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DOI: 10.4103/kleuhsj.kleuhsj_13_22

Evaluation and comparison of antibacterial effects of a licorice gel and chlorhexidine gel on *Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans,* and *Tannerella forsythia* – An *in vitro* study

Alpana Andrews, Neelamma Shetti

Abstract:

INTRODUCTION: Periodontitis is known to cause inflammation and progressive breakdown of tooth-supporting structures. The treatment for periodontitis includes scaling and root planing that effectively removes the disease causing plaque and antimicrobial agents such as chlorhexidine (CHX) used as an adjunct to maintain the healthy state of tissues. However, these antimicrobial agents have unpleasant side effects that have led researchers toward discovering, isolating, and developing natural herbal plant extracts as effective antimicrobial agents. Hence, the purpose of the study was to assess and compare the antimicrobial activity of the one such herbal plant extract *Glycyrrhiza glabra* (licorice) gel and CHX gel on periopathogens.

MATERIALS AND METHODS: The hydroalcoholic root extract of *G. glabra* (licorice) was prepared through maceration, evaporation, and lyophilization to obtain dried crude extract. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *G. glabra* extract against *Porphyromonas gingivalis*, *Aggregatibacter Actinomycetemcomitans,* and *Tannerella forsythia* were determined using broth dilution method and streaking on blood agar plates. The gel was then prepared accordingly using Carbopol 940. The antibacterial activity of the prepared *G. glabra* gel was tested and compared to CHX gel using the agar well diffusion assay.

RESULTS: The MIC of *G. glabra* extract was found to be 15 mg against *A. actinomycetamcomitans* and *T. forsythia* and 7.5 mg against *P. gingivalis.* The MBC of the *G. glabra* extract was 30 mg for all three organisms. The antibacterial effects of the prepared licorice gel were assessed using agar well diffusion assay, and it showed that 100 μl of prepared licorice gel had a greater effect on *A. actinomycetamcomitans* and *T. forsythia* but not *P. gingivalis.*

CONCLUSION: Licorice extract showed inhibitory activity against *P. gingivalis*, *A. actinomycetamcomitans,* and *T. forsythia*.

Keywords:

Dental plaque, *Glycyrrhiza glabra*, herbal extract, licorice, periodontitis

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Received: 03 January 2022,
Revised: 09 March 2022,
Accepted: 10 April 2022,
Published: 21 January 2023

How to cite this article: Andrews A, Shetti N. Evaluation and comparison of antibacterial effects of a licorice gel and Chlorhexidine gel on *Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans,* and *Tannerella forsythia* – An *in vitro* study. Indian J Health Sci Biomed Res 2023;16:48-53.

Introduction

Periodontitis is an oral disease that is known to cause inflammation of tooth-supporting structures due to the presence of plaque (dental biofilm). It results in progressive breakdown of the periodontal ligament and its supporting tissues.[1] Untreated periodontitis can lead to formation of deep periodontal pockets and progressive loss of alveolar bone which causes tooth loosening eventually leading to tooth loss. Epidemiological studies have determined a high prevalence of this multifactorial, polymicrobial infection among the Indian population.[2]

Dental plaque is, in simple terms, a structured organic biofilm that is found on a tooth's surface, and it normally maintains a homeostatic relationship with the human host.[3] The periodontal disease is initiated when there is a disturbance in this microbial homeostasis that causes a shift toward acidophilic anaerobic bacterial species in the biofilm.

The frontline treatment for periodontitis includes scaling and root planing (SRP). Although SRP is an indispensable phase of periodontal therapy, it single handedly is unable to eliminate the tissue invading pathogens completely indicating the need for adjuvant antimicrobial therapy. Chlorhexidine (CHX) gluconate is a gold standard potent antimicrobial agent used widely as a disinfectant in intraoral applications. However, it is known to have several adverse effects that include tooth staining, xerostomia, dysgeusia, and precipitation of phosphate and calcium ions on the tooth surface. There have been reports documenting the development of antimicrobial resistance to CHX prompting clinicians to now prescribe this drug judiciously.[4]

Over the years, application of natural, herbal substances in dentistry has been gaining importance mainly to avoid the development of microbial resistance to and side effects of synthetic allopathic medications. There has been a shift from modern medicine to traditional medicine as they are economical and have medicinal value and are known to have fewer side effects.[5]

One such herb with potent antibacterial and anti-inflammatory properties is licorice/liquorice. The two varieties of licorice studied extensively for orodental diseases is *Glycyrrhiza uralensis* (Chinese licorice) and *Glycyrrhiza glabra* (European licorice). *G. glabra* is a sweet-tasting root with a high medicinal value and is used commonly in ayurvedic herbal preparations.[6] Liquorice extracts and their bioactive phytoconstituents have been demonstrated in several clinical studies to have an effect on both oral microbes and the host immune response involved in common oro-dental diseases such as dental caries, periodontal diseases, and candidiasis.[5]

Hence, the aim of the current study was to assess and compare the antimicrobial activity of the licorice gel and CHX gel on *Porphyromonas gingivalis*, *Aggregatibacter actinomycetamcomitans,* and *Tannerella forsythia.*

Materials and Methods

Ethical Clearance was obtained from ethical committee of KLE V.K Institute of Dental Sciences, Belagavi with Ref no MDC 2019/1310 dated 07/07/2019 after which the study was conducted in the following way.

Methodology of extract preparation

The entire root of the *G. glabra* plant [Figure 1] was collected, washed by tap water, and air-dried at room temperature before being ground into powder with the assistance of mechanical grinder. The powder was subjected to extraction by maceration in 95% ethanol. Approximately 300 g of the powdered licorice was soaked in 1050 ml of ethanol and 450 ml of water (1:5) for 72 h at room temperature. Initially, the extracts were filtered through Whatman No. 1 filter paper, followed by a 0.45-m membrane filter (Sigma). The filtrate obtained was then subjected to evaporation at room temperature in the rotary evaporator (BUCHI Rotavapor R114). In order to verify the sterility of the dried extract, it was irradiated with UV light overnight and plated on nutrient agar.

The crude extracts were further lyophilized to obtain dried crude extract which was kept at room temperature in a dry place until used for testing. Stock solution of extract was prepared by dissolving 300 mg of dried crude extract in dimethyl sulfoxide saline (DMSO) at pH 7.0 prepared with concentration of 60 mg/ml and kept at 4°C protected from light before being used.

Inoculum preparation

Inoculum preparation was carried out in brain–heart infusion (BHI) broth. Standard bacterial colonies of the same morphological type of *A. actinomycetamcomitans, P. gingivalis,* and *T. forsythia* were taken from a cultured agar plate. Further, each colony was picked with the help of sterile loop, and the grown bacteria were transferred to a Falcon tube having 5 mL of BHI broth. This broth culture was further incubated at 37°C for 8–14 h till it matched the turbidity of the 0.5 McFarland standards. The actively growing bacterial inoculum's turbid appearance was adjusted with that of the broth to obtain a final turbidity of 0.5 McFarland standards.

Broth dilution method (resazurin) for determining minimum inhibitory concentration

Initially, ten wells were selected from 96-well plates for broth dilution method. Total of 100 µl of broth was added to all the 8 wells; in the first well, 100 µl of extract was added and serially diluted to required concentrations up to the tenth well. Further, 20-µl bacterial inoculum was added to all the ten wells; separately, ten wells were used for positive and negative controls. The 96-well plates were kept for incubation in McIntosh and Filde's anaerobic jar, and resazurin reagent was added after 48 h and observed after 4 h for probable color change. Any color change from blue/violet to slight pink/pink/magenta was recorded as minimum inhibitory concentration (MIC) of emulsion. The results were recorded by taking quality photographs [Figure 2].

Note: Separate 96 well plates used for each bacteria and extract.

Commercially available CHX gel (Hexigel©) was used in the study in the control group.

Figure 1: Whole roots of licorice

Figure 3: Prepared licorice gel

Gel preparation

One-gram Carbopol was dispersed in 50 ml of water overnight with the aid of a magnetic stirrer at 1200 rpm at room temperature to get a homogenous composition of 2% Carbopol. To this, 0.5 ml of propyl paraben was added and 5 ml of this preparation was further diluted to 1%. Then, to this solution, 300 mg of licorice extract was added to get 60 mg/ml concentration of drug. In another beaker, 10-mg potassium sorbate was mixed with 5 ml of hot water for 30 min using a magnetic stirrer. To this mixture, 5 ml of the Carbopol-extract mix was added and the mixture was stirred for 5–10 min after which 1 drop of triethanolamine was added to neutralize the acidic Carbopol. This solution was further stirred slowly until a gel consistency was obtained. The final concentration of *G. glabra* in the prepared gel was 30 mg/ml [Figure 3].

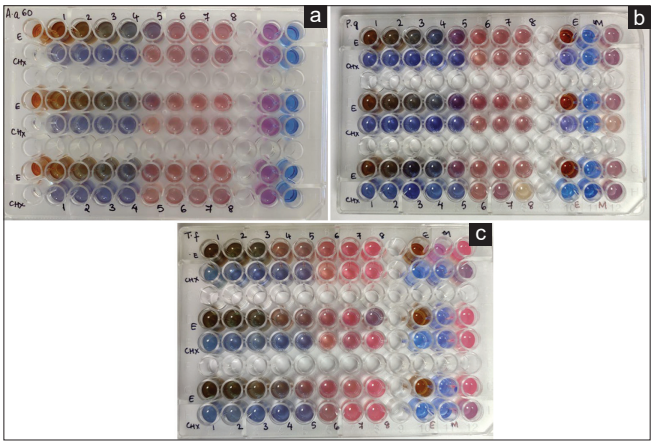

Figure 2: (a) Minimum inhibitory concentration of *Aggregatibacter actinomycetamcomitans*. (b) Minimum inhibitory concentration of *Porphyromonas gingivalis*. (c) Minimum inhibitory concentration of *Tannerella forsythia*

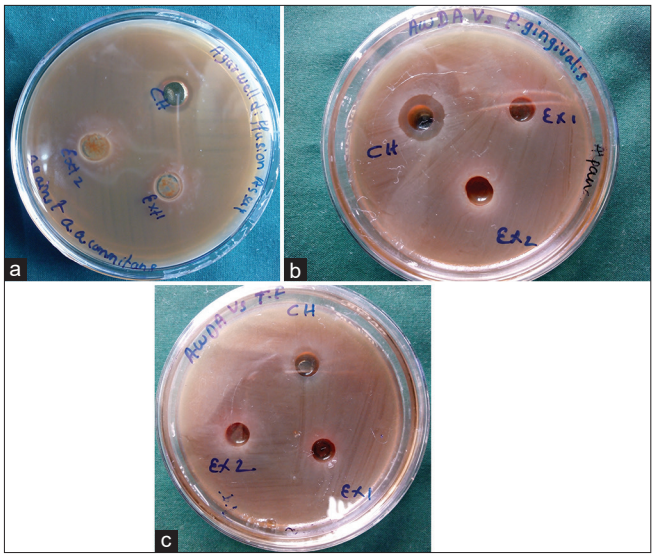

Figure 4: (a) Minimum bactericidal concentration of *Aggregatibacter actinomycetamcomitans*. (b) Minimum bactericidal concentration of *Porphyromonas gingivalis*. (c) Minimum bactericidal concentration of *Tannerella forsythia*

Agar well diffusion assay

The agar well diffusion assay was performed on bacteriological agar plates prepared. The BHI agar with blood (sterilized) was prepared, and left undisturbed for 10–15 min at room temperature to allow it to solidify. The bacterial broth cultures of *A. actinomycetamcomitans*, *P. gingivalis*, and *T. forsythia* were taken (0.5 McFarland's) and spread on prepared BHI agar plates (100 µl) with sterile cotton spreader, and left undisturbed for 15 min at room temperature. Then, using a cork borer of 8 mm size, wells were made in the agar plates and sample reagents (100-µl CHX, prepared extract gel, and 50 µl of prepared extract gel) were added into the respective wells and the plates were observed for diffusion after being placed in 37°C, CO_2 incubator (desiccator jar) for 24–72 h of incubation.

The plates were observed for growth pattern and results were noted against CHX as standard [Figure 4].

Results

Antibacterial susceptibility tests

The antibacterial effects of *G. glabra L.* extract were evaluated using broth dilution assay (resazurin) for minimum inhibitory concentration [Table 1] and agar plate assay for minimum bactericidal concentration [Table 2]. These tests were conducted on standard ATCC strains of three anaerobic bacteria, *P. gingivalis*, *A. actinomycetemcomitans*, and *T. forsythia* that were revived from the repository of the research centre for the purpose of the study.

The control group for all the tests was CHX extract/gel, a known gold standard antimicrobial agent used to treat periodontal infections. The test group for the MIC and MBC test was *G. glabra L.* (licorice) extract, while for the well diffusion assay, it was 50 µl of the prepared licorice gel and 100 µl of the prepared licorice gel.

Furthermore, the antibacterial effects of the prepared licorice gel were assessed against the same organisms through the agar well diffusion assay. The results of the agar well diffusion assay are listed in Table 3.

The intergroup comparison between the CHX group and licorice extract groups was analyzed using unpaired *t*-test while the intragroup comparison (licorice extract 50 µl v/s licorice extract 100 µl) was performed using one-way analysis of variance test. The results of this inter- and intragroup comparisons are listed in Table 4.

Discussion

Oral infections are considered a serious public health problem around the world. Periodontitis is an oral infection which begins at the gingival tissue level and which, if left untreated, penetrates into the deeper tissues and alters the bone homeostasis causing tooth loss. Periodontal disease has a multifactorial polymicrobial origin. The important etiologic factor responsible for development of periodontitis is the organized bacterial biofilm found on the tooth surfaces. Due to intrinsic changes in the local environment and host immune response, there is a shift of Gram-positive microbiological population connected to periodontal health to a predominant Gram-negative microbial population related to periodontal disease in dental plaque biofilm. The most common causative pathogens in the disease causing plaque are *P. gingivalis*, *A. actinomycetamcomitans*, and *T. forsythia*.[7]

Mulethi (*G. glabra*) is considerably significant in terms of its medicinal and aromatic values. This plant, being a member of the Fabaceae family (Genus *Glycyrrhiza*), is native to South-East Europe and South-West Asia, including Iran).[6] Licorice is composed of more than 20 triterpenoids and nearly 300 flavonoids. Among them, there are several chief bioactive components, which possess significant antiviral and antibacterial properties. These are, to name a few, glycyrrhizin, 18α-glycyrrhetinic acid, liquiritigenin, licochalcone E, licochalcone A, and glabridin. The root of *G. glabra* is an effective expectorant that has been used since classical times. Particularly in Ayurveda, licorice has been extensively used in the preparation of tooth powders and is commonly known as Jastimadhu/Mulethi regionally.[8]

Shivaprasad compared SRP with local drug delivery (LDD) of licorice gel for the treatment of periodontitis from a clinical and microbiological perspective. They found greater reduction of gingival index scores, bleeding index scores, probing pocket depth, clinical attachment level, and *P. gingivalis* in the test group demonstrating the potential of licorice gel as LDD agent for treatment of periodontal pockets.[9]

In our study, a hydroalcoholic extract of *G. glabra* (licorice) was prepared which was then evaluated for its antibacterial activity. MIC of licorice extract (determined using broth dilution assay) against *A. actinomycetamcomitans* and *T. forsythia* was found to be 15 mg, while for *P. gingivalis*, it was 7.5 mg [Table 1]. The MBC of the extract (determined using agar plate assay) was 30 mg for all three organisms [Table 2]. This showed that the phytochemical constituents of

Table 1: Minimum inhibitory concentration of *Glycyrrhiza glabra L.* extract

Samples	MIC (mg)					
	Aggregatibacter actinomycetamcomitans	Average	*Porphyromonas gingivalis*	Average	*Tannerella forsythia*	Average
Glycyrrhiza glabra L. (extract)	15	15 mg	7.5	7.5 mg	15	15 mg
	15		7.5		15	
	15		7.5		15	

MIC: Minimum inhibitory concentration

Table 2: Minimum bactericidal concentration of *Glycyrrhiza glabra* extract

Samples	MBC (mg)					
	Aggregatibacter actinomycetamcomitans	Average	*Porphyromonas gingivalis*	Average	*Tannerella forsythia*	Average
Glycyrrhiza glabra L.	30	30 mg	30	30 mg	30	30 mg
	30		30		30	
	30		30		30	

MBC: Minimum bactericidal concentration

Table 3: Agar well diffusion assay of licorice gel and chlorhexidine gel against *Aggregatibacter actinomycetamcomitans, Porphyromonas gingivalis*, and *Tannerella forsythia*

Samples	*Aggregatibacter actinomycetamcomitans* (mm)	*Porphyromonas gingivalis* (mm)	*Tannerella forsythia* (mm)
Chlorhexidine	11	14	10
Extract (50 µl)	10	10	10
Extract (100 µl)	12	11	11

Table 4: Inter- and intragroup comparison of chlorhexidine group and licorice extract groups

	t-test	*P*
Chlorhexidine versus extract 50 µl	1.4	0.3
Chlorhexidine versus extract 100 µl	0.3	0.826
Extract 50 µl versus extract 100 µl	4.0	0.008*

**P* value <0.05 = statistically significant

G. glabra show antibacterial activity. The antibacterial effects of the prepared licorice gel were assessed using agar well diffusion assay, and it showed that 100 µl of prepared licorice gel had a greater effect on *A. actinomycetamcomitans* and *T. forsythia* in comparison to the control group (CHX) [Table 3], but the results were not significant statistically [Table 4]. For *P. gingivalis*, the control group performed better than the test group (prepared licorice gel). Between the two quantities of gel taken, the results for the 100-µl group were found to be significant statistically in contrast to the results obtained by 50-µl group [Table 4]. This shows that the 100 µl of the prepared *G. glabra* extract gel showed significant antibacterial effect. Hence, *G. glabra* can be a suitable antibacterial alternative to CHX to treat periodontal disease.

Conclusion

The present study supports the hypothesis that *G. glabra* root extract is a useful antibacterial agent against oral pathogens to fight periodontal disease. The findings of the current study conclude that G. glabra can discourage the growth of *P. gingivalis*, *A. actinomycetemcomitans*, and *T. forsythia*. The plant extracts exhibit potential to be used as a LDD agent.

Further research is needed to develop and formulate a licorice extract LDD system which is designed to treat periodontal pockets. The result of this research requires to be further corroborated with long-term prospective *in vivo* clinical trials.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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