, the suspension was centrifuged three times. The resulting extract was filtered through a Watman filter No. 4. The resulting propolis filtrate was a clear, dark brown liquid, which was further subjected to a vaporization process and stored in a dark flask at 4 °C until use. In this way, solvents that may have toxic effects were eliminated and at the same time the active components of propolis were preserved.

Antimicrobial activity

The essential oils and propolis solutions were individually tested against specific bacteria. The bacteria were cultured overnight at 37 °C in Mueller Hinton broth (HiMedia, Mumbai, India.), pH = 7.4.

Determination of the minimum inhibitory concentration (MIC)

The antimicrobial activity of propolis and essential oils was investigated by the agar dilution method [10]. Serial dilutions of essential oils were prepared in plates, and the assay plates were estimated to contain 100, 50, 25 and 12.5 µg/mL of active essential oils. Dilutions for propolis were 50, 25, 12.5 and 6.3 µg/mL of active propolis solutions. Inoculates were applied to blood

Table 1. Minimum inhibitory concentrations of four propolis solutions for some oral bacteria.

Bacteria	Propolis I	Propolis II	Propolis III	Propolis IV
	µg/mL	µg/mL	µg/mL	µg/mL
A. odontolyticus	12.5	12.5	12.5	12.5
S. mitis	12.5	12.5	12.5	12.5
S. sanguis	12.5	12.5	12.5	12.5
E. corrodens	12.5	12.5	12.5	12.5
F. nucleatum	12.5	12.5	12.5	12.5
L. acidophilus	12.5	12.5	6.3	12.5
S. mutans	12.5	12.5	12.5	12.5

agar surfaces (Liofilchem Roseto degli Abruzzi, Italy), producing approximately 10^6 µg/mL of bacteria. All plates were incubated for 48-72 hours under anaerobic conditions. MIC was taken as the lowest concentration of essential oil and propolis that produced no visible bacterial growth as compared to the control growth. The oils and propolis were tested in triplicates.

Results

The MIC values of propolis solutions ranged from 6.3 to 12.5 µg/mL. Solutions I, II and IV, dissolved in benzene, diethyl ether and methyl chloride, respectively, displayed for all tested bacteria the MICs of 12.5 µg/mL. In contrast, fraction III dissolved in acetone displayed the MIC of 6.3 µg/mL only for *L. acidophilus* (Table 1).

The antibacterial activities of *Salvia officinalis* and *Satureja kitaibelii* essential oils were tested against *S. mutans* and *P. gingivalis*. At the MIC of 12.5 µg/mL, essential oils of *Salvia officinalis* and *Satureja kitaibelii* were effective against *S. mutans* and *P. gingivalis*, respectively. For the latter, the MIC of *Salvia officinalis* essential oil was twice higher (25 µg/mL) (Table 2).

Table 2. Minimum inhibitory concentrations of essential oils tested against P. gingivalis and S. mutans.

Bacteria	Plants	
	Salvia officinalis	***Satureja kitaibelii***
	µg/mL	**µg/mL**
P. gingivalis	25	12.5
S. mutans	12.5	-*

*- not performed.

Discussion

Because of its complex chemical composition, many biological activities have been attributed to the ethanolic extract of propolis, while some of propolis flavonoids are considered to be antimicrobial agents [11,12]. In the present study, MIC values of propolis solutions I (dissolved in benzene), II (dissolved in diethyl ether) and IV (dissolved in methyl chloride) were 12.5 µg/mL for all investigated strains. In contrast, MIC values of propolis III (dissolved in acetone) were the same for most of the bacteria, except for *L. acidophilus* (6.3 µg/mL). Confirming the findings of the previous study, about anti-caries properties of propolis type-3 and type-12 [5], Hayacibara *et al*. demonstrated that chloroform fraction, and especially hexane fraction of both propolis types, were the most effective extracts [13]. In general, the hexane fraction from both propolis types rich in flavonoids, showed the most potent antibacterial and anti-GTFs activity *in vitro* [13]. Further, it was interesting to note from earlier works that propolis ethanolic extract RS2 with the highest concentrations of flavonoids, demonstrated both a higher antimicrobial activity and inhibition of glucosyltransferase activity [12].

The results of Gebara *et al*. [14] showed that propolis extract demonstrated antimicrobial activity *in vitro* not only against some periodontophatic bacteria (*F. nucleatum*, *P. gingivalis*, *P. intermedia*, *P. melaninogenica*, *A. actinomycetemcomitans* and *C. gingivalis*), but also against some organisms able to cause superinfection (*S. aureus*, *P. aeruginosa*, *E. coli* and *Candida albicans*). Interestingly, the MIC of propolis was 0.25 µg/mL for *Fusobacterium nucleatum,* which meant that the tested microorganism was susceptible to propolis at a lower MIC than the strain in the present study (12.5 µg/mL). Regarding susceptibility of the tested microorganisms to propolis, it was worth mentioning that they seemed to be more susceptible to propolis than to some antibiotics [14,15].

Similar to the results of present study, Koo *et al*. [6], Topcuoglu *et al*. [16] and Kim *et al*. [17] reported a greater anti-*Streptococcus mutans* effect, with minimum inhibitory concentrations of 14–35 µg/mL of propolis.

Considerable variability of the chemical composition of propolis (due to its geographical distribution) may be a limitation in terms of its quality control, comparability and effect reproducibility [18]. That could affect the determination of MIC values which depends on technical details that may vary between laboratories and on bacterial inherent virulence and susceptibility [19].

Four different propolis solutions exhibited equal effectiveness against investigated strains in the study, but one of them – the one dissolved in acetone – had the outstanding MIC of 6.3 µg/mL only for *L. acidophilus*. Comparing the antimicrobial effect of Egyptian propolis with propolis from New Zealand on *S. mutans* and *Lactobacillus* spp., the propolis hexane fraction from New Zealand was reported to have the strongest antimicrobial action [20]. Although it was capable of inhibiting the development of cariogenic bacteria *Lactobacillus fermentum*, the activity of Chilean propolis was variable and depended on the chemical composition of the propolis used [21].

The exhibited antimicrobial activity of the essential oils is supposed to be due to the synergism of the compounds [22]. The antimicrobial activities of essential oils of different *Satureja* species have been extensively studied because of their very low minimal

inhibitory concentrations [23]. The minimal inhibitory concentrations of *S. kitaibelii* essential oil ranged from 0.097 μg/mL (*C. albicans*) to 25 μg/mL (*Enterococcus faecalis*) [8]. The same MIC of 12.5 μg/mL for *P. gingivalis* and *P. aeruginosa* was established in the present and in one earlier work [8], respectively. Moreover, for other *Satureja* species, many authors reported antibacterial effectiveness of *S. hortensis* [24,25] and *Satureja intermedia* [26] against cariogenic bacteria *F. nucleatum*, *S. mutans*, *S. salivarius* and *S. sanguis*.

Contemporary investigations have confirmed antibacterial activity of *S. officinalis* essential oils against *S. mutans* [27]. In order to develop novel and effective agents against oral bacteria responsible for dental caries, Moreira *et al.* [28] emphasized that manool and manool-rich *S. officinalis* extract (SODH2) were important and selective plant-derived products that could be potentially used in the control of caries disease. A very promising anti-*Streptococcus mutans* effect with MIC values of 6.24 μg/mL of manool and especially of 15.68 μg/mL of SODH2 has been obtained, which is similar to the results of this work (12.5 μg/mL). Regarding minimal inhibitory concentrations of essential oil, the results of the present study for *P. gingivalis* (25 μg/mL) corresponded to those for *S. salivarius* and *S. sobrinus* (24.96 μg/mL) [28].

Conclusions

This study showed a positive inhibitory influence of different propolis solutions and essential oils on the growth of investigated oral microorganisms. The observed reduction in oral flora counts may provide an alternative preventive and therapeutic approach for individuals at high risk for dental caries and other oral diseases.

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