Inhibitory effects of different medicinal plants on the growth of some oral microbiome members

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Summary

Oral infections and dental caries are still considered as a serious public health problem. Using the method of minimum inhibitory concentrations (MICs), the aim of this study was to investigate the inhibitory effects of 16 plants on the growth of *Actinomyces odontolyticus*, *Streptococcus mitis*, *Streptococcus sanguinis*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Lactobacillus acidophilus* and *Streptococcus mutans*. The most effective were ethanol extracts of *Rosmarinus officinalis*, *Salvia officinalis*, *Thymus vulgaris*, *Calendula officinalis* and *Hypericum perforatum*. The least efficiency was observed for *Acorus calamus* extract. Plant extracts could be used in oral health as therapeutic and prophylactic approach or in balancing oral microbiota.

Keywords: medicinal plants, oral cavity bacteria, antimicrobial activity

The human oral microbiota consists of 500 to 700 common oral species of bacteria (19, 9). Nearly 300 bacterial species have been isolated and named belonging to several phyla, including *Bacteroidetes*, *Firmicutes*, *Tenericutes*, *Actinobacteria*, *Proteobacteria*, *Euryarchaeota*, *Chlamydiae*, and *Spirochaetes* (26). Molecular methods using 16S rRNA studies identified approximately 600 species or phylotypes (24). When oral microbiota is balanced its presence in the oral cavity is protective against local and systemic diseases. Since oral microbiota is influenced by many factors, such as underlying diseases, food, pH, aging etc., they can lead to normal microbiota being replaced by etiological agents of pathological processes (15). Some of the members of oral cavity microbiota, such as *Streptococcus mutans* and *Lactobacillus* spp., play a role in the etiology of caries, others are important in the etiology of gingivitis and various paradontopathies, and even in various types of oral pyogenic processes, such as abscess and osteomyelitis (9). In addition, oral cavity microorganisms have been shown to cause a number of systemic diseases, including cardiovascular diseases (6), ischemic stroke (16), pregnancy complications such as preterm birth (22), diabetes (11), and pneumonia (3). In traditional

medicine, medicinal herbs are used in balancing oral microbiota (13).

In the present study, we investigated the inhibition properties of 16 plant ethanol extracts against standard strains of cariogenic bacteria: *Streptococcus mutans* (*S. mutans*), *Streptococcus mitis* (*S. mitis*), *Streptococcus sanguinis* (*S. sanguinis*), *Lactobacillus acidophilus* (*L. acidophilus*) and bacteria involved in paradontopathy: *Actinomyces odontolyticus* (*A. odontolyticus*), *Eikenella corrodens* (*E. corrodens*), *Fusobacterium nucleatum* (*F. nucleatum*). Ethanol extracts were made from medicinal plants often used in Serbian ethnomedicine.

Material and methods

Collection of plant material. The following medicinal plants were collected in northern Serbia: the free-growing plants *Aesculus hippocastanum*, *Artemisia absinthium*, *Capsella bursa-pastoris*, *Hypericum perforatum* and *Thymus vulgaris*, and the cultivated plants *Achillea millefolium*, *Calendula officinalis*, *Malva mauritanica*, *Origanum majorana*, *Plantago lanceolata*, *Sinapis alba*, *Tilia cordata*, *Teucrium montanum*, *Acorus calamus*, as well as *Salvia officinalis* and *Rosmarinus officinalis* which are both free-growing and cultivated medicinal plants.

Bacterial strains. Investigated strains were: *A. odontolyticus* ATCC 17929, *S. mitis* ATCC 6249, *S. sanguinis* ATCC 10556, *E. corrodens* ATCC 23834, *F. nucleatum* ATCC 25586, *L. acidophilus* ATCC 4356 and *S. mutans* ATCC 25175 (Microbiologics).

Preparation of ethanolic extracts. Dry plants (150 g) were milled into fine powder with an electric blender. Extraction was done in a percolator, using 500 ml of (70%) ethanol, with low-pressure evaporation following extraction. The content of the extract was left for at least 16 h. The amount of the extract was six times the volume of the starting drug (1 : 6 extract). The extract was stored and finally passed through a 0.22 µm filter (Millipore, Billerica, MA). After the dry substance had been obtained in an evaporator, the extracts were kept at 4°C until use (22).

Antimicrobial activity. The ethanol extracts were individually tested against specific bacterium. Bacteria were cultured overnight at 37°C in Mueller Hinton broth (HiMedia), pH = 7.4.

Determination of the minimum inhibitory concentration (MIC). The method applied for the evaluation of the antimicrobial activity was minimum inhibitory concentration (MIC) by the method of agar dilution (CLSI, 2010.). Serial dilutions of plant extracts were prepared in plates, and the assay plates were estimated to contain 300, 150, 75 and 37.5 µg/ml of active extracts. Inocula were applied on surfaces of blood agar (Liofilchem) giving approximately 10⁶ µg/ml of bacteria. All plates were incubated for about 48 to 72 h under anaerobic conditions at 37°C. MIC was taken as the lowest concentration of extract that produced no visible bacterial growth as compared to the control growth. The extracts were tested in triplicates.

Results and discussion

Extracts of *Rosmarinus officinalis*, *Salvia officinalis*, *Thymus vulgaris*, *Calendula officinalis* and *Hypericum perforatum* were efficient against most investigated bacteria (5/5), (5/5), (5/6), (5/6) and (4/5), respectively, with MICs of 37.5 µg/ml. *Acorus calamus* extract had the lowest efficiency of MICs = 300 µg/ml for most oral bacteria (6/7) except for *L. acidophilus* (MIC = 75 µg/ml). *Aesculus hippocastanum* extract had high MICs for *F. nucleatum* and *S. mutans* (300 µg/ml). Moderate efficiency is shown against *A. odontolyticus*, *S. sanguinis* and *L. acidophilus* (75 µg/ml), but the exception was *E. corrodens* with MIC of 37.5 µg/ml. Among oral streptococci, *S. mutans* was the most resistant strain to examined plant extracts, except to *T. vulgaris*, *R. officinalis* and *S. officinalis* extracts (Tab. 1). *F. nucleatum* expressed the highest sensitivity to *T. vulgaris*, *R. officinalis*, *H. perforatum*, *S. officinalis* and *C. officinalis* extracts (37.5 µg/ml) and less sensitivity to *A. calamus*, *Tilia cordata*, *A. hippocastanum* and *Capsella bursa-pastoris* (300 µg/ml). *A. odontolyticus* were the most sensitive bacteria with MICs of 37.5 µg/ml to extracts of: *Sinapis alba*, *T. vulgaris*, *R. officinalis*, *H. perforatum*, *Teucrium montanum*, *Artemisia absinthium*, *Plantago lanceolata*, *S. officinalis*, *C. officinalis*, *C. bursa-pastoris* and *Origanum majorana*, but less sensitive to *Malva mauritanica* and *A. hippocastanum* (75 µg/ml). These bacteria expressed the least sensitivity to *T. cordata*, *Achillea millefolium* (150 µg/ml) and *A. calamus* extracts (300 µg/ml).

The human oral microbiota is based on a substantial

number of microorganisms with complex interactions, which at the same time represent normal microbiota of the human cavity and reservoir for bacteria that participate in the etiology of local and systemic disease.

The ethanol extracts of *Rosmarinus officinalis*, *Salvia officinalis*, *Thymus vulgaris*, *Calendula officinalis* and *Hypericum perforatum* were the most efficient in this research, with MICs of 37.5 µg/ml for majority of investigated bacteria. On the other hand, *Acorus calamus* extract had the highest MICs = 300 µg/ml for most oral pathogens. Of all tested strains, *S. mutans* were the most resistant bacteria.

Shruthi and Geetha (29) showed that *T. vulgaris* extracts had very good antibacterial activity against *S. sanguinis* and *S. mutans*. Results in previous studies in which methanolic extracts and aqueous extracts of *T. vulgaris* showed no effect either on *S. mutans* or *S. sanguinis* (4) could

Tab. 1. MICs (µg/ml) of 16 herbal extracts for some members of oral microbiota

Bacteria \ Plants	<i>A. odontolyticus</i>	<i>S. mitis</i>	<i>S. sanguinis</i>	<i>E. corrodens</i>	<i>F. nucleatum</i>	<i>L. acidophilus</i>	<i>S. mutans</i>
<i>Sinapis alba</i>	37.5	> 300	75	75	75	150	300
<i>Thymus vulgaris</i>	37.5	> 300	37.5	37.5	37.5	75	37.5
<i>Rosmarinus officinalis</i>	37.5	> 300	37.5	37.5	37.5	> 300	37.5
<i>Hypericum perforatum</i>	37.5	> 300	37.5	37.5	37.5	> 300	300
<i>Teucrium montanum</i>	37.5	> 300	37.5	37.5	75	300	75
<i>Artemisia absinthium</i>	37.5	> 300	37.5	75	75	150	300
<i>Plantago lanceolata</i>	37.5	> 300	75	37.5	75	75	75
<i>Salvia officinalis</i>	37.5	> 300	37.5	37.5	37.5	> 300	37.5
<i>Calendula officinalis</i>	37.5	> 300	37.5	37.5	37.5	37.5	150
<i>Acorus calamus</i>	300	300	300	300	300	75	300
<i>Malva mauritanica</i>	75	> 300	300	75	150	75	300
<i>Tilia cordata</i>	150	> 300	150	150	300	150	> 300
<i>Aesculus hippocastanum</i>	75	> 300	75	37.5	300	75	300
<i>Capsella bursa-pastoris</i>	37.5	> 300	300	75	300	300	300
<i>Origanum majorana</i>	37.5	150	37.5	37.5	150	75	150
<i>Achillea millefolium</i>	150	300	150	150	150	300	300

be explained by the use of different solvents. Clinical investigation on effects of *T. vulgaris* ethanolic extract on pulpitis, indicated for pulpothomy, supported experimental findings on the antimicrobial effect of this plant on oral microbiota (2).

In contrast to our results, Lauk et al. (18) documented a lower inhibitory activity ($MIC \geq 2048$ mg/L) of *C. officinalis* methanol extracts against parodontopathic bacteria *F. nucleatum*, *E. corrodens* and *A. odontolyticus*.

The present study showed an inhibitory concentration of *S. officinalis* at 37.5 µg/ml for *S. mutans*. Moreover, significant reduction in the *S. mutans* colony count has been recorded *in vivo* evaluations as well as in the agar diffusion method (7, 32, 17).

Antimicrobial activity of *R. officinalis* L. propylene glycol extract showed a significant reduction in colony forming units per milliliter (CFU/ml) in all biofilms at MIC of 200 mg/ml (23, 30). Also, some studies revealed that dentifrice containing alcoholic *R. officinalis* extract had antimicrobial activity similar to commercially available herbal dentifrice against some streptococci (32).

In our research, the effect of *Achillea millefolium* extract showed moderate activity against *A. odontolyticus*, *S. sanguinis*, *E. corrodens* and *F. nucleatum*, with MICs of 150 µg/ml, while its activity against *S. mitis*, *L. acidophilus* and *S. mutans* was low with MICs of 300 µg/ml. These results, although there are some differences in MICs, are in accordance with previous studies revealing the activity of *A. millefolium* extract as a possible supplement in preparations against oral bacteria. On the other hand, *S. mitis* was the most susceptible of the tested organisms to the herbal mixture extract of *Juniperus communis*, *Urtica dioica* and *A. millefolium*, with an MIC value of 1 mg/ml (35).

At the MICs of 37.5 µg/ml and of 75 µg/ml, in the present study *Artemisia absinthium* ethanol extract expressed very good antimicrobial activity against *A. odontolyticus* and *S. sanguinis* and good activity against *E. corrodens* and *F. nucleatum*, respectively. However, we observed weak activity against *L. acidophilus* (MIC = 150 µg/ml) and especially weak activity against *S. mutans* (MIC = 300 µg/ml) which is similar to results of Vieira et al. (34) (MIC = 250 µg/ml), although there was differences in the used preparations.

Soleimanpour et al. (31) found no resistance in investigated strains (*S. mutans* and *S. sanguinis*) to the antibacterial activity of *Capsella bursa-pastoris* ethanol extracts and its combination with *Glycyrrhiza glabra*. Since our results are the opposite, showing the highest values of MICs (300 µg/ml) for *S. mutans* and *S. sanguinis* as well as for *F. nucleatum* and *L. acidophilus*, it could be suggested that mixed extract was more effective against all bacteria, indicating its synergistic effect.

The assessment of *Aesculus hippocastanum* anti-bacterial activity found aqueous and ethanolic extracts being effective against tested oral bacteria (*S. mutans*, *S. sanguinis* and *L. acidophilus*), while the ethanol extract was more effective against *S. mutans* and *S. sanguinis* (27). In our research the MIC of *A. hippocastanum* ethanol extract against *S. mutans* was 300 µg/ml and for *S. sanguinis* and *L. acidophilus* 75 µg/ml, which is considerably different in comparison to previous studies.

A. calamus L. is described as an incredible herb because of its medicinal characteristics (14). However, there is little evidence of its activity against oral bacteria. While the *A. calamus* ethanol extract was active against all the investigated bacterial strains, aqueous extract was completely inactive against some investigated Gram-negative bacteria and active at a high concentration only against some Gram-positive bacteria (20). We registered *L. acidophilus* (MIC=75 µg/ml) to be the most sensitive bacteria to *A. calamus* ethanol extract, while MICs for all other investigated bacteria were 300 µg/ml.

Most of the studies revealed good antibacterial activity of *H. perforatum* ethanol extract against different strains of pathogenic bacteria (5, 21), as well as against *Bifidobacterium animalis* and *L. plantarum* (25). In our study, ethanol extract of *H. perforatum* possessed very good antibacterial activity against all investigated bacteria (MIC = 37.5 µg/ml) except for *S. mutans* (300 µg/ml).

Of the tested bacteria in the present investigation, the most resistant strains (MIC = 150 µg/ml) to *Origanum majorana* ethanol extract were *S. mitis*, *S. mutans* and *F. nucleatum*. Previous studies of Hajlaoui et al. (12) confirmed higher MICs of *O. majorana* essential oil for *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Vibrio parahaemolyticus*. In contrast, Chaudhry et al. (8) have shown a great antibacterial potential of *O. majorana* essential oil and aqueous infusion against *Citrobacter* spp. and *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Enterobacter aerogenes*, respectively.

Because of the significant decrease in streptococci in the oral cavity, some authors concluded that *Plantago lanceolata* extract could be a natural anticariogenic agent through its antimicrobial effect and useful in the proliferation control of cariogenic flora (10). In our research, the strongest activities of *P. lanceolata* ethanol extracts were observed against *A. odontolyticus* and *E. corrodens* (MIC = 37.5 µg/ml), while *S. mutans* and *S. sanguinis* had MICs of 75 µg/ml as did *L. acidophilus* and *F. nucleatum*. On the other hand, ethanol extract of *P. major* did not show any activity against primary plaque colonizers or periodontal pathogens (28).

In our investigation, ethanol extracts of *Teucrium montanum* and of *Sinapis alba* showed good antimicrobial activities against the investigated oral microbiota, with the exception of *L. acidophilus* and *S. mutans* (MIC = 300 µg/ml), respectively.

As determined by the disc diffusion method, the crude ethanol extracts as well as its fractions of *Malva parviflora* have shown good antibacterial effect against *Staphylococcus aureus* and *Escherichia coli* (1). The activity of *M. mauritanica* ethanol extract in our study against oral bacteria was good, except for *S. sanguinis* and *S. mutans* with MICs of 300 µg/ml.

The strong antimicrobial activity of *Tilia cordata* was found against *Listeria ivanovii*, but the highest activity of methanolic extract was measured against *Pseudomonas aeruginosa* with disc diffusion method (33). Our investigation of *T. cordatata* ethanol extract efficiency showed a moderate activity against *A. odontolyticus*, *S. sanguinis*, *E. corrodens* and *L. acidophilus* with MICs of 150 µg/ml and a weak effect on *F. nucleatum* with MIC of 300 µg/ml.

Ethanol extracts of *Rosmarinus officinalis*, *Salvia officinalis*, *Thymus vulgaris*, *Calendula officinalis* and *Hypericum perforatum* were shown to be the most effective against investigated bacteria, while *Acorus calamus* extract displayed a minimum of efficacy. However, the most resistant bacteria among oral streptococci and investigated anaerobes were *S. mutans* strains. The results from this study indicate that plant extracts could be used in oral health as a therapeutic and prophylactic approach or in balancing oral microbiota.

References

- Abdel-Ghani A. E., Hanaa H. M., El-Shazl A. M.: Phytochemical and biological study of *Malva parviflora* L. grown in Egypt. *Zagazig J. Pharm. Sci.* 2013, 22, 17-25.
- Alolofi H., El-Sayed M., Taha T.: Clinical and radiographical evaluation of propolis and *Thymus vulgaris* extracts compared with formocresol pulpotomy in human primary molars. *BDJOPEN* 2016, 2, 16005.
- Awano S., Ansai T., Takata Y., Soh I., Akifusa S., Hamasaki T., Yoshida A., Sonoki K., Fujisawa K., Takehara T.: Oral health and mortality risk from pneumonia in the elderly. *J. Dent. Res.* 2008, 87, 334-339.
- Babpour E., Angaji S., Angaj S. M.: Antimicrobial effects of four medicinal plants on dental plaque. *J. Med. Plants Res.* 2009, 3, 132-137.
- Barbagallo C., Chisari G.: Antimicrobial activity of three *Hypericum* species. *Fitoterapia* 1987, 58, 175-177.
- Beck J. D., Offenbacher S.: Systemic effects of periodontitis: epidemiology of periodontal disease and cardiovascular disease. *J. Periodontol.* 2005, 76, 2089-2100.
- Beheshti-Rouy M., Azarsina M., Rezaie-Soufi L., Alikhani M. Y., Roshanaie G., Komaki S.: The antibacterial effect of sage extract (*Salvia officinalis*) mouthwash against *Streptococcus mutans* in dental plaque: a randomized clinical trial. *Iran. J. Microbiol.* 2015, 7, 173-177.
- Chaudhry N. M. A., Saeed S., Tariq P.: Antibacterial effects of oregano (*Origanum vulgare*) against Gram negative bacilli. *Pak. J. Bot.* 2007, 39, 609-613.
- Dewhirst F. E., Chen T., Izard J., Paster B. J., Tanner A. C., Yu W. H., Lakshmanan A., Wade W. G.: The human oral microbiome. *J. Bacteriol.* 2010, 192, 5002-5017.
- Ferrazzano G. F., Cantile T., Roberto L., Ingenito A., Catania M. R., Roscetto E., Palumbo G., Zarrelli A., Pollio A.: Determination of the in vitro and in vivo antimicrobial activity on salivary *Streptococci* and *Lactobacilli* and chemical characterisation of the phenolic content of a *Plantago lanceolata* infusion. *Biomed. Res. Int.* 2015, 2015, 286817.
- Genco R. J., Grossi S. G., Ho A., Nishimura F., Murayama Y.: A proposed model linking inflammation to obesity, diabetes, and periodontal infections. *J. Periodontol.* 2005, 76, 2075-2084.
- Hajlaoui H., Mighri H., Gharsallah M. A. N., Kadri A.: Chemical composition and in vitro evaluation of antioxidant, antimicrobial, cytotoxicity and anti-acetylcholinesterase properties of Tunisian *Origanum majorana* L. essential oil. *Microbial. Pathogenesis* 2016, 95, 86-94.
- Haque M. M., Alsareii S. A.: A review of the therapeutic effects of using miswak (*Salvadora Persica*) on oral health. *Saudi Med. J.* 2015, 36, 530-543.
- Imam H., Riaz Z., Azhar M., Sofi G., Hussain A.: Sweet flag (*Acorus calamus* Linn.): an incredible medicinal herb. *Int. J. Green Pharm.* 2013, 7, 288-296.
- Iwami Y.: Rate-limiting steps of the glycolytic pathway in the oral bacteria *Streptococcus mutans* and *Streptococcus sanguis* and the influence of acidic pH on the glucose metabolism. *Arch. Oral Biol.* 1980, 25, 163-169.
- Joshiyura K. J., Hung H. C., Rimm E. B., Willett W. C., Ascherio A.: Periodontal disease, tooth loss, and incidence of ischemic stroke. *Stroke* 2003, 34, 47-52.
- Kemanshah H., Hashemi K. S. S., Arami S., Mirsalehian A., Kamalinejad M., Karimi M., Jabalameli F.: Comparison of antibacterial effect of hydroalcoholic extract of four plants against cariogenic microorganisms by two in vitro methods. *JBUMS* 2011, 13, 21-29.
- Lauk L., Lo Bue A. M., Milazzo I., Rapisarda A., Blandino G.: Antibacterial activity of medicinal plant extracts against periodontopathogenic bacteria. *Phytother. Res.* 2003, 17, 599-604.
- Lederberg J., McCray A. T.: 'Ome sweet' omics – a genealogical treasury of words. *Scientist* 2001, 15, 8-10.
- Manikandan S., Devi R. S., Srikumar R., Thangaraj R., Ayyappan R., Jegadeesh R., Lakshmanan H.: In vitro antibacterial activity of aqueous and ethanolic extracts of *Acorus calamus*. *Int. J. Appl. Biol. Pharm. Technol.* 2010, 1, 1072-1075.
- Molochko V. A., Lastochkina T. M., Krylov I. A., Brangulis K. A.: The anti-staphylococcal properties of plant extracts in relation to their prospective use as therapeutic and prophylactic formulations for the skin. *Vestn. Dermatol. Vener.* 1990, 8, 54-56.
- Offenbacher S., Jared H. L., O'Reilly P. G., Wells S. R., Salvi G. E., Lawrence H. P., Socransky S. S., Beck J. D.: Potential pathogenic mechanisms of periodontitis associated pregnancy complications. *Ann. Periodontol.* 1998, 3, 233-250.
- Oliveira J. R. de, Jesus D. de, Figueira L. W., Oliveira F. E. de, Soares C. P., Camargo S. E. A., Jorge A. O. C., Oliveira L. D. de: Biological activities of *Rosmarinus officinalis* L. (rosemary) extract as analyzed in microorganisms and cells. *Exp. Biol. Med.* (Maywood) 2017, 242, 625-634.
- Paster B. J., Boches S. K., Galvin J. L., Ericson R. E., Lau C. N., Levanos V. A., Sahasrabudhe A., Dewhirst F. E.: Bacterial diversity in human subgingival plaque. *J. Bacteriol.* 2001, 183, 3770-3783.
- Reichling J., Weseler A., Saller R.: A current review of the antimicrobial activity of *Hypericum perforatum* L. *Pharmacopsychiatry* 2001, 34, 116-118.
- Reynolds-Campbell G., Nicholson A., Thoms-Rodriguez C. A.: Oral bacterial infections: Diagnosis and management. *Dent. Clin. North. Am.* 2017, 61, 305-318.
- Roy A., Geetha R. V., Lakshmi T.: In vitro evaluation of antibacterial activity of aqueous and ethanolic extracts of *Aesculus hippocastanum* on oral microbes. *Asian J. Pharm. Clin. Res.* 2011, 4, 90-92.
- Sharma H., Yunus G. Y., Mohapatra A. K., Kulshrestha R., Agrawal R., Kalra M.: Antimicrobial efficacy of three medicinal plants *Glycyrrhiza glabra*, *Ficus religiosa*, and *Plantago major* on inhibiting primary plaque colonizers and periodontal pathogens: An in vitro study. *Indian J. Dent. Res.* 2016, 27, 200-204.
- Shruthi D., Geetha R. V.: Effect of herbal extracts in prevention of dental caries – an in vitro study. *Int. J. Drug Dev. Res.* 2018, 10, 20-21.
- Silva B. N. de, Nakassugi L. P., Faggion P. O. J., Kohiyama C. Y., Mossini S. A., Grespan R., Nerilo S. B., Mallmann C. A., Alves A. F. B., Machinski M. Jr.: Antifungal activity and inhibition of fumonisin production by *Rosmarinus officinalis* L. essential oil in *Fusarium verticillioides* (Sacc.) Nirenberg. *Food Chem.* 2015, 166, 330-336.
- Soleimanpour S., Sedighinia F. S., Afshar A. S., Zarif R., Asili J., Ghazvini K.: Synergistic antibacterial activity of *Capsella bursa-pastoris* and *Glycyrrhiza glabra* against oral pathogens. *Jundishapur J. Microbiol.* 2013, 6, e7262.
- Valones M. A., Higino J. S., Souza P. R., Crovella S., Caldas A. F. Jr, Carvalho A. A.: Dentifrice containing extract of *Rosmarinus officinalis* Linn.: an antimicrobial evaluation. *Braz. Dent. J.* 2016, 27, 497-501.
- Vatřák A., Kolesárová A., Vuković N., Rovná K., Petrová J., Vimmerová V., Hleba L., Mellen M., Kačániová M.: Antimicrobial activity of medicinal plants against different strains of bacteria. *J. Microbiol. Biotech. Food Sci.* 2014, 3, 174-176.
- Vieira T. M., Dias H. J., Medeiros T. C. T., Grundmann C. O., Groppo M., Heleno V. C. G., Martins C. H. G., Cunha W. R., Crotti A. E. M., Silva E. O.: Chemical composition and antimicrobial activity of the essential oil of *Artemisia absinthium* Asteraceae leaves. *JEOP* 2017, 20, 123-131.
- Weijden G. A. van der, Timmer C. J., Timmerman M. F., Reijerse E., Mantel M. S., Velden U. van der: The effect of herbal extracts in the experimental mouthrinse on established plaque and gingivitis. *J. Clin. Periodontol.* 1998, 25, 399-403.

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