ORIGINAL ARTICLE
ORIGINALNI RAD
DOI: https://doi.org/10.2298/SGS2501003T
UDC: 615.322:582.991; 612.313

Antimicrobial effect of *Sphilantes acmella* on *Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis* and *Candida albicans* in oral cavity

Zoran Tambur[1], Stevan Avramov[1,2], Ema Aleksić[1], Anica Joksić[1], Uroš Dimitrijević[1], Vladimir Biočanin[1]

[1]University Business Academy in Novi Sad, Faculty of Stomatology, Pančevo, Serbia;
[2]University of Belgrade, National Institute of the Republic of Serbia, Siniša Stankovic Institute for Biological Research, Belgrade, Serbia

SUMMARY

Introduction Due to the increasing tolerance to antibiotics, the treatment of disorders of the microbial community in the oral cavity represents a major challenge. An alternative substance that can partially or completely replace existing antibacterial agents could be products derived from plants. *Spilanthes acmella* has been shown to be effective in the treatment of diseases of the oral cavity. The aim of this study is to investigate the potential antimicrobial efficacy of the plant *Spilanthes acmella* against *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans*, which are frequently associated with diseases of the oral cavity, and to compare this effect with conventional therapies.

Material and methods Three groups of samples were analyzed: the flower heads, the leaves and the remaining aerial parts (the stem and twigs).

Results The results showed that the flower extract showed no inhibitory effect on the bacteria tested. The extract from the stem and twigs showed an inhibitory effect on *Candida albicans*. The leaf extract showed the best inhibitory effect against *Streptococcus mutans*.

Conclusion Considering that chlorhexidine is the only solution with a proven antiplaque effect, we can conclude from these results that the synergistic effect of these two solutions provides the best results in the chemical control of dental plaque and could be part of the protocol in the prevention of both dental caries and periodontal disease in the future.

Keywords: plant extracts; antimicrobial activity; oral cavity; minimum inhibitory concentrations

INTRODUCTION

The human oral microbiome is an extremely complex and extraordinary group of microorganisms that form diverse associations in the mouth and harbor more than 700 species, including harmless symbionts, commensals and opportunistic pathogens [1, 2]. When the oral microbiome is disturbed, dysbiosis occurs in which disease-promoting bacteria gain the upper hand and cause oral diseases [3]. In addition, the role of the oral microbiome in a number of non-oral diseases such as pancreatic cancer, diabetes mellitus and endocarditis has been uncovered [4]. The microorganisms that make up the oral microbiome are usually organized in biofilms, commonly referred to as dental plaque. Oral pathogenic biofilms have been recognized as a predisposing factor for various oral infections, including dental caries, gingivitis, periodontitis and peri-implantitis [5, 6]. Dental caries is considered the most prevalent infectious disease in the world and one of the most frequent oral diseases, affecting 60–90% of schoolchildren and the vast majority of adults [7]. About 5–15% of the general population is affected by severe periodontitis [8]. Disruption of microbial community dynamics plays an important role in the etiology of gingivitis and the development of periodontitis [9].

Streptococcus mutans is considered to be the most important etiological factor in the development of dental caries [10]. In the early stages of caries formation, the combined action of enzymes released by *Strep. mutans* (glucosyltransferase and fructosyltransferase) and the adhesion-promoting substances (glucans) secreted by *Candida albicans* form cariogenic plaque biofilms on tooth surfaces. This provides habitats for the growth of the etiological bacteria [11, 12]. Of particular concern is the observation that there are strains of *Strep. mutans* that are resistant to various antibiotics and also to the presence of fluoride [13, 14].

Staphylococcus aureus is the most pathogenic member of the genus and the causative agent of a variety of diseases ranging from superficial skin abscesses and food poisoning to life-threatening conditions such as bacteremia, necrotic pneumonia in children and endocarditis [15]. *Staph. aureus* is thought to benefit from the presence of *C. albicans* in the oral cavity [16, 17]. The formation of such a specific microbial community associates *Staph. aureus* with several risks, such as the development of oral diseases (e.g. caries,

Address for correspondence: Zoran TAMBUR, University Business Academy in Novi Sad, Faculty of Stomatology, Žarka Zrenjanina 179, 26000 Pančevo, Serbia; *zoran.tambur@stomatoloski.rs*

halitosis, periodontitis, oral cancer, systemic infections), and may even influence the progression of several systemic diseases such as osteoporosis, atherosclerosis, diabetes, cardiovascular disease and ischemic cardiomyopathy [18]. The widespread use of prophylactic antibiotics in dentistry has been associated with the emergence of antibiotic resistance in several commensal microorganisms, including *Staph. aureus* [19].

Enterococcus faecalis is a facultative anaerobic gram-positive bacterium in the human oral cavity that is mainly responsible for various oral pathologies, in particular dental caries, dental abscesses, periodontal infections, apical periodontitis and persistent endodontic infections [20]. The ability of *E. faecalis* to grow as a biofilm on the walls of root canals and as a monoinfection in treated canals without synergistic support from other bacteria leads to a high resistance to antimicrobial agents [21, 22]. Clinical isolates of *E. faecalis* recovered from root canal and periodontal infections may exhibit antimicrobial resistance to conventional treatments recommended for dental procedures [23].

The opportunistic pathogen *Candida albicans* is the most frequently isolated fungus in oral infections [24]. The results of the meta-analysis by Edit et al. [25] indicate that individuals with the presence of *Candida* spp. have a higher prevalence of dental caries than individuals without these microorganisms in the oral cavity. The presence of *C. albicans* in the oral cavity has been associated with other oral diseases, such as denture stomatitis, oral cancer as well as the failure of endodontic treatments [26, 27, 28]. Over the past two decades, conventional antimicrobials have proven increasingly ineffective in controlling *C. albicans* [4, 29, 30].

Chlorhexidine is the most popular and widely accepted oral antibacterial agent and is considered the gold standard [31]. However, it cannot be used long-term as it has various side effects, such as brown discoloration, taste disturbances, oral mucosal lesions, parotid gland swelling, increased supragingival plaque formation and sometimes an unacceptable taste [32, 33]. Another problem with the use of chlorhexidine is the development of antimicrobial resistance, which is a serious adverse effect [34, 35].

Due to these side effects, it is important to find an alternative substance that can partially or completely replace the existing antibacterial agents. One of the ways to find new promising drugs is to thoroughly investigate herbs and herbal remedies used in the traditional medicine of different countries [36, 37, 38].

One of these herbs is *Spilanthes acmella* L., which is native to Brazil and Peru, but can be introduced as an annual plant in continental climates. This herb is popularly known as a toothache plant as it has an anesthetic effect when the leaves and flowers are chewed [39]. *S. acmella* is an herb with ascending, cylindrical, hairy stems that grows up to 40–60 cm tall and belongs to the *Asteraceae* family. Plant extracts, formulations and bioactive ingredients of *Spilanthes* species have been shown to have a wide range of potential applications in the pharmaceutical industry [40]. *S. acmella* has been shown to be effective in traditional medicine for the treatment of rheumatism, fever and influenza, tuberculosis, rabies, malaria and scurvy [41]. It has antinociceptive, antimicrobial, anti-inflammatory, anesthetic, analgesic, antifungal, antimutagenic, diuretic and immunostimulant effects [42, 43, 44]. It has also been shown to be effective in the treatment of diseases of the oral cavity such as dentalgia, periodontitis and ulcers of the oral mucosa [40, 45]. Alkamides are considered the predominant phytochemicals in the genus *Spilanthes* and the most important alkamide found is spilanthol. Despite numerous studies on the biological significance of this metabolite, there are only a few commercial spilanthol-based products for pharmacological purposes [46].

The aim of this study is to investigate the potential antimicrobial efficacy of the flower heads, the leaves and the stem and twigs of the *S. acmella* plant against typical infectious pathogens in the human oral cavity: *Strep. mutans*, *Staph. aureus*, *E. faecalis* and *C. albicans*. This study will also analyze the comparison with conventional therapies (chlorhexidine). Given the origin of this plant and its limited distribution, such studies have not yet been carried out in European countries as far as we know.

METHODS

Plant material and preparation of the extract

The plant material was obtained in July 2023 from *S. acmella* plants grown from seed (Tuinzaden.eu, Weesperstraat 94d, 1112AP Dieme, The Netherlands). Three different groups of samples were analyzed. The first consisted of the flower heads, the second of the leaves and the third of the remaining aerial parts (the stem and twigs). All parts of the plant material that were torn, crumpled and with dark edges were removed.

The plant parts were thoroughly washed under running tap water. The raw materials were disinfected by immersion in a solution containing 100 ppm (mg/L) of free residual chlorine for 10 minutes [47]. The final rinse was done with distilled water. The fresh samples were dried in an oven at 50°C to remove all water. To obtain a fine and homogeneous powder, the dried mass of flower heads, leaves and remaining aerial parts were macerated separately with liquid nitrogen and a porcelain pestle and mortar. These extracts obtained by maceration were dissolved in methanol (HPLC grade, Merck) (1:20) at room temperature. The *S. acmella* plant material was then left in the bath sonicator for ten minutes. The methanolic extract was then centrifuged for 15 minutes at 5000 rpm in a refrigerated centrifuge (Sigma 4K 15C, Sigma Laborzentrifugen GmbH Osterode am Harz, Germany). The centrifuged aliquot (supernatant) was collected. The residue was centrifuged again in a methanol solution to obtain a larger amount of the extract. The residue was then discarded and the entire methanolic solution was collected and filtered. The solutions were concentrated to dryness under reduced pressure in a rotary evaporator (Buchi Rotavapor, Flawil, Switzerland). The vials containing the extracts were wrapped in aluminum foil and stored in a freezer at -30°C until analysis of potential antimicrobial activity.

Microorganisms used in research

The strains tested were gram-positive facultative anaerobes Strep. mutans ATCC 25175, E. faecalis ATCC 14506, Staph. aureus ATCC 25923 and C. albicans ATCC 10231, which were cultured in the presence of oxygen. All strains were grown overnight at 37 °C in Miller-Hinton broth (HiMedia, Mumbai, Maharashtra, India) with a standard pH of 7.4 for this broth.

Determination of minimum inhibitory concentrations

The method used to evaluate the antimicrobial activity was the minimum inhibitory concentration (MIC) by the tryptic soy agar dilution method (EUCAST, 2024) [48]. During the cooling of the substrate before it hardens, plant extracts are added in the appropriate concentration. Serial dilutions of the plant extracts were prepared in plates with concentrations of 6.25, 12.5, 25, 50, 100, 200 and 400 μg/ml. The MIC was defined as the lowest concentration of the extract that caused no visible bacterial growth compared to the control growth.

There was no intra-observer variation as the investigator inoculating the media for the antimicrobial activity test obtained identical results for three measurements at three time points.

The comparison was made with a standard solution of 0.12% chlorhexidine (Galenika, Zemun, Belgrade, Serbia). Serial dilutions of chlorhexidine were prepared in plates with concentrations of 6.25, 12.5, 25, 50, 100, 200 and 400 μl/ml.

RESULTS

The effect of different concentrations of chlorhexidine was investigated on four types of microorganisms. Chlorhexidine had an inhibitory effect on the tested strains of all three types of bacteria (*Strep. mutans*, *E. faecalis* and *Staph. aureus*) as well as on *C. albicans*. The inhibitory effects were achieved even at the lowest chlorhexidine concentration used (6.25 μg/mL). The extract from the flowerheads of *S. acmella* showed no inhibitory effect on *C. albicans* in our experiment in all tested concentrations (Table 1). We found that the extract from the stem and twigs of *S. acmella* had an inhibitory effect on *C. albicans* at 50 μg/mL as well as at higher concentrations (Table 2).

The leaf extract of *S. acmella* showed a highly effective inhibitory activity against the bacterium *Strep. mutans* (MIC = 6.3 μg/mL) (Table 2).

In our study, we found no significant effect of *S. acmella* extract on *E. faecalis*. The flower of *S. acmella* showed no inhibitory effect on the tested bacteria – MIC > 400 μg/mL (Table 1 and Table 2). Our studies did not confirm any significant effect of *S. acmella* extracts on *S. aureus* (Table 1 and Table 2).

Table 1. Determination of the minimum inhibitory concentrations of chlorhexidine and extracts from flower heads of *S. acmella* plants for *Streptococcus mutans*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans*
+ growth of microorganisms;
- inhibition of the growth of microorganisms
Tabela 1. Određivanje minimalnih inhibitornih koncentracija hlorheksidina i ekstrakta iz cvasti biljaka *S. acmella* za *Streptococcus mutans*, *Enterococcus faecalis*, *Staphilococcus aureus* i *Candida albicans*
+ rast mikroorganizama;
- inhibicija rasta mikroorganizama

	Streptococcus mutans	Enterococcus faecalis	Staphylococcus aureus	Candida albicans
Chlorhexidine / Hlorheksidin				
400 μg/mL	-	-	-	-
200 μg/mL	-	-	-	-
100 μg/mL	-	-	-	-
50 μg/mL	-	-	-	-
25 μg/mL	-	-	-	-
12.5 μg/mL	-	-	-	-
6.25 μg/mL	-	-	-	-
S. acmella flowerheads / *S. acmella* cvasti				
400 μg/mL	+	+	+	+
200 μg/mL	+	+	+	+
100 μg/mL	+	+	+	+
50 μg/mL	+	+	+	+
25 μg/mL	+	+	+	+
12.5 μg/mL	+	+	+	+
6.25 μg/mL	+	+	+	+

Table 2. Determination of the minimum inhibitory concentrations of extracts from stems, twigs and leaves of *S. acmella* plants for *Streptococcus mutans*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans*. + growth of microorganisms; - inhibition of the growth of microorganisms
Tabela 2. Određivanje minimalnih inhibitornih koncentracija ekstrakata iz stabljika, grančica i listova biljaka *S. acmella* za *Streptococcus mutans*, *Enterococcus faecalis*, *Staphilococcus aureus* i *Candida albicans*
+ rast mikroorganizama;
- inhibicija rasta mikroorganizama

	Streptococcus mutans	Enterococcus faecalis	Staphylococcus aureus	Candida albicans
S. acmella stem and twigs / *S. acmella* stabljike i grančice				
400 μg/mL	+	+	+	-
200 μg/mL	+	+	+	-
100 μg/mL	+	+	+	-
50 μg/mL	+	+	+	-
25 μg/mL	+	+	+	+
12.5 μg/mL	+	+	+	+
6.25 μg/mL	+	+	+	+
S. acmella leaves / *S. acmella* listovi				
400 μg/mL	-	+	+	+
200 μg/mL	-	+	+	+
100 μg/mL	-	+	+	+
50 μg/mL	-	+	+	+
25 μg/mL	-	+	+	+
12.5 μg/mL	-	+	+	+
6.25 μg/mL	-	+	+	+

DISCUSSION

The increasing resistance of pathogens to conventional antibiotics and the undesirable side effects of existing therapies have made traditional medicinal plants an attractive source for screening for antimicrobial agents. In line with these efforts, the present study aimed to investigate the antimicrobial activities of crude methanolic extracts of above ground parts of *S. acmella* against four important dental pathogens: *Strep. mutans*, *E. faecalis*, *Staph. aureus* and *C. albicans*. The aim was also to compare these herbal effects with conventional therapies using chlorhexidine. Numerous studies have already investigated the antibacterial effect of *S. acmella*, but they often lead to contradictory results.

A significant antimicrobial effect of chlorhexidine observed in our study was also reported by Balagopal and Arjunkumar [32], Leyes Borrajo et al. [49] and Jiang et al. [50]. Brookes et al. [35] also cite several studies in their meta-analysis confirming that chlorhexidine is a potent antimicrobial agent. The antifungal effects of chlorhexidine relate to the prevention of biofilm formation [35] and disruption of the structure or cell membrane of *C. albicans* [50]. Although chlorhexidine gluconate is often used to reduce and inhibit biofilm formation, it has some disadvantages. For example, it has some negative effects on the tissues of the oral cavity and also contributes to some negative systemic changes [33, 51]. There is also evidence that some populations of *C. albicans* may persist after the application of chlorhexidine and form a multi-tolerant subpopulation, which may reduce the efficacy of chlorhexidine over time [52]. In these cases, the combined effect of chlorhexidine with some other substances can be of great help. Since the extract from the aerial parts of *S. acmella* has been shown to have an inhibitory effect on *C. albicans* even at low concentrations, we can conclude that it could be a good adjuvant in the treatment of candidiasis, especially when resistant strains are suspected to be present. This is of particular importance as, to our knowledge, no antifungal resistance to spilanthol, the main bioactive metabolite of *S. acmella*, has been demonstrated.

It has been described that *Strep. mutans* communicates closely with *C. albicans* in a complex bidirectional interaction that is involved in biofilm formation [53]. Several reports have shown that the dental microbiome is not only responsible for major disease outbreaks in the oral cavity, but may also play a role in systemic disease [54, 55]. These findings make the control of oral microorganisms an important issue. In this context, the biofilm in dental plaque is a major obstacle to such control as it protects pathogenic bacteria from antibiotics [56, 57]. Since the prevention of biofilm formation is centered on the eradication of *Strep. mutans*, it is important to have effective means to control the presence of this bacterium in the oral cavity. The highly effective bactericidal effect that the extract of *S. acmella* has on *Strep. mutans* offers the possibility of using this plant as an independent or additional medicinal agent that does not have the negative side effects observed with conventional agents [58].

The persistence of *E. faecalis* is problematic for endodontic treatment and new effective antimicrobial agents are clearly needed [59]. Regarding the efficacy of *S. acmella* against this bacteria, different results have been reported. For example, in contrast to our results, Sathyaprasad et al. [60] observed that extracts from the flower heads of *S. acmella* exhibited a statistically broader zone of inhibition compared to Ca(OH)2. Dube et al. [61] investigated the antibacterial efficacy of *Spilanthes calva* De Candolle against *E. faecalis*. From this *in vitro* study, it was concluded that the differences in efficacy between the root extracts and the commonly used root canal irrigants depended on the concentration of the herbal extracts [61]. The differences between the results may be caused by non-standardized conditions for plant cultivation as well as different conditions for the preparation of the plant material or chemical extraction methods.

Staph. aureus is a common gram-positive bacterium in the human mucosal microbiota [62], which is strongly associated with biofilm-related infections [63]. The established biofilms of *Staph. aureus* is highly tolerant to common antimicrobial treatments [64, 65]. Jahan et al. [66] and Thakur et al. [67] found a significant bactericidal effect of the aerial parts of *S. acmella*. Although our results showed no direct effect of *S. acmella* extract on *Staph. aureus*, the possible indirect effect through its action on *C. albicans* could be of importance. When oral immunity is weakened (diabetes mellitus, HIV, cancer, use of corticosteroids, etc.), oropharyngeal candidiasis may develop, overcoming the oral epithelial barrier. If *Staph. aureus* is also present; it can invade together with *C. albicans* and utilize the phagocytes present in the tissue to spread to the draining lymph nodes [68]. Thus, by suppressing the development of candidiasis, *S. acmella* extract may reduce the likelihood of some of the harmful effects of *Staph. aureus* as well as *E. faecalis* and some other bacteria capable for bloodstream infections.

These results justify the use of *S. acmella* for the treatment of various infectious oral diseases caused by the investigated microorganisms. The results of the present work are preliminary and further investigations are required to determine the actual nature of the bioactive compounds which may be present in the different parts of the plant. The *in vitro* observations of the herbal products seem promising, but preclinical and clinical studies are needed to evaluate the biocompatibility and safety factor before they can be conclusively recommended as antimicrobial solutions and drugs. By analyzing the potential mechanism of action and comparing it with conventional therapies, we hope to further explore the potential of *S. acmella* as a valuable addition to the antimicrobial armamentarium.

ACKNOWLEDGMENT

We would like to thank Branislav Šiler for preparing plant material.

CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest.

REFERENCES

1. Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. J Oral Maxillofac Pathol. 2019;23(1):122–8. [DOI: 10.4103/jomfp.JOMFP_304_18] [PMID: 31110428]
2. Caselli E, Fabbri C, D'Accolti M, Soffritti I, Bassi C, Mazzacane S, et al. Defining the oral microbiome by whole-genome sequencing and resistome analysis: The complexity of the healthy picture. BMC Microbiol. 2020;20(1):120. [DOI: 10.1186/s12866-020-01801-y] [PMID: 32423437]
3. Kilian M, Chapple ILC, Hannig M, Marsh PD, Meuric V, Pedersen AML, et al. The oral microbiome- An update for oral healthcare professionals. Br Dent J. 2016;221(10):657–6. [DOI: 10.1038/sj.bdj.2016.865] [PMID: 27857087]
4. Lee Y, Puumala E, Robbins N, Cowen LE. Antifungal Drug Resistance: Molecular Mechanisms in *Candida albicans* and Beyond. Chem Rev. 2021;121(6):3390–441. [DOI: 10.1021/acs.chemrev.0c00199] [PMID: 32441527]
5. Roberts AP, Mullany P. Oral biofilms: A reservoir of transferable, bacterial, antimicrobial resistance. Expert Rev Anti Infect Ther. 2010;8(12):1441–50. [DOI: 10.1586/eri.10.106] [PMID: 21133668]
6. Chawhuaveang DD, Yu OY, Yin IX, Lam WY, Mei ML, Chu CH. Acquired salivary pellicle and oral diseases: A literature review. J Dent Sci. 2021;16(1):523–9. [DOI: 10.1016/j.jds.2020.10.007] [PMID: 33384841]
7. Petersen PE, Lennon MA. Effective use of fluorides for the prevention of dental caries in the 21st century: the WHO approach. Community Dent Oral Epidemiol. 2004;32(5):319–21. [DOI: 10.1111/j.1600-0528.2004.00175.x] [PMID: 15341615]
8. Fontanive V, Abegg C, Tsakos G, Oliveira M. The association between clinical oral health and general quality of life: A population-based study of individuals aged 50-74 in Southern Brazil. Community Dent Oral Epidemiol. 2013;41(2):154–62. [DOI: 10.1111/j.1600-0528.2012.00742.x] [PMID: 22900534]
9. Valm AM. The Structure of dental plaque microbial communities in the transition from health to dental caries and periodontal disease. J Mol Biol. 2019;431(16):2957–69. [DOI: 10.1016/j.jmb.2019.05.016] [PMID: 31103772]
10. Ranganathan V, Akhila CH. *Streptococcus mutans*: has it become prime perpetrator for oral manifestations. J Microbiol Exp. 2019;7:207–13. [DOI: 10.15406/jmen.2019.07.00261]
11. Falsetta ML, Klein MI, Colonne PM, Scott-Anne K, Gregoire S, Pai CH, et al. Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes virulence of plaque biofilms in vivo. Infect Immun. 2014;82(5):1968–71. [DOI: 10.1128/IAI.00087-14] [PMID: 24566629]
12. Choi HA, Cheong DE, Lim HD, Kim WH, Ham MH, Oh MH, et al. Antimicrobial and anti-biofilm activities of the methanol extracts of medicinal plants against dental pathogens *Streptococcus mutans* and *Candida albicans*. J Microbiol Biotechnol. 2017;27(7):1242–8. [DOI: 10.4014/jmb.1701.01026] [PMID: 28478657]
13. Liao Y, Brandt BW, Li J, Crielaard W, Van Loveren C, Deng DM. Fluoride resistance in *Streptococcus mutans*: a mini review. J Oral Microbiol. 2017;9(1):1344509. [DOI: 10.1080/20002297.2017.1344509] [PMID: 28748043]
14. Alhasani AH, Ishag RA, Al-Akwa AAY, Al-Shamahy HA, Al-labani MAA. Association between the *Streptococcus mutans* biofilm formation and dental caries experience and antibiotics resistance in adult females. Universal J Pharm Res. 2020;56:1–3. [DOI: 10.22270/ujpr.v5i6.507]
15. Shaw L, Golonka E, Potempa J, Foster SJ. The role and regulation of the extracellular proteases of *Staphylococcus aureus*. Microbiology. 2004;150(Pt 1):217–8. [DOI: 10.1099/mic.0.26634-0] [PMID: 14702415]
16. Peters BM, Ovchinnikova ES, Krom BP, Schlecht LM, Zhou H, Hoyer LL, et al. *Staphylococcus aureus* adherence to *Candida albicans* hyphae is mediated by the hyphal adhesin Als3p. Microbiology. 2012;158 (Pt 12):2975–86. [DOI: 10.1099/mic.0.062109-0] [PMID: 22918893]
17. Schlecht LM, Peters BM, Krom BP, Freiberg JA, Hansch GM, Filler SG, et al. Systemic *Staphylococcus aureus* infection mediated by *Candida albicans* hyphal invasion of mucosal tissue. Microbiology. 2015;161 (Pt 1):168–81. [DOI: 10.1099/mic.0.083485-0] [PMID: 25332378]
18. Kozak M, Pawlik A. The Role of the Oral Microbiome in the Development of Diseases. Int J Mol Sci. 2023;24(6):5231. [DOI: 10.3390/ijms24065231] [PMID: 36982305]
19. Campos J, Pires MF, Sousa M, Campos C, da Costa CFA, Sampaio-Maia B. Unveiling the relevance of the oral cavity as a *Staphylococcus aureus* colonization site and potential source of antimicrobial resistance. Pathogens. 2023;12(6):765. [DOI: 10.3390/pathogens12060765] [PMID: 37375455]
20. Rôças IN, Siqueira JF, Santos KR. Association of *Enterococcus faecalis* with different forms of periradicular diseases. J Endod. 2004;30(5):315–20. [DOI: 10.1097/00004770-200405000-00004] [PMID: 15107642]
21. Estrela C, Silva JA, de Alencar AH, Leles CR, Decurcio DA. Efficacy of sodium hypochlorite and chlorhexidine against *Enterococcus faecalis* – a systematic review. J Appl Oral Sci. 2008;16(6):364–8. [DOI: 10.1590/s1678-77572008000600002] [PMID: 19082392]
22. Murad CF, Sassone LM, Souza MC, Fidel RAS, Fidel S, Junior RH. Antimicrobial activity of sodium hypochlorite, chlorhexidine and MTAD® against *Enterococcus faecalis* biofilm on human dentin matrix in vitro. Rev Bras Odontol. 2012;9(2):143–50. [DOI: 10.21726/rsbo.v9i2.979]
23. Rams TE, Feik D, Mortensen JE, Degener JE, van Winkelhoff AJ. Antibiotic susceptibility of periodontal *Enterococcus faecalis*. J Periodontol. 2013;84(7):1026–33. [DOI: 10.1902/jop.2012.120050] [PMID: 23106507]
24. Sharma N, Bhatia S, Sodhi AS, Batra N. Oral microbiome and health. AIMS Microbiol. 2018;4(1):42–66. [DOI: 10.3934/microbiol.2018.1.42] [PMID: 31294203]
25. Edit G, Waltermann EDM, Hilgert JB, Arthur RA. Candida and dental caries in children, adolescents and adults: A systematic review and meta-analysis. Arch Oral Biol. 2020;119:104876. [DOI: 10.1016/j.archoralbio.2020.104876] [PMID: 32905885]
26. Grover C, Dhawan P, Mehta D, Nautiyal M. Denture stomatitis: a review. J Prosthet Implant Dent. 2022;5:68–73. [DOI: 10.55231/jpid.2022.v05.i02.01]
27. Deeiam K, Pankam J, Sresumatchai V, Visedketkan P, Jindavech W, Rungraungrayabkul D, et al. Presence of *Candida* and its associated factors in participants attending oral cancer screening in the lower northeastern area of Thailand. BMC Oral Health. 2023;23(1):527. [DOI: 10.1186/s12903-023-03198-2] [PMID: 37507787]
28. Kumar J, Sharma R, Sharma M, Prabhavathi V, Paul J, Chowdary CD. Presence of *Candida albicans* in root canals of teeth with apical periodontitis and evaluation of their possible role in failure of endodontic treatment. J Int Oral Health. 2015;7(2):42–5. [PMID: 25859106]
29. Robbins N, Caplan T, Cowen LE. Molecular Evolution of Antifungal Drug Resistance. Annu Rev Microbiol. 2017;71:753–75. [DOI: 10.1146/annurev-micro-030117-020345] [PMID: 28886681]
30. Revie NM, Iyer KR, Robbins N, Cowen LE. Antifungal drug resistance: evolution, mechanisms and impact. Curr Opin Microbiol. 2018;45:70–6. [DOI: 10.1016/j.mib.2018.02.005] [PMID: 29547801]
31. Mathur S, Mathur T, Srivastava R, Khatri R. Chlorhexidine: The gold standard in chemical plaque control. Natl J Physiol Pharm Pharm. 2011;1:45–50.
32. Balagopal S, Arjunkumar R. Chlorhexidine: the gold standard antiplaque agent. J Pharm Sci. 2013;5(12):270–4.
33. Poppolo DR, Ouanounou A. Chlorhexidine in dentistry: pharmacology, uses, and adverse effects. Int Dent J. 2022;72(3):269–77. [DOI: 10.1016/j.identj.2022.01.005] [PMID: 35287956]
34. Cieplik F, Jakubovics NS, Buchalla W, Maisch T, Hellwig, Al-Ahmad A. Resistance toward chlorhexidine in oral bacteria - Is There Cause for Concern? Front Microbiol. 2019;10:587. [DOI: 10.3389/fmicb.2019.00587] [PMID: 30967854]
35. Brookes ZLS, Bescos R, Belfield LA, Ali K, Roberts A. Current uses of chlorhexidine for management of oral disease: a narrative review. J Dent. 2020;103:103497. [DOI: 10.1016/j.jdent.2020.103497] [PMID: 33075450]
36. Prabu GR, Gnanamani A, Sadulla S. Guaijaverin – a plant flavonoid as potential antiplaque agent against *Streptococcus mutans*.

J Appl Microbiol. 2006;101(2):487–95. [DOI: 10.1111/j.1365-2672.2006.02912.x] [PMID: 16882158]
37. Kouidhi B, Mohammed AY, Qurashi A, Chaieb K. Drug resistance of bacterial dental biofilm and the potential use of natural compounds as alternative for prevention and treatment. Microb Pathog. 2015;80:39–49. [DOI: 10.1016/j.micpath.2015.02.007] [PMID: 25708507]
38. Hickl J, Argyropoulou A, Sakavitsi E, Halabalaki M, Al-Ahmad A, Hellwig E, et al. Mediterranean herb extracts inhibit microbial growth of representative oral microorganisms and biofilm formation of *Streptococcus mutans*. PLoS One. 2018;13(12):e0207574. [DOI: 10.1371/journal.pone.0207574] [PMID: 30540782]
39. Janse RK. The systematics of *Acmella* (Asteraceae-Heliantheae). Syst Bot Monogr. 1985;8:115. [DOI: 10.2307/25027614]
40. Paulraj J, Govindarajan R, Palpu P. The Genus *Spilanthes* ethnopharmacology, phytochemistry, and pharmacological properties: a review. Adv Pharmacol Pharm Sci. 2013;2013:510298. [DOI: 10.1155/2013/510298] [PMID: 24454346]
41. Storey C, Salem JI. Lay use of amazonian plants for the treatment of tuberculosis. Acta Amaz. 1997;27:175–2. [DOI: 10.1590/1809-43921997273182]
42. Dias AMA, Santos P, Seabra IJ, Júnior RNC, Braga MEM, De Sousa HC. Spilanthol from *Spilanthes acmella* flowers, leaves and stems obtained by selective supercritical carbon dioxide extraction. J Supercri Fluids. 2012;61:62–30. [DOI: 10.1016/j.supflu.2011.09.020]
43. 43. Arif M, Juyal D, Joshi A. A review on pharmacognostic and phytochemical study of a plant *Spilanthes acmella* Murr. Pharm Innov. 2017;6:172–7.
44. Abdul Rahim R, Jayusman PA, Muhammad N, Mohamed N, Lim V, Ahmad NH, et al. Potential antioxidant and anti-Inflammatory effects of *Spilanthes acmella* and its health beneficial effects: a review. Int J Environ Res Public Health. 2021;18(7):3532. [DOI: 10.3390/ijerph18073532] [PMID: 33805420]
45. Peretti P, Rodrigues ET, de Souza Junior BM, Bezerra Roberto JAC, Fernandez E, De Sousa FFO, et al. Spilanthol content of *Acmella oleracea* subtypes and their bactericide and antibioflm activities against *Streptococcus mutans*. S Afr J Bot. 2021;143:17–24. [DOI: 10.1016/j.sajb.2021.08.001]
46. Silveira N, Sandjo L, Biavatti MW. Spilanthol-containing products: A patent review (1996–2016). Trends Food Sci Technol. 2018;74:107–11. [DOI: 10.1016/j.tifs.2018.02.012]
47. Barbosa AF, Pereira CSS, Mendes ME, RC Junior, Carvalho MG, Maia J, et al. Spilanthol content in the extract obtained by supercritical CO2 at different storage times of *Acmella oleracea* L. J Food Process Eng. 2017;40:1–7. [DOI: 10.1111/jfpe.12441]
48. European Committee on Antimicrobial Susceptibility Testing (EUCAST). https://www.eucast.org (2024). Accessed 14 Nov. 2024.
49. Leyes Borrajo JL, Garcia VL, Lopez CG, Rodriguez-Nunez I, Garcia FM, Gallas TM. Efficacy of chlorhexidine mouthrinses with and without alcohol: a clinical study. J Periodontol. 2002;73(3):317–21. [DOI: 10.1902/jop.2002.73.3.317] [PMID: 11924590]
50. Jiang Q, Deng Y, Li S, Yang D, Tao L. Sub-lethal concentrations of chlorhexidine inhibit *Candida albicans* growth by disrupting ROS and metal ion homeostasis. J Oral Microbiol. 2023;15(1):2278937. [DOI: 10.1080/20002297.2023.2278937] [PMID: 38415078]
51. Bescos R, Ashworth A, Cutler C, Brookes ZL, Belfield L, Rodiles A, et al. Effects of Chlorhexidine mouthwash on the oral microbiome. Sci Rep. 2020;10(1):5254. [DOI: 10.1038/s41598-020-61912-4] [PMID: 32210245]
52. LaFleur MD, Kumamoto CA, Lewis K. *Candida albicans* biofilms produce antifungal-tolerant persister cells. Antimicrob Agents Chemother. 2006;50(11):3839–46. [DOI: 10.1128/AAC.00684-06] [PMID: 16923951]
53. Koo H, Bowen WH. *Candida albicans* and *Streptococcus mutans*: A potential synergistic alliance to cause virulent tooth decay in children. Future Microbiol. 2014;9(12):1295–7. [DOI: 10.2217/fmb.14.92] [PMID: 25517895]
54. Li XJ, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. Clin Microbiol Rev. 2000;13(4):547–8. [DOI: 10.1128/CMR.13.4.547] [PMID: 11023956]
55. Douglas LJ. *Candida* biofilms and their role in infection. Trends Microbiol. 2003;11(1):30–6. [DOI: 10.1016/s0966-842x(02)00002-1] [PMID: 12526852]
56. Jolivet-Gougeon A, Bonnaure-Mallet M. Biofilms as a mechanism of bacterial resistance. Drug Discov Today Technol. 2014;11:49–56. [DOI: 10.1016/s0966-842x(02)00002-1] [PMID: 12526852]
57. Singh A, Amod A, Pandey P, Bose P, Pingali MS, Shivalkar S, et al. Bacterial biofilm infections, their resistance to antibiotics therapy and current treatment strategies. Biomed Mater. 2022;17(2). [DOI: 10.1088/1748-605X/ac50f6] [PMID: 35105823]
58. Shobana G. Anti-bacterial efficacy of *Spilanthes acmella* on salivary mutans Streptococci in 15-17 years old school students in Madurai District: A Randomized controlled trial. MDS thesis, Best Dental Science College, Madurai, 2017.
59. Basrani B, Tjäderhane L, Santos JM, Pascon E, Grad H, Lawrence HP, et al. Efficacy of chlorhexidine-and calcium hydroxide-containing medicaments against *Enterococcus faecalis* in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;96(5):618–24. [DOI: 10.1016/s1079-2104(03)00166-5] [PMID: 14600699]
60. Sathyaprasad S, Bijosh KJ, Chandra HS. Antimicrobial and antifungal efficacy of *Spilanthes acmella* as an intracanal medicament in comparison to calcium hydroxide: An: in vitro: study. Indian J Dent Res. 2015;26(5):528–32. [DOI: 10.4103/0970-9290.172081] [PMID: 26672426]
61. Dube M, Kumari M, Pandya M, Dube S, Trivedi P. Comparative evaluation of antibacterial efficacy of *Spilanthes calva* DC root extract, sodium hypochlorite, chlorhexidine and doxycycline at different concentrations on *Enterococcus faecalis* – An in-vitro study. Endodontology. 2013;25:63–2. [DOI: 10.4103/0970-7212.352317]
62. Otto M. Staphylococci in the human microbiome: the role of host and interbacterial interactions. Curr Opin Microbiol. 2020;53:71–7. [DOI: 10.1016/j.mib.2020.03.003] [PMID: 32298967]
63. Al-Mebairik NF, El-Kersh TA, Al-Sheikh YA, Marie MAM. A review of virulence factors, pathogenesis, and antibiotic resistance in *Staphylococcus aureus*. Rev Med Microbiol. 2016;27:50–6. [DOI: 10.1097/MRM.0000000000000067]
64. Jones SM, Morgan M, Humphrey TJ, Lappin-Scott H. Effect of vancomycin and rifampicin on methicillin-resistant *Staphylococcus aureus* biofilms. Lancet. 2001;357(9249):40–1. [DOI: 10.1016/S0140-6736(00)03572-8] [PMID: 11197363]
65. Colombo APV, do Souto RM, Araújo LL, Espíndola LCP, Hartenbach FARR, Magalhães CB, et al. Antimicrobial resistance and virulence of subgingival staphylococci isolated from periodontal health and diseases. Sci Rep. 2023;13(1):11613. [DOI: 10.1038/s41598-023-38599-4] [PMID: 37463947]
66. Jahan N, Khatoon R, Ahmad S, Shahzad A. Evaluation of antibacterial potential of medicinal plant *Spilanthes acmella* Murr. and its in vitro raised callus against resistant organisms especially those harbouring bla genes. J App Pharm Sci. 2013;3:119–24. [DOI: 10.7324/JAPS.2013.31021]
67. Thakur S, Anand S, Ved P. Studies on antibacterial and antioxidant activity of different extracts of *Spilanthes acmella* L. Plant Arch. 2019;1:1711–7.
68. Pasman R, Krom BP, Zaat SAJ, Brul S. The role of the oral immune system in oropharyngeal candidiasis- facilitated invasion and dissemination of *Staphylococcus aureus*. Front Oral Health. 2022;3:851786. [DOI: 10.3389/froh.2022.851786] [PMID: 35464779]

Received: 06.12.2024 • Accepted: 09.01.2025

Antimikrobni efekat *Spilanthes acmella* na *Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis* i *Candida albicans* u usnoj duplji

Zoran Tambur[1], Stevan Avramov[1,2], Ema Aleksić[1], Anica Joksić[1], Uroš Dimitrijević[1], Vladimir Biočanin[1]

[1]Univerzitet Privredna akademija u Novom Sadu, Stomatološki fakultet u Pančevu, Pančevo, Srbija;
[2]Univerzitet u Beogradu, Institut od nacionalnog značaja za Republiku Srbiju, Institut za biološka istraživanja „Siniša Stanković“, Beograd, Srbija

SAŽETAK

Uvod Zbog sve veće tolerancije mikroorganizama na antibiotike, lečenje poremećaja mikrobne zajednice u usnoj duplji predstavlja veliki izazov. Alternativne supstance koje mogu delimično ili potpuno zameniti postojeće antibakterijske agense mogu biti proizvodi dobijeni od biljaka. *Spilanthes acmella* pokazala se efikasnom u lečenju bolesti usne duplje.

Cilj ovog istraživanja bio je da se ispita potencijalna antimikrobna efikasnost biljke *Spilanthes acmella* protiv *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis* i *Candida albicans*, mikroorganizama koji se često povezuju sa oboljenjima usne duplje, kao i da se ovaj efekat uporedi sa konvencionalnim terapijama.

Materijal i metode Analizirane su tri grupe uzoraka: cvetne glavice, listovi i preostali nadzemni delovi (stabljika i grančice).

Rezultati Rezultati su pokazali da ekstrakt cveta nije ispoljio inhibitorni efekat na testirane bakterije. Ekstrakt iz stabljike i grančica pokazao je inhibitorni efekat na *Candida albicans*. Ekstrakt lista pokazao je najbolji inhibitorni efekat protiv *Streptococcus mutans*.

Zaključak S obzirom na to da je hlorheksidin jedino sredstvo sa dokazanim antiplak efektom, iz dobijenih rezultata može se zaključiti da sinergistički efekat ova dva rastvora daje najbolje rezultate u hemijskoj kontroli zubnog plaka i može biti deo protokola za prevenciju kako zubnog karijesa, tako i parodontalnih bolesti u budućnosti.

Ključne reči: biljni ekstrakti; antimikrobna aktivnost; usna duplja; minimalna inhibitorna koncentracija

UVOD

Ljudski oralni mikrobiom je izuzetno složena grupa mikroorganizama koji formiraju različite veze u ustima i sadrže više od 700 vrsta, uključujući bezopasne simbionte, komensale i oportunističke patogene [1, 2]. Kada je oralni mikrobiom poremećen, dolazi do disbioze u kojoj patogene bakterije preuzimaju prevlast i izazivaju oralne bolesti [3]. Pored toga, otkrivena je uloga oralnog mikrobioma u brojnim neoralnim bolestima, kao što su rak pankreasa, dijabetes melitus i endokarditis [4]. Mikroorganizmi koji čine oralni mikrobiom obično su organizovani u biofilm, koji se naziva zubni plak. Oralni patogeni biofilma su prepoznati kao predisponirajući faktor za različite oralne infekcije, uključujući karijes, gingivitis, parodontitis i periimplantitis [5, 6]. Zubni karijes se smatra najrasprostranjenijom zaraznom bolešću u svetu i jednom od najčešćih oralnih bolesti, koja pogađa 60–90% školske dece i veliku većinu odraslih osoba [7]. Teškim parodontitisom pogođeno je 5–15% opšte populacije [8]. Poremećaj dinamike mikrobne zajednice igra važnu ulogu u etiologiji gingivitisa i nastanku parodontitisa [9].

Streptococcus mutans se smatra najvažnijim etiološkim faktorom u nastanku karijesa zuba [10]. U ranim fazama formiranja karijesa, kombinovano delovanje enzima koje oslobađa *Strep. mutans* (glukoziltransferaza i fruktoziltransferaza) i supstance koje podstiču adheziju (glukani) koje sintetiše *Candida albicans* formiraju biofilm kariogenog plaka na površini zuba. Time se stvaraju uslovi za rast etioloških bakterija [11, 12]. Posebno je zabrinjavajuće postojanje sojeva *Strep. mutans* koji su otporni na različite antibiotike, kao i na prisustvo fluora [13, 14].

Staphylococcus aureus je najpatogeniji član roda i uzročnik raznih bolesti – od površinskih apscesa kože i trovanja hranom do stanja opasnih po život kao što su bakteremija, nekrotična pneumonija kod dece i endokarditis [15]. *Staph. aureus* ima koristi od prisustva *C. albicans* u usnoj duplji [16, 17]. Formiranje takve specifične mikrobne zajednice povezuje *Staph. aureus* sa nekoliko rizika, kao što je razvoj oralnih bolesti (npr. karijes, halitoza, parodontitis, oralni karcinom, sistemske infekcije), a može uticati i na progresiju nekih sistemskih bolesti kao što su osteoporoza, ateroskleroza, dijabetes, kardiovaskularne bolesti i ishemijska kardiomiopatija [18]. Široka upotreba profilaktičkih antibiotika u stomatologiji povezana je sa pojavom rezistencije na antibiotike kod nekoliko komensalnih mikroorganizama, uključujući *Staph. aureus* [19].

Enterococcus faecalis je fakultativna anaerobna, gram-pozitivna bakterija u usnoj duplji koja je uglavnom odgovorna za različite oralne patologije, posebno zubni karijes, zubne apscese, parodontalne infekcije, apikalni parodontitis i perzistentne endodontske infekcije [20]. Sposobnost *E. faecalis* da formira biofilm na zidovima kanala korena zuba i to kao monoinfekcija u tretiranim kanalima, bez sinergističke podrške drugih bakterija, dovodi do visoke rezistencije na antimikrobne agense [21, 22]. Klinički izolati *E. faecalis* dobijeni iz inficiranih kanala korena zuba kao i parodontalne infekcije mogu pokazati antimikrobnu rezistenciju na konvencionalne tretmane preporučene za stomatološke procedure [23].

Oportunistički patogen *C. albicans* je najčešće izolovana gljiva u oralnim infekcijama [24]. Rezultati metaanalize Edit i saradnika [25] ukazuju da osobe sa prisustvom *Candida* spp. u usnoj duplji imaju veću prevalenciju zubnog karijesa nego osobe bez ovih mikroorganizama. Prisustvo *C. albicans* u usnoj duplji je povezano sa drugim oralnim oboljenjima, kao što su stomatitis proteza, oralni karcinom, kao i neuspešna endodontska lečenja [26, 27, 28]. Tokom poslednje dve decenije, konvencionalni antimikrobni lekovi pokazuju se kao sve neefikasniji u kontroli *C. albicans* [4, 29, 30].

Hlorheksidin je najčešće korišćen i široko prihvaćen oralni antibakterijski agens i smatra se zlatnim standardom [31]. Ipak, njegova dugotrajna upotreba nije preporučljiva zbog brojnih neželjenih efekata: bojenje zuba u smeđu boju, poremećaj čula ukusa, lezije oralne sluzokože, oticanje parotidnih žlezda, povećano formiranje supragingivalnog plaka i ponekad neprijatan ukus [32, 33]. Drugi problem sa upotrebom hlorheksidina je razvoj antimikrobne rezistencije, što predstavlja ozbiljan neželjeni efekat [34, 35].

Zbog ovih neželjenih efekata, važno je pronaći alternativne supstance koje mogu delimično ili potpuno zameniti postojeće antibakterijske agense. Jedan od načina za pronalaženje novih obećavajućih lekova je temeljno istraživanje biljaka i biljnih lekova koji se koriste u tradicionalnoj medicini različitih zemalja [36, 37, 38].

Jedna od ovih biljaka je *Spilanthes acmella* L., poreklom iz Brazila i Perua, koja se može gajiti kao jednogodišnja biljka u kontinentalnoj klimi. Ova biljka je u narodu poznata kao biljka protiv zubobolje jer ima anestetički efekat kada se žvaću listovi i cvetovi [39]. *S. acmella* je biljka sa uzlaznim, cilindričnim, dlakavim stabljikama koja naraste 40–60 cm visine i pripada porodici Asteraceae. Pokazalo se da biljni ekstrakti i bioaktivni sastojci vrsta roda *Spilanthes* imaju širok spektar potencijalnih primena u farmaceutskoj industriji [40]. *S. acmella* se pokazala efikasnom u tradicionalnoj medicini za lečenje reumatizma, groznice i gripa, tuberkuloze, besnila, malarije i skorbuta [41]. Ima antinociceptivna, antimikrobna, antiinflamatorna, anestetička, analgetička, antifungalna, antimutagena, diuretička i imunostimulativna dejstva [42, 43, 44]. Takođe se pokazala efikasnom u lečenju bolesti usne duplje, kao što su dentalne bolesti, parodontitis i čirevi na oralnoj sluzokoži [40, 45]. Alkamidi se smatraju dominantnim fitokemikalijama u rodu *Spilanthes*, a najvažniji pronađeni alkamid je spilantol. Uprkos brojnim studijama o biološkom značaju ovog metabolita, postoji samo nekoliko komercijalnih proizvoda na bazi spilantola za farmakološke svrhe [46].

Cilj ove studije je da se ispita potencijalna antimikrobna efikasnost cvetova, listova, stabljike i grančica biljke *S. acmella* protiv čestih infektivnih patogena u usnoj duplji čoveka: *Strep. mutans*, *Staph. aureus*, *E. faecalis* i *C. albicans*. U ovoj studiji će se takođe porediti efekti biljnog ekstrakta sa efektima konvencionalne terapije hlorheksidinom. S obzirom na poreklo ove biljke i njenu ograničenu rasprostranjenost, ovakva istraživanja, koliko nam je poznato, još uvek nisu sprovedena u evropskim zemljama.

METODE

Biljni materijal i priprema ekstrakta

Biljni materijal je dobijen u julu 2023. od biljaka *S. acmella* uzgojenih iz semena (Tuinzaden.eu, Veesperstraat 94d, 1112AP Dieme, Holandija). Analizirane su tri različite grupe uzoraka. Prvi se sastojao od glavičastih cvasti, drugi od listova, a treći od preostalih nadzemnih delova (stabljika i grančice). Uklonjeni su svi delovi biljnog materijala koji su bili pocepani, zgužvani ili tamnih ivica.

Korišćeni delovi biljaka su temeljno oprani pod tekućom vodom. Materijal je dezinfikovan uranjanjem u rastvor koji sadrži 100 ppm (mg/L) slobodnog hlora tokom deset minuta [47]. Završno ispiranje je obavljeno destilovanom vodom. Sveži uzorci su sušeni u pećnici na 50 °C kako bi se uklonila voda. Da bi se dobio fini i homogeni prah, osušena masa cvasti, listova i preostalih nadzemnih delova odvojeno su usitnjavani korišćenjem tečnog azota u porcelanskom avanu. Ekstrakti dobijeni usitnjavanjem rastvoreni su u metanolu (HPLC kvaliteta, Merck) (1 : 20) na sobnoj temperaturi. Biljni materijal *S. acmella* je zatim tretiran u sonifikatoru deset minuta. Metanolni ekstrakt je zatim centrifugiran 15 minuta na 5000 rpm u hlađenoj centrifugi (Sigma 4K 15C, Sigma Laborzentrifugen GmbH Osterode am Harz, Nemačka). Centrifugirani supernatant je sakupljen, a ostatak je ponovo centrifugiran u rastvoru metanola da bi se dobila veća količina ekstrakta. Preostali deo je zatim odbačen, a ceo metanolni rastvor je sakupljen i filtriran. Rastvori su koncentrovani do suve mase pod sniženim pritiskom u rotacionom isparivaču (Buchi Rotavapor, Flavil, Švajcarska). Bočice sa ekstraktima su umotane u aluminijumsku foliju i čuvane u zamrzivaču na -30 °C do analize potencijalne antimikrobne aktivnosti.

Mikroorganizmi koji se koriste u istraživanju

Testirani sojevi su bili gram-pozitivni fakultativni anaerobi: *Strep. mutans* ATCC 25175, *E. faecalis* ATCC 14506, *Staph. aureus* ATCC 25923 i *C. albicans* ATCC 10231, koji su kultivisani u prisustvu kiseonika. Svi sojevi su uzgajani preko noći na 37 °C u Miller-Hinton medijumu (HiMedia, Mumbai, Indija) sa standardnom pH od 7,4 za ovaj medijum.

Određivanje minimalnih inhibitornih koncentracija (MIC)

Metoda korišćena za procenu antimikrobne aktivnosti bila je minimalna inhibitorna koncentracija (MIC) metodom razblaživanja triptičnog sojinog agara (EUCAST, 2024) [48]. Prilikom hlađenja, pre nego što očvrsne, u podlogu su dodavani biljni ekstrakti u odgovarajućoj koncentraciji. Serijska razblaženja biljnih ekstrakata pripremana su u koncentracijama od 6,25, 12,5, 25, 50, 100, 200 i 400 μg/ml. MIC je definisan kao najniža koncentracija ekstrakta koja nije izazvala vidljiv rast bakterija u poređenju sa kontrolnim rastom.

Poređenje je vršeno sa standardnim rastvorom 0,12% hlorheksidina (Galenika, Zemun). Serijska razblaženja hlorheksidina su pripremljena, slično biljnim ekstraktima, u koncentracijama od 6,25, 12,5, 25, 50, 100, 200 i 400 μg/ml.

Nije bilo varijabilnosti merenja „unutar posmatrača" pošto je istraživač koji je inokulirao medijume za test antimikrobne aktivnosti dobio identične rezultate za tri merenja u tri vremenska perioda.

REZULTATI

Ispitivano je dejstvo različitih koncentracija hlorheksidina na četiri vrste mikroorganizama. Hlorheksidin je imao inhibitorni efekat na ispitivane sojeve sve tri vrste bakterija (*Strep. mutans*, *E. faecalis* i *Staph. aureus*) kao i na *C. albicans*. Inhibitorni efekti su postignuti čak i pri najnižoj korišćenoj koncentraciji hlorheksidina (6,25 μg/mL). Ekstrakt cvasti *S. acmella* nije pokazao inhibitorni efekat na *C. albicans* u našem eksperimentu u svim ispitivanim koncentracijama (Tabela 1). Potvrđeno je da ekstrakt iz stabljike i grančica *S. acmella* ima inhibitorni efekat na *C. albicans* pri 50 μg/mL, kao i pri svim većim koncentracijama (Tabela 2).

Ekstrakt lista *S. acmella* pokazao je značajnu inhibitornu aktivnost protiv bakterije *Strep. mutans* (MIC = 6,3 μg/mL) (Tabela 2).

U našoj studiji nismo pronašli značajan efekat ekstrakta *S. acmella* na *E. faecalis*. Cvetovi *S. acmella* nisu pokazali inhibitorni efekat na ispitivane bakterije – MIC > 400 μg/mL (Tabela 1 i Tabela 2). Naše studije nisu potvrdile značajan efekat ekstrakta *S. acmella* na *S. aureus* (Tabela 1 i Tabela 2).

DISKUSIJA

Sve veća otpornost patogena na konvencionalne antibiotike i neželjeni efekti postojećih terapija učinili su tradicionalne lekovite biljke privlačnim izvorom za skrining antimikrobnih agenasa. U skladu sa ovim trendovima, ova studija je imala za cilj da istraži antimikrobnu aktivnost metanolnih ekstrakata nadzemnih delova *S. acmella* na četiri važna dentalna patogena: *Strep. mutans*, *E. faecalis*, *Staph. aureus* i *C. albicans*. Cilj je takođe bio da se uporede ovi efekti ekstrakta biljaka sa konvencionalnom terapijom korišćenjem hlorheksidina. Brojne studije su već ispitivale antibakterijski efekat *S. acmella*, ali rezultati nisu bili jednoznačni.

Značajan antimikrobni efekat hlorheksidina dobijen u ovoj studiji takođe je potvrđen u radovima koje su objavili Balagopal i Arjunkumar [32], Leyes Borrajo i saradnici [49] i Jiang i saradnici [50]. Brookes i saradnici [35] takođe navode nekoliko studija u svojoj metaanalizi koje potvrđuju da je hlorheksidin moćan antimikrobni agens. Antifungalni efekti hlorheksidina odnose se na prevenciju formiranja biofilma [35] i narušavanje strukture ćelijske membrane *C. albicans* [50]. Iako se hlorheksidin-glukonat često koristi za smanjenje i inhibiciju formiranja biofilma, on ima izvesne nedostatke. Na primer, može negativno uticati na tkiva usne duplje i doprineti nekim negativnim sistemskim promenama [33, 51]. Takođe postoje dokazi da neke populacije *C. albicans* mogu da opstanu nakon primene hlorheksidina i formiraju multitolerantnu subpopulaciju, što može tokom vremena smanjiti efikasnost hlorheksidina [52]. U ovim slučajevima, kombinovano dejstvo hlorheksidina sa nekim drugim supstancama može biti od velike pomoći. Pošto se pokazalo da ekstrakt nadzemnih delova *S. acmella* čak i u niskim koncentracijama deluje inhibitorno na *C. albicans*, može se zaključiti da bi ekstrakt ove biljke mogao biti dobar adjuvans u lečenju kandidijaze, posebno kada se sumnja na prisustvo rezistentnih sojeva. Ovo je od posebnog značaja jer, prema našim saznanjima, nije pokazana antifungalna otpornost na spilantol, glavni bioaktivni metabolit *S. acmella*.

Dokazano je da *Strep. mutans* blisko komunicira sa *C. albicans* u složenoj dvosmernoj interakciji koja je uključena u formiranje biofilma [53]. Više istraživanja je pokazalo da dentalni mikrobiom nije odgovoran samo za pojavu bolesti u usnoj duplji već može imati važnu ulogu u nastanku sistemskih bolesti [54, 55]. Ovi rezultati ukazuju da je kontrola oralnih mikroorganizama veoma značajna. U tom kontekstu, biofilm u zubnom plaku je glavna prepreka takvoj kontroli jer štiti patogene bakterije od antibiotika [56, 57]. Pošto je prevencija stvaranja biofilma usredsređena na uklanjanje *Strep. mutans*, važno je imati efikasna sredstva za kontrolu prisustva ove bakterije u usnoj duplji. Veoma efikasan baktericidni efekat koji ekstrakt *S. acmella* ima na *Strep. mutans* nudi mogućnost upotrebe ove biljke kao samostalnog ili dodatnog lekovitog sredstva koje nema neželjene efekte uočene kod konvencionalnih agenasa [58].

Perzistentnost *E. faecalis* je problematična za endodontsko lečenje i jasno je da su potrebni novi efikasni antimikrobni agensi [59]. Što se tiče efikasnosti *S. acmella* protiv ove bakterije, zabeleženi su različiti rezultati. Na primer, za razliku od naših rezultata, Sathiaprasad i saradnici [60] primetili su da ekstrakti cvetova *S. acmella* pokazuju širu zonu inhibicije u poređenju sa $Ca(OH)_2$. Dube i saradnici [61] istraživali su antibakterijsku efikasnost *Spilanthes calva* De Candolle protiv *E. faecalis*. Iz ove *in vitro* studije, zaključeno je da razlike efikasnosti između ekstrakata korena ove biljke i uobičajenih sredstava za irigaciju kanala korena zavise od koncentracije biljnih ekstrakata [61]. Razlike između rezultata mogu biti uzrokovane nestandardizovanim uslovima za uzgoj biljaka, kao i različitim uslovima za pripremu biljnog materijala ili hemijskim metodama ekstrakcije.

Staph. aureus je uobičajena gram-pozitivna bakterija u mikrobioti ljudske sluzokože [62] i značajan je faktor infekcija povezanih sa biofilmom [63]. Formirani biofilm *Staph. aureus* je veoma otporan na uobičajene antimikrobne tretmane [64, 65]. Jahan i saradnici [66] i Thakur i saradnici [67] utvrdili su značajan baktericidni efekat nadzemnih delova *S. acmella* protiv ove bakterije. Iako naši rezultati nisu pokazali direktan efekat ekstrakta *S. acmella* na *Staph. aureus*, mogući indirektni efekat preko njegovog delovanja na *C. albicans* mogao bi biti značajan. Kada je oralni imunitet oslabljen (dijabetes melitus, HIV, kancer, upotreba kortikosteroida itd.), može se razviti orofaringealna kandidijaza, koja prolazi kroz oralnu epitelnu barijeru. Ako je pri tome prisutan i *Staph. aureus*, on može zajedno sa *C. albicans* da iskoristi fagocite prisutne u tkivu i proširi se na drenažne limfne čvorove [68]. Dakle, suzbijanjem razvoja kandidijaze, ekstrakt *S. acmella* može smanjiti verovatnoću štetnih efekata *Staph. aureus*, ali i *E. faecalis* i nekih drugih bakterija sposobnih za infekcije putem krvotoka.

Rezultati ovog istraživanja opravdavaju primenu *S. acmella* za lečenje različitih oralnih bolesti izazvanih ispitivanim mikroorganizmima. Rezultati ovog rada su preliminarni i potrebna su dalja istraživanja kako bi se utvrdio sadržaj svih bioaktivnih jedinjenja koja mogu biti prisutna u različitim delovima biljke. *In vitro* zapažanja o ovim biljnim proizvodima su obećavajuća, ali su potrebne pretkliničke i kliničke studije kako bi se procenili faktori biokompatibilnosti i bezbednosti pre nego što se mogu definitivno preporučiti kao antimikrobna rešenja i lekovi. Nadamo se da će analizom potencijalnih mehanizama delovanja i upoređivanjem sa drugim konvencionalnim terapijama biti bolje istražen potencijal *S. acmella* kao korisnog dodatka antimikrobnim tretmanima.