JoP Journal of Pharmacopuncture
Journal of Pharmacopuncture 2023;26(2):139-146
pISSN: 2093-6966 • eISSN: 2234-6856
https://doi.org/10.3831/KPI.2023.26.2.139
Original Article
Check for updates

Antibacterial and Anti-Glucosyltransferase Activity of *Verbascum speciosum* Against Cariogenic Streptococci

Shabnam Pourmoslemi[1], Amir Larki-Harchegani[2]*, Sajad Daneshyar[2], Dara Dastan[3], Amir Nili-Ahmadabadi[2], Mina Jazaeri[4]

[1]Department of Pharmaceutics, Medicinal Plants and Natural Products Research Center, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran
[2]Department of Pharmacology and Toxicology, Medicinal Plants and Natural Products Research Center, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran
[3]Department of Pharmacognosy, Medicinal Plants and Natural Products Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
[4]Department of Oral Medicine, Hamadan Dental Faculty, Hamadan University of Medical Sciences, Hamadan, Iran

Received September 14, 2022
Reviewed November 17, 2022
Accepted March 16, 2023

***Corresponding Author**
Amir Larki-Harchegani
Department of Pharmacology and Toxicology, School of Pharmacy, Hamadan University of Medical Sciences, Shahid Fahmideh Blvd., In front of Mardom Park, Third floor, room number 415, Hamadan 65178-38678, Iran
Tel: +98-813-838-1591
E-mail: dr.amir.larki@gmail.com

Objectives: Dental caries is a prevalent chronic human infection worldwide and several plants have shown anticariogenic properties through antibacterial activity against oral pathogens. The present study aimed to assess anticariogenic activity of *Verbascum speciosum*, in search of novel agents for the prevention and treatment of dental caries.
Methods: Hydro-alcoholic extracts from flowers and total aerial parts of the plant were prepared by maceration. Antibacterial activity of the extracts against *Streptococcus mutans* (ATCC 35668) and *Streptococcus sobrinus* (ATCC 27607) was investigated by agar diffusion and microdilution techniques. Inhibitory concentration-fifty values of the flowers' extract against *Streptococcus mutans* glucosyltransferase enzymes were determined. The total flavonoid content of the extracts was determined using an aluminum chloride reaction.
Results: *Verbascum speciosum* flowers' extract showed significantly higher flavonoid content and antibacterial activity; with minimum inhibitory concentrations of 100 and 200 μg/mL for *Streptococcus mutans* and *Streptococcus sobrinus*, respectively. The extract inhibited the synthesis of glucan by cell-associated and extracellular glucosyltransferase enzymes in a dose-dependent manner with higher activity against the extracellular enzyme.
Conclusion: This study indicated effective anticariogenic activity of Verbascum speciosum flowers extract. This extract can be considered as an alternative to current anticaries therapies or an additive to dental care products.

Keywords: dental caries, glucan, *Streptococcus mutans*, *Streptococcus sobrinus*, *Verbascum speciosum*

INTRODUCTION

Dental caries is one of the most prevalent chronic human infections worldwide. It begins with bacterial adherence to the tooth surface and formation of dental plaque/biofilms. Cariogenic bacteria within these dental plaques produce acid by metabolizing carbohydrates. Persistent acidification drops the plaque pH under the critical pH 5.5, which destroys enamel surfaces. Demineralization of enamel and degradation of dentine organic matrix leads to development of carries and inflammation of pulp that can end with tooth loss [1, 2].

Among various microorganisms, mutans streptococci, consisting mainly of *Streptococcus mutans* and *Streptococcus sobrinus*, are the principal oral microorganisms involved in the initiation and development of dental caries [3]. Epidemiological studies have shown a direct correlation between salivary levels

of these microorganisms and the number of decayed teeth. These microorganisms can easily adhere to enamel surfaces and form a high cell density biofilm through irreversible interactions between bacterial cells. Their other virulence factors are acidogenicity, acid tolerance, and synthesis of water-insoluble glucan from sucrose [4].

Glucans are polymers of individual glucose units synthesized from sucrose by the enzymatic action of glucosyltransferase (GTF). They facilitate bacterial aggregation in stable biofilms and favour their adhesion to the tooth enamel by binding to hydroxyapatite minerals [5, 6]. Both *S. mutans* and *S. sobrinus* synthesize extracellular GTFs that promote bacterial adherence via glucan synthesis in the presence of sucrose. *S. mutans* also produces significant amounts of insoluble glucan by cell-associated GTFs [7].

Phytochemicals possess antibacterial properties against a wide range of microorganisms [8, 9]. They can prevent and treat dental caries without the associated adverse effects and risk of the development of antibiotic resistant bacterial strains associated with conventional antibiotics [10]. Antibacterial effects on oral pathogens and inhibition of bacterial GTFs for glucan synthesis are the main mechanisms whereby natural products prevent biofilm formation and the development of dental caries [6].

Verbascum L. is the largest genus of the Scrophulariaceae family, with 360 different species native to eastern Europe and western Asia [11, 12]. *Verbascum* species contain a wide range of compounds such as flavonoids, saponins, alkaloids, and glycosides. They have traditionally have been used as expectorants, mucolytics, analgesics, and antiviral and antiseptic agents [13]. A notable member of this genus, *Verbascum speciosum*, was traditionally used to treat wounds, cuts, and skin disorders [14]. Previous studies have also reported on the remarkable antifungal and antibacterial activity of this plant against *Escherichia coli*, *Bacillus cereus*, *bacillus subtilis*, *Haemophilus Influenza*, and *Pseudomonas aeruginosa* [14, 15].

The aim of this study was to investigate anticariogenic activity of different parts of *V. speciosum* against two cariogenic streptococci, *S. mutans* and *S. sobrinus*. Initial susceptibility testing was followed by determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extracts against these bacteria. The extracts were also investigated for total flavonoid content. The inhibitory effect of the extract obtained from flowers of the plant on *S. mutans* extracellular and cell-associated GTF enzymes for glucan production was also investigated.

MATERIALS AND METHODS

1. Drugs and chemicals

Amoxicillin reference standard powder was kindly provided by The Accredited Laboratory for Food and Drug Quality Control (Tehran, Iran). Ethanol, sodium hydrogen monobasic, sodium hydrogen phosphate dibasic, sucrose, aluminum chloride, and sodium acetate were supplied from Merck (Dartmouth, Germany). Quercetin reference standard, dextran T10, sodium azide, and dialysis bags (15,000 cutoff) were from Sigma-Aldrich (St Louis, MO, USA).

2. Preparation of *V. speciosum* extracts

V. speciosum was collected at the flowering stage in June 2019 from Hamedan, Iran. A voucher specimen of this plant (voucher number PMP-1803) is available in the Herbarium of the School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran. Plant materials were dried at room temperature in the dark and ground using a mortar. 100 g of flowers and combined aerial parts of the plant were extracted separately in 2,000 mL ethanol/water (70:30 v/v) using the maceration method [16]. Plant powder and hydro-alcoholic solvent were placed in a 5 L Erlenmeyer flask and kept in darkness for 3 days with occasional mixing. Extraction was repeated three times on the initial plant material and extracts were combined. After removing the solvent under decreased pressure at 50℃, a waxy material was obtained. Extracts were further dried in an electric oven at 40℃ and ground in a mortar to a fine powder [17]. After weighing, the percentage of dry hydroalcoholic extract obtained from 100 g of plant material sample was 26.7 g; extraction yield was thus calculated as 26.7% (W/W).

3. Bacterial strains and growth media

Standard strains of *S. mutans* (ATCC 35668) and *S. sobrinus* (ATCC 27607) were obtained from the Persian Type Culture Collection of the biotechnology department at the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. Blood Agar Base supplemented with 5% sheep blood and Tryptic Soy Broth (TSB) and incubation at 37℃ for 24 h were used for growing bacteria throughout the study. Nutrient

 https://doi.org/10.3831/KPI.2023.26.2.139

Agar (NA) and Brain Heart Infusion (BHI) broth were used for susceptibility testing and determination of MIC and MBC. Isolates were maintained in TSB containing 15% glycerol at –80℃ until use.

4. Disc diffusion susceptibility tests

Susceptibility testing was carried out by the standard disc diffusion method for various concentrations of both extracts prepared from flowers and combined aerial parts of *V. speciosum*. NA plates were inoculated by spreading 100 μL of *S. mutans* and *S. sobrinus* suspensions at half McFarland (1.5×10^8 cfu/mL) bacterial concentration. Sterile blank discs (6 mm diameter) were impregnated with extract solutions in DMSO at 10, 25, 50, and 100 mg/mL concentrations. Impregnated discs were placed on the inoculated plates and plates were incubated at 37℃ for 24 h. A 10 μg/mL amoxicillin solution (AMX) and DMSO were investigated as positive and negative controls, respectively. Antimicrobial activity was evaluated by measuring inhibition zone diameters at 0.1 mm accuracy. This test was conducted in three separate experiments, each one in triplicate.

5. Determination of MIC and MBC

MIC of *V. speciosum* flower extract and total extract against *S. mutans* and *S. sobrinus* was determined on sterile 96 well microdilution plates according to the Clinical and Laboratory Standards Institute (CLSI) Guidelines [18]. 100 mg/mL extract stock solution in DMSO was further diluted in normal saline to a series of concentrations. 100 μL of each extract dilution was mixed with 100 μL BHI broth medium inoculated by bacterial suspension (containing 1.5×10^6 cfu/mL) in an eight well row of the microdilution plate. Final concentrations of the extract in the microdilution wells were 0.025, 0.05, 0.1, 0.2, 0.5, 1, 2.5, 5, 10, 15, and 20 mg/mL. Four wells were not treated with extract in order to permit maximum bacterial growth, and four others containing uninoculated medium were used as a control for monitoring of aseptic conditions. After incubation of the plates at 37℃ for 24 h, the concentration at which no growth was observed was designated as the MIC. Bacterial growth was investigated by measuring the absorbance of the contents of the wells before and after incubation at 550 nm using an automatic micro plate reader.

Determination of MBC was performed by adding 100 μL from the constituents of the wells not showing microbial growth in the MIC test to nutrient-supplemented agar plates. After incubation at 37℃ for 24 h, the lowest concentration of the extracts that did not form any colonies was designated as the MBC. These tests were conducted in three separate experiments, each one in eight replicates.

6. GTF inhibition activity

1) Preparation of *S. mutans* GTF enzymes

Extracellular and cell-associated GTF enzymes of *S. mutans* were obtained from culture in BHI broth. Suitable numbers of *S. mutans* bacteria were cultured in 1 L of BHI broth in 37℃ for 24 h. Broth culture was divided in 50 mL falcon tubes and centrifuged at 9,000 rpm for 15 minutes to separate supernatant and bacterial cells.

Supernatant was salted out by adding ammonium sulfate to achieve 50% saturation. After stirring for 1 h, extracellular GTF was collected by centrifugation at 9,000 rpm for 15 minutes. Precipitated enzyme was dissolved in 10 mL of 10 mM sodium phosphate buffer (pH = 6.5) and dialyzed against the same buffer in order to remove excess ammonium sulfate.

Cell-associated GTF was obtained from *S. mutans* cells separated from the broth culture. Bacterial cells were washed three times with 10 mM sodium phosphate buffer (pH = 6.5) by centrifugation, in order to remove the remaining culture medium. The centrifuged pellet was dispersed in 30 mL of 8 M urea solution as the extraction fluid and kept at 25℃ for 1 h with occasional shaking. Bacterial cells were separated by centrifugation at 9,000 rpm for 15 minutes. Extraction of cell-associated GTF from *S. mutans* cells was repeated three times and supernatants were mixed. Cell associated GTF was obtained by 50% saturation ammonium sulfate precipitation of supernatant mixture and subsequent dialysis of the precipitate against 10 mM sodium phosphate buffer, pH = 6.5 [7]. The dialyzed solutions were used as crude GTF enzymes and kept at 4℃ until use.

2) Inhibitory activity against *S. mutans* GTF enzymes

Six different dilutions (0.05, 0.1. 0.25, 0.5, 1, 2 mg/mL) of *V. speciosum* extract in 10 mM sodium phosphate buffer (pH = 6.5), containing 1% (w/v) sucrose, 0.5% (w/v) Dextran T10, and 0.02% (w/v) sodium azide were prepared. Two mL from each dilution was mixed with 2 mL of extracellular and cell-associated GTF crude enzymes separately, and incubated at 37℃ for 18 h [19]. Insoluble glucan synthesis in the presence of different concentrations of the extract was determined by mea-

suring the absorbance of assay mixtures at 500 nm against the blank mixtures that were prepared in the same manner as the assay mixtures without incubation. Enzyme activity percent at each concentration was determined according to Equation (1). In this equation, A represents absorbance of the assay mixture and A_0 represents absorbance of the control that was prepared by the same method as the assay mixture without adding *V. speciosum* extract.

$$\% \text{ Enzyme activity} = (A/A_0) \times 100 \qquad \text{Equation (1)}$$

7. Total flavonoid content

The aluminum chloride colorimetric method was used for determining total amounts of flavonoid compounds in the extracts obtained from flowers and total aerial parts of *V. speciosum*. Two hundred μL of 10 mg/mL solution of the extract was mixed with 1.5 mL methanol, 0.5 mL of 1 M sodium acetate, and 100 μL of 10% (w/v) aluminum chloride solution. After adding 2.8 mL distilled water to the above mixture and incubating at room temperature for 1 h, absorbance was measured at 415 nm against the blank that was prepared by replacing aluminum chloride with an equal volume of distilled water [20]. Total flavonoid content was determined using the plot of absorbance against various concentrations of the reference compound quercetin. The result was expressed as mg quercetin equivalent per g of dry weight.

8. Statistical analysis

Statistical analysis was performed in SPSS 16.0 software, using one-way ANOVA followed by Tukey's test to compare multiple groups. IC50 determination and plotting were performed in GraphPad Prism (version 8.3).

RESULTS

1. Extracts from *V. speciosum*

Maceration extraction from 100 g of dried aerial parts and flowers of *V. speciosum* yielded 4.7 g and 3.3 g dried extracts, respectively. Extracts were dark green powders which were stored in airtight containers in the refrigerator until use.

2. Antibacterial activity of *V. speciosum* extracts against *S. mutans* and *S. sobrinus*

Antibacterial activity of hydro-alcoholic extracts prepared from flowers and total aerial parts of *V. speciosum* against *S. mutans* and *S. sobrinus* was investigated using the disc diffusion susceptibility test. Fig. 1 shows a diagram of diameters of inhibition zones obtained for four different concentrations of the extracts along with 10 μg/mL amoxicillin solution used as a positive control. Results of this test showed poor antibacterial activity of the aerial parts' extract on both microorganisms. Inhibition zones were observed only at 50 and 100 mg/mL concentrations of this extract, without a significant difference between different concentrations or against different microorganisms. On the other hand, the extract obtained from flowers of the plant showed dose-dependent antibacterial activity against both microorganisms. *S. mutans* was more susceptible to the flower extract compared with *S. sobrinus* at every investigated concentration ($p < 0.05$).

DMSO did not show any antibacterial effect, while amoxicillin showed apparent antibacterial activity against both microor-

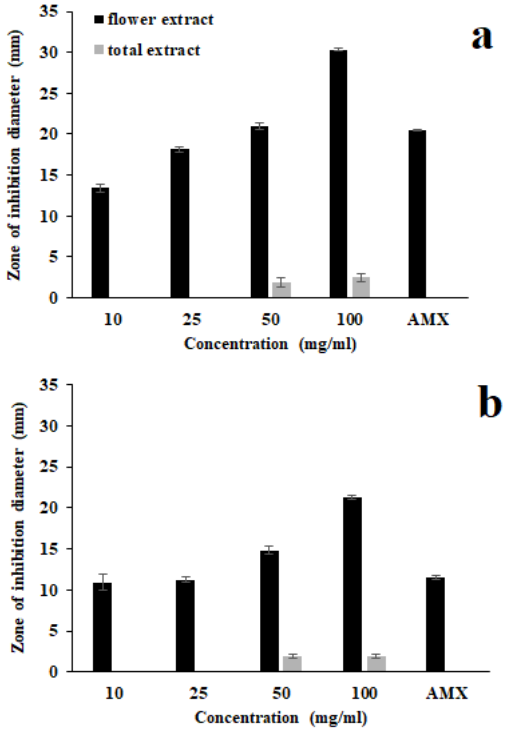

Figure 1. Average diameter of inhibition zones obtained from *V. speciosum* extracts and 10 μg/mL amoxicillin solution (AMX) on (a) *S. mutans* and (b) *S. sobrinus*. Data are expressed as mean of inhibition zones obtained in three separate days; error bars show SD.

 https://doi.org/10.3831/KPI.2023.26.2.139

ganisms with higher activity against *S. mutans* ($p < 0.05$).

3. MIC and MBC of *V. speciosum* extracts on *S. mutans* and *S. sobrinus*

Table 1 shows estimated values for MIC and MBC for the extracts obtained from flowers and aerial parts of *V. speciosum*. These tests were conducted in three separate experiments and results that were repeatable in at least two replicates were reported as MIC and MBC.

4. Inhibitory activity of *V. speciosum* flower extract on *S. mutans* GTF enzymes

Fig. 2 shows plots of enzyme activity percent of *S. mutans* GTF enzymes in the presence of different concentrations of *V. speciosum* flower extract. Enzyme activity in the control sample was assumed to be 100% and incorporated in the plot data against a very small number as extract concentration, as a zero value cannot be plotted in logarithmic charts. Concentrations of the extract that lowered activity of cell-associated and extracellular GTF enzymes by 50% (IC50) were estimated as 160 and 70 μg/mL, respectively.

5. Total flavonoid content of *V. speciosum* extracts

Total flavonoid content of *V. speciosum* flower extracts and total aerial part extracts were calculated as 1.45 and 0.38 mg quercetin/g dry weight.

DISCUSSION

Several plant extracts have been shown to exert antibacterial effects on cariogenic mutans streptococci, and are also capable of inhibiting bacterial GTFs, the critical enzymes in adherent glucan production and subsequent plaque formation [5-7, 19]. Previous studies have shown that plants belonging to the Verbascum genus possess promising antibacterial properties. In line with these findings, the present study was conducted to investigate anticariogenic properties of *V. speciosum*, the most prevalent species of this genus in Hamadan province, Iran [21, 22].

Hydro-alcoholic extracts from the flowers and aerial parts of *V. speciosum* were prepared separately and investigated for antibacterial properties against *S. mutans* and *S. sobrinus*. Results of antimicrobial susceptibility tests, shown in Fig. 1, indicate that the flowers possess greater antibacterial activity than the

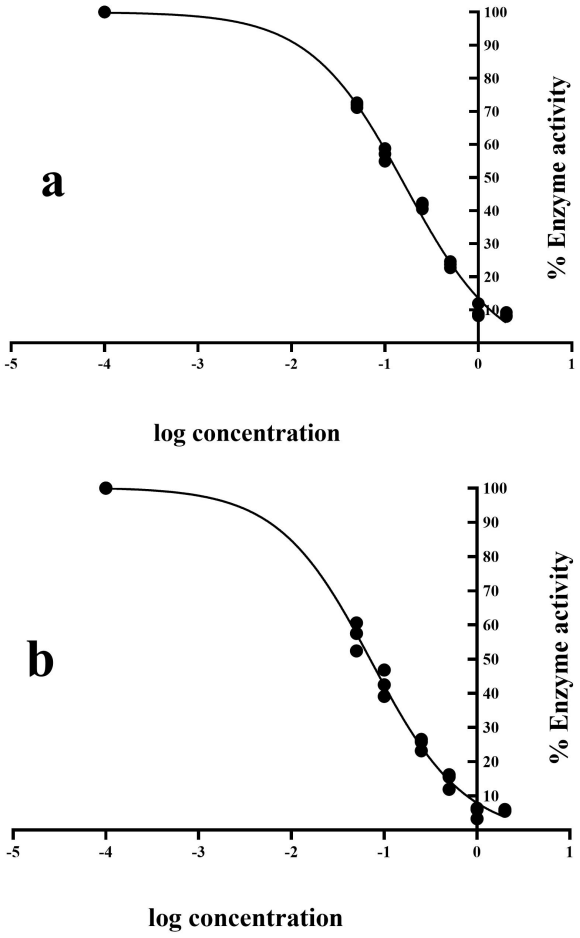

Figure 2. Plots of (a) cell associated and (b) extracellular enzyme activity percent in the presence of different concentrations of *V. speciosum* flowers' extract.

Table 1. MIC and MBC of *V. speciosum* extracts against *S. mutans* and *S. sobrinus*

	Flowers extract		Aerial parts extract	
	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)
S. mutans	100	200	5,000	15,000
S. sobrinus	200	1,000	5,000	ND[¶]

[¶]Not determined in the range of concentrations investigated in the present study.

aerial parts. This observation agrees with findings of a previous study that showed higher antibacterial activity for extract of *Verbascum viedemannianum* flowers compared with the extract obtained from the leaves [23]. Flavonoids seem to be the most important antibacterial constituent of the flower extract.

Dental caries is one of the most widespread and costly biofilm-mediated oral infectious diseases, affecting people of all ages worldwide. Among the several hundred bacterial species that constitute dental plaque, *Streptococcus mutans* (*S. mutans*) is a principal causative agent of caries [24].

As polyphenolic compounds, flavonoids encourage the accumulation of free radicals such as superoxides [25]. The presence of flavonoids confers antibacterial, antifungal, and even antitumorigenic effects on plants enriched in such compounds [25]. Antimicrobial activities in particular are attributable to phenolic compounds, flavonoids, and phenylethanoids [26, 27]. Saponins are glycosides with high molecular weight, and their most important physical attribute is that they produce foams when dissolved in water. Saponins are biologically active compounds that act as a defensive agent against pathogens [28]. Studies have shown that saponins are highly toxic to fungi [25]. It is therefore likely that many of the antimicrobial effects observed in Verbascum spp. stem from the presence of a variety of glycosides and alkaloids in these plants [14]. Verbascum phenolic compounds have also received considerable attention for their antioxidant properties. These compounds, including flavonoids in the form of crude extract or isolated pure ingredients, are suggested to be even more powerful antioxidants than vitamins C, E, and β-carotene [29].

HPLC-DAD analysis has shown that different species of Verbascum plant found in Iran are enriched in flavonoids and active phenolic compounds in different parts of the plant, especially the aerial organs [30]. Verbascum species contain caffeic acid (0.022 g/kg^{-1} DW), chlorogenic acid (2.649 g/kg^{-1} DW), p-coumaric acid (0.253 g/kg^{-1} DW), apigenin (0.066 g/kg^{-1} DW), gallic acid (0.134 g/kg^{-1} DW), rutin (5.254 g/kg^{-1} DW), quercetin (1.303 g/kg^{-1} DW), and cinnamic acid (1.031 g/kg^{-1} DW) [30].

A previous study on the flavonoid content of three Verbascum species showed that *V. speciosum* had higher flavonoid content than the other studied species [15]. Antibacterial properties are most likely due to the higher concentration of these compounds in flowers relative to other aerial parts. Flavonoids exert their anticariogenic activity through both antibacterial and GTF inhibitory activities [31, 32].

Susceptibility tests also indicated higher antibacterial activity of *V. speciosum* flower extract on *S. mutans* compared with *S. sobrinus*. *S. mutans* was also more susceptible to the 10 μg/mL amoxicillin solution used as a positive control. These results were consistent with the lower MIC and MBC values found in *S. mutans*. Different susceptibility of these microorganisms to antibacterial agents was reported previously by de Araújo et al. [33]. In this study, *S. mutans* showed two-fold higher susceptibility to *Anacardium occidentale* extract than *S. sobrinus*, while *S. sobrinus* was more susceptible to 0.12% chlorhexidine solution [33].

As previously mentioned, inhibition of mutans Streptococci GTF enzymes can enhance the anticariogenic potential of plant extracts. These bacteria produce both cell-associated and extracellular GTF enzymes responsible for insoluble glucan synthesis [7]. The presence of water-insoluble, adherent glucan in saliva and tooth enamel surfaces promotes accumulation of mutans streptococci and plaque formation [6]. It is noteworthy that antibacterial and GTF inhibitory activities are not always concomitant. Several compounds with antibacterial activity against cariogenic microorganisms do not effectively lower glucan synthesis. Conversely, other bioflavonoids such as apigenin, found in plants and propolis, disrupt formation and accumulation of *S. mutans* biofilms without killing the organism [34].

A comparison of the IC50 values of *V. speciosum* flower extract for *S. mutans* GTF enzymes indicated higher inhibitory activity of the extract against the extracellular GTF enzyme. Previous studies have reported a wide range of IC50 values for plant extracts or their pure constituents on *S. mutans* GTF enzymes. For example, apigenin inhibited both cell-associated and extracellular GTF enzymes within an IC50 range of 16-26 μg/mL [34]. In another study, IC50 values of two green tea polyphenols for extracellular and cell-associated GTF enzymes of *S. mutans* were 250 and 500 μg/mL, respectively [35]. Results of this study showed that *V. speciosum* flower extract was a highly effective inhibitor of *S. mutans* GTF enzymes. Further, the extract lowered glucan synthesis by both GTF enzyme types at concentrations around its MIC for *S. mutans*, with higher activity against the extracellular enzymes. From the dose-dependent inhibitory activity of the extract on glucan synthesis, it can be assumed that irreversible bonds between the active ingredients of the extract and GTF enzymes leads to decreased glucan synthesis.

 https://doi.org/10.3831/KPI.2023.26.2.139

CONCLUSION

The results presented here demonstrate that *V. speciosum* flower extract possesses strong anticariogenic activity. Specifically, this effect can be attributed to antibacterial activity against cariogenic mutans Streptococci and inhibition of glucan synthesis by the GTF enzymes present in these organisms. This extract can be considered as an alternative to current anticaries therapies or as an additive to dental care products.

ACKNOWLEDGEMENTS

We would like to declare our special thanks to the Research Affairs of Hamedan University of Medical Sciences for financial support. The authors wish to thank Ms. Sahar Foroughinia for her excellent technical assistance.

ETHICAL APPROVAL

All procedures in this study were done in agreement with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration. The research was approved by the Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1396.302).

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

GUARANTOR

Dr. Amir Larki-Harchegani accept full responsibility for this performed study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest, financial or other, exists.

FUNDING

This research has been financially supported by vice-chancellor for research and technology, Hamadan University of Medical Sciences, Hamadan, Iran (Grant No.9606073549).

ORCID

Shabnam Pourmoslemi, https://orcid.org/0000-0002-9014-4619
Amir Larki-Harchegani, https://orcid.org/0000-0001-9014-6772
Sajad Daneshyar, https://orcid.org/0000-0002-7553-3843
Dara Dastan, https://orcid.org/0000-0002-7208-208X
Amir Nili-Ahmadabadi, https://orcid.org/0000-0001-9072-8318
Mina Jazaeri, https://orcid.org/0000-0003-0896-7788

REFERENCES

1. Wu CY, Su TY, Wang MY, Yang SF, Mar K, Hung SL. Inhibitory effects of tea catechin epigallocatechin-3-gallate against biofilms formed from Streptococcus mutans and a probiotic lactobacillus strain. Arch Oral Biol. 2018;94:69-77.
2. Yang Y, Liu S, He Y, Chen Z, Li M. Effect of LongZhang Gargle on biofilm formation and acidogenicity of Streptococcus mutans in vitro. Biomed Res Int. 2016;2016:5829823.
3. Law V, Seow WK, Townsend G. Factors influencing oral colonization of mutans streptococci in young children. Aust Dent J. 2007;52(2):93-100; quiz 159.
4. Nishikawara F, Katsumura S, Ando A, Tamaki Y, Nakamura Y, Sato K, et al. Correlation of cariogenic bacteria and dental caries in adults. J Oral Sci. 2006;48(4):245-51.
5. Bhagavathy S, Mahendiran C, Kanchana R. Identification of glucosyl transferase inhibitors from Psidium guajava against *Streptococcus mutans* in dental caries. J Tradit Complement Med. 2018;9(2):124-37.
6. Veloz JJ, Saavedra N, Alvear M, Zambrano T, Barrientos L, Salazar LA. Polyphenol-rich extract from propolis reduces the expression and activity of Streptococcus mutans glucosyltransferases at subinhibitory concentrations. Biomed Res Int. 2016; 2016:4302706.
7. Hamada S, Horikoshi T, Minami T, Okahashi N, Koga T. Purification and characterization of cell-associated glucosyltransferase synthesizing water-insoluble glucan from serotype C Streptococcus mutans. J Gen Microbiol. 1989;135(Pt 2):335-44.
8. Dorman HJ, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol. 2000; 88(2):308-16.
9. Jazaeri M, Pakdek F, Rezaei-Soufi L, Abdolsamadi H, Rafieian N. Cariostatic effect of green tea in comparison with common anticariogenic agents: an in vitro study. J Dent Res Dent Clin Dent Prospects. 2015;9(1):44-8.
10. Al-Bayati FA, Sulaiman KD. In vitro antimicrobial activity of *Salvadora persica* L. extracts against some isolated oral pathogens in Iraq. Turk J Biol. 2008;32(1):57-62.

11. Karavelioğulları FA, Ocak A, Ekici M, Cabi E. *Verbascum eskisehirensis* sp. nov. (Scrophulariaceae) from central Anatolia, Turkey. Nord J Bot. 2009;27(3):222-7.
12. Sotoodeh A, Civeyrel L, Attar F. *Verbascum shahsavarensis* (Scrophulariaceae), a new species for flora of Iran. Phytotaxa. 2015;203(1):76-80.
13. Escobar FM, Sabini MC, Zanon SM, Tonn CE, Sabini LI. Antiviral effect and mode of action of methanolic extract of Verbascum thapsus L. on pseudorabies virus (strain RC/79). Nat Prod Res. 2012;26(17):1621-5.
14. Amirnia R, Khoshnoud H, Alahyary P, Ghiyasi M, Tajbakhsh M, Valizadegan O. Antimicrobial activity of Verbascum speciosum against three bacteria strains. Fresenius Environ Bull. 2011; 20(3):690-3.
15. Karamian R, Ghasemlou F. Total phenolic content, antioxidant and antibacterial activities of three verbascum species from Iran. JMPB. 2013;2(1):43-51.
16. Dastan D, Karimi S, Larki-Harchegani A, Nili-Ahmadabadi A. Protective effects of Allium hirtifolium Boiss extract on cadmium-induced renal failure in rats. Environ Sci Pollut Res Int. 2019;26(18):18886-92.
17. Amidi N, Moradkhani S, Sedaghat M, Khiripour N, Larki-Harchegani A, Zadkhosh N, et al. Effect of green tea on inflammation and oxidative stress in cisplatin-induced experimental liver function. J Herbmed Pharmacol. 2016;5(3):99-102.
18. Cockerill F. Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement. Wayne (PA): Clinical and Laboratory Standards Institute; 2011.
19. Shimamura A, Tsumori H, Mukasa H. Purification and properties of Streptococcus mutans extracellular glucosyltransferase. Biochim Biophys Acta. 1982;702(1):72-80.
20. Kerdar T, Moradkhani S, Dastan D. Phytochemical and biological studies of *Scrophularia striata* from Ilam. Jundishapur J Nat Pharm Prod. 2018;13(3):e62705.
21. Dulger G, Tutenocakli T, Dulger B. Antimicrobial potential of the leaves of common mullein (*Verbascum thapsus* L., Scrophulariaceae) on microorganisms isolated from urinary tract infections. J Med Plants Stud. 2015;3(2):86-9.
22. Prakash V, Rana S, Sagar A. Studies on antibacterial activity of *Verbascum Thapsus*. J Med Plants Stud. 2016;4(3):101-3.
23. Barbour EK, Al Sharif M, Sagherian VK, Habre AN, Talhouk RS, Talhouk SN. Screening of selected indigenous plants of Lebanon for antimicrobial activity. J Ethnopharmacol. 2004;93(1):1-7.
24. Chen H, Xie S, Gao J, He L, Luo W, Tang Y, et al. Flavonoid baicalein suppresses oral biofilms and protects enamel hardness to combat dental caries. Int J Mol Sci. 2022;23(18):10593.
25. Jamshidi-Kia F, Lorigooini Z, Asgari S, Saeidi K. Iranian species of *Verbascum*: a review of botany, phytochemistry, and pharmacological effects. Toxin Rev. 2019;38(4):255-62.
26. Asgary S, Afshani MR, Sahebkar A, Keshvari M, Taheri M, Jahanian E, et al. Improvement of hypertension, endothelial function and systemic inflammation following short-term supplementation with red beet (Beta vulgaris L.) juice: a randomized crossover pilot study. J Hum Hypertens. 2016;30(10):627-32.
27. Bagheri Z, Azizi A, Oshvandi K, Mohammadi Y, Larki-Harchegani A. The effect of comfrey on enoxaparin-induced bruise in patients with acute coronary syndrome: a randomised clinical trial. J Pharmacopuncture. 2021;24(4):196-205.
28. Francis G, Kerem Z, Makkar HP, Becker K. The biological action of saponins in animal systems: a review. Br J Nutr. 2002;88(6): 587-605.
29. Okasha YM, Fathy FI, Soliman FM, Fayek NM. The untargeted phytochemical profile of *Verbascum thapsus* L. with potent antiviral, antibacterial and anticancer activities. South Afr J Bot. 2023;156:334-41.
30. Amini S, Hassani A, Alirezalu A, Maleki R. Phenolic and flavonoid compounds and antioxidant activity in flowers of nine endemic Verbascum species from Iran. J Sci Food Agric. 2022; 102(8):3250-8.
31. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26(5):343-56. Erratum in: Int J Antimicrob Agents. 2006;27(2):181.
32. Ferrazzano GF, Amato I, Ingenito A, Zarrelli A, Pinto G, Pollio A. Plant polyphenols and their anti-cariogenic properties: a review. Molecules. 2011;16(2):1486-507.
33. de Araújo JSC, de Castilho ARF, Lira AB, Pereira AV, de Azevêdo TKB, de Brito Costa EMM, et al. Antibacterial activity against cariogenic bacteria and cytotoxic and genotoxic potential of Anacardium occidentale L. and Anadenanthera macrocarpa (Benth.) Brenan extracts. Arch Oral Biol. 2018;85:113-9.
34. Koo H, Seils J, Abranches J, Burne RA, Bowen WH, Quivey RG Jr. Influence of apigenin on gtf gene expression in Streptococcus mutans UA159. Antimicrob Agents Chemother. 2006;50(2):542-6.
35. Sakanaka S, Sato T, Kim M, Yamamoto T. Inhibitory effects of green tea polyphenols on glucan synthesis and cellular adherence of cariogenic Streptococci. Agric Biol Chem. 1990;54(11):2925-9.