"TO ASSESS AND COMPARE THE ANTIMICROBIAL ACTIVITY OF LICORICE GEL AND CHLORHEXIDINE GEL ON PORPHYROMONAS GINGIVALIS, AGGREGATIBACTER ACTINOMYCETAMCOMITANS AND TANNERELLA FORSYTHIA - AN IN-VITRO STUDY"

By

Dr ALPANA ANDREWS

REG NO.IK0219001

Dissertation

Submitted to KLE Academy of Higher Education and Research (KAHER), Belagavi

In Partial Fulfillment of the Requirements for the Degree Of

MASTER OF DENTAL SURGERY

In

PERIODONTICS
(Branch II)

Under the Guidance of

Dr. Neelamma Shetti M.D.S

DEPARTMENT OF PERIODONTICS
KAHER'S KLE VISHWANATH KATTI
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2019 -2022

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I, hereby declare, that the dissertation ***"To Assess And Compare The Antimicrobial Activity of Licorice Gel And Chlorhexidine Gel On Porphyromonas Gingivalis, Aggregatibacter Actinomycetamcomitans And Tannerella Forsythia-an in-vitro study"*** *is a bonafide and genuine research work carried out by me under the guidance of* ***Dr. NEELAMMA SHETTI*** *M.D.S Reader, Department of Periodontics, KAHER KLE V.K Institute of Dental Sciences, Belagavi*

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*I would not be here today without the blessings and love of the **Almighty** and my parents **Col R.S Andrews, Mrs Veronica Andrews**, my brother **Abhijeet Andrews**, my new extended family **Cmde Rajan Mathew, Mrs Alice Mathew** and my dear husband **Dr Benjamin Barsouma Mathew**. Your faith in me has kept me going forward. I truly owe my success to you all.*

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Lecturer and ***Dr Shruti Karvekar*** ***M.D.S*** *Lecturer, Dept of Periodontics, KLE V.K Institute of Dental Sciences, Belagavi for their invaluable advice and support. They all have been invaluable sources of learning.*

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Last but not the least, I would like to thank ***Mr Anand*** *and* ***Mr Arun*** *who patiently managed and helped in the formatting and printing of this dissertation.*

May God continue to bless us and keep us walking in the light of His Love.

Date:

Place: Belagavi ***Dr. ALPANA ANDREWS***

LIST OF ABBREVIATIONS

CHX	Chlorhexidine
MIC	Mean inhibitory concentration
MBC	Mean bactericidal concentration

ABSTRACT

INTRODUCTION:

Periodontitis is an oral disease that is known to cause inflammation of tooth supporting structures due to presence of plaque (dental biofilm). It results in progressive breakdown of the periodontal ligament and its supporting tissues. It is caused by the accumulation of dental plaque which is a structurally and functionally well-organized biofilm that normally maintains a homeostatic relationship with the human host. A disturbance in this balance causes a microbial shift from commensal to pathogenic periodontal pathogens that mark the beginning of periodontal disease. The frontline treatment for periodontitis includes scaling and root planning (SRP) that effectively removes the plaque and restores the periodontium to a healthy state and antimicrobial agents such as Chlorhexidine that are often used as an adjunct to SRP to aid and maintain the healthy state of tissues. However these antimicrobial agents have side effects such as alteration of taste, discoloration of teeth and development of antimicrobial resistance. Hence, there has been a shift in research towards discovering, isolating and developing natural herbal plant extracts as effective antimicrobial agents.

The purpose of the current study is to assess and compare the antimicrobial activity of the one such herbal plant extract *Glycyrrhiza glabra* (licorice) gel and chlorhexidine gel on *Porphyromonas gingivalis*, *Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia.*

AIM:

To assess and compare the antimicrobial activity of the licorice gel and chlorhexidine gel on *Porphyromonas gingivalis*, *Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia.*

MATERIALS AND METHODS:

This is an experimental in-vitro microbial study. The hydroalcoholic root extract of *Glycyrrhiza glabra* (licorice) was prepared through maceration. The extract was then filtered using Whatman No.1 filter paper and using the rotary evaporator, the filtrate was further evaporated at room temperature. The crude extract was further lyophilized to obtain dried crude extract which was kept at room temperature until further use.

MIC and MBC of the *G.glabra* extract against standard bacterial strains of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia* (revived from the repository of the research centre) was 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 in two quantities was tested and compared to Chlorhexidine gel using the agar well diffusion assay

RESULTS:

The MIC of *G.glabra* extract against *Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia* was found to be 15 mg while for *Porphyromonas gingivalis* it was 7.5 mg. 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 *Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia* when contrasted with the control group but the results were found not to be statistically significant. For *Porphyromonas gingivalis,* the control group performed better than the test group. Between the two quantities of gel taken, the results for the 100 µl group were found to be significant statistically when compared to the 50 µl group.

CONCLUSION:

- The MIC of licorice extract against *Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia* is 15 mg while for *Porphyromonas gingivalis* it was 7.5 mg.
- The MBC of the licorice extract was 30 mg for all three organisms.
- 100 µl of prepared licorice gel had a greater effect on *Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia* but not on *Porphyromonas gingivalis.*
- Between the two quantities of gel taken, the results for the 100 µl group were significant statistically as opposed to those of the 50 µl group

KEYWORDS: Dental plaque, *Glycyrrhiza glabra,* Herbal extract, Licorice, Periodontitis

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXURES

INTRODUCTION

Periodontitis is an oral disease that is known to cause inflammation of tooth supporting structures due to 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 amongst the Indian population. [2] It is the disconcerting presence of the disease that drove researchers into finding an efficient treatment protocol that restores the periodontium to a healthy state.

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 towards acid loving anaerobic bacterial species in the biofilm.

Periodontitis involves primary colonizers such as Streptococcus species inhabiting the dental plaque biofilm which creates a favourable environment for attachment of secondary colonizers such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia*. These anaerobic secondary colonizers are key periodontal pathogens that are known to cause destruction of the periodontium. [1]

The frontline treatment for periodontitis includes scaling and root planning (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 gluconate (CHX) is a gold standard potent antimicrobial agent used widely as a disinfectant in intraoral applications. At lesser concentrations (0.02%-0.06%) there is displacement of Ca2+, Mg2+ ions and K+ ions from the cell wall due to CHX that alters the cell permeability of the bacterial membrane resulting in a bacteriostatic effect. At higher concentrations (>0.1%) CHX causes leakage of the cellular components out of the cell, resulting in cell lysis and death (bactericidal) effect. 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). Glycyrrhiza glabra is a sweet tasting root with a high medicinal value and is used commonly in Ayurvedic herbal preparations. By-products of liquorice roots metabolism (flavonoids, coumarins) have shown positive results in treating various diseases such as atherosclerosis, carcinogenic and tubercular infections, ulcers of the

gastric mucosa, immunodeficient conditions, hepatitis and other bacterial infections. [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 like dental caries, periodontal diseases, and candidiasis.

In the roots of Glycyrrhiza, the compound Glycyrrhizin (a triterpene saponin) is found in a high concentration. The concentration ranges from 2.5 and 9%, depending on the source and method used to prepare the drug. Glycyrrhizin, a diglucuronide of glycyrrhetinic acid, has been found to be the drug's active principle, responsible for most, if not all, of its positive effects. [5] In-vitro, major isoflavans such as Licoricidin and Licorisoflavan A have inhibited volatile sulphur production, protease activity and growth of *Porphyromonas gingivalis*. It is known that lipochalcone A inhibits the growth of *P. gingivalis* biofilms, along with the host immune response, which can lead to periodontitis. *P. gingivalis*-associated lipopolysaccharide-induced vascular permeability is markedly reduced by 18 α-glycyrrhetinic acid by suppressing nuclear factor κB-dependent endothelial IL- 8 production, suggesting a therapeutic potential for treating *P. gingivalis*-related vascular diseases.[7] The capacity of licorice to lessen development of dental plaque adds to its role in periodontitis management.[8]

Local drug delivery systems (LDD) aim to enhance the effectiveness of the drug at a local site through sustained release and vastly improve periodontal outcomes. Different drugs such as doxycycline, minocycline, metronidazole and chlorhexidine have been used in LDD. These systems can be in the form of a chip, microsphere, gel or patch. Gels are easily manufactured and offer ease of use. They

are not easily washed away from the gingival sulcus, which is the preferred site for local drug delivery in periodontal disease.

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

AIM AND OBJECTIVES

AIM OF THE STUDY

- To assess and compare the antimicrobial activity of the licorice gel and chlorhexidine gel on *Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia*.

OBJECTIVES OF STUDY

- To assess antimicrobial activity of licorice gel on *Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia*.
- To assess the antimicrobial activity of chlorhexidine gel on *Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia.*
- To compare the antimicrobial activity of licorice gel and chlorhexidine gel on *Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia*.

REVIEW OF LITERATURE

1. Suttipalin Suwannakul et al (2017) [9] investigated the antimicrobial and anti-proteolytic activities of Glycyrrhiza glabra root extract on *Porphyromonas gingivalis* in both planktonics and biofilm cells. The MIC and MBC of licorice root extract against *P.gingivalis* planktons was found to be 62.5µg/ml and 125µg/ml respectively. The time kill assay showed the licorice extract had an inhibitory effect on *P.gingivalis* growth at the concentration 2 and 4 folds of MBC value. The biofilm assay demonstrated that licorice extract was able to inhibit the formation of planktonic biofilms dose-dependently. They also observed that licorice root extract could inhibit both Rgp- and Kgp-proteinase proteinase activities of *P.gingivalis* whole cells at the sub-inhibitory concentration. The authors concluded that Licorice extract had the capability to inhibit *P.gingivalis* associated biofilm formation and it could eradicate the established biofilm as it exhibited the inhibitory effect on *P.gingivalis* cell surface proteinases activities which is necessary for the nutrient acquisition and growth and adhesion on host cells.

2. Shivaprasad BM et al (2017) [8] compared scaling and root planing with local drug delivery (LDD) of licorice gel for the treatment of periodontitis from a clinical and microbiological perspective. Thirty patients having chronic periodontitis were recruited for the study. The control group received scaling and root planing (SRP) alone while the test group received locally delivered licorice gel in addition to SRP. In addition to clinical parameters recorded at baseline, 15 days and one month post treatment, the reduction in the levels of *P.gingivalis* at follow-up visits was also assessed through microbiological analysis. The study

confirmed greater reduction of gingival index, bleeding index, probing pocket depth, clinical attachment level and *P.gingivalis* in the test group when compared to the control sites. The authors concluded that subgingivally delivered licorice as an adjunct to scaling and root planing in the treatment of chronic periodontitis showed anticipative results and required more in-vitro and in-vivo research focusing on the anti-inflammatory/anti-microbial effects of liquorice

3. Sunil Lingaraj Ajagannanavar et al (2014) [5] assessed the anti-bacterial effect of licorice root extract in-vitro against *S. mutans* and *L. acidophilus* against Chlorhexidine. The authors found the mean inhibitory concentration of licorice root extract in ethanolic and aqueous forms against *S. mutans* and *L.acidophilus* was 25% and 12.5%, at 48 hours respectively while the average inhibition zone of the aqueous and alcoholic forms of licorice extracts against *S. mutans* was found to be 22.8 mm and 26.7 mm at 48 hours, respectively. The average inhibition zone of the aqueous and alcoholic forms of licorice extracts against *L. acidophilus* was seen as 14.4 mm and 15.1 mm, at 48 hours respectively. The median inhibition zone of the Chlorhexidine against *S. mutans* and *L. acidophilus* was noted as 20.5mm and 13.2 mm, respectively at 48 hours. The authors were able to clearly demonstrate that alcoholic form of licorice root extract inhibited the growth of *S. mutans* and *L. acidophilus* better when compared to the aqueous form of licorice extract and Chlorhexidine.

4. Su-Ryun Kim et al (2012) [10] evaluated the effect of 18 α-glycyrrhetinic acid (18α-GA), a natural triterpenoid compound derived from licorice root extract, on *P. gingivalis* lipopolysaccharide (LPS)-induced vascular permeability. In both in-vivo and in-vitro experiments, they observed LPS-induced endothelial permeability was significantly inhibited by 18 α-GA. 18 α -GA reduced

endothelial gap formation induced by *P. gingivalis* LPS and decreased endothelial permeability through modulation of IL-8 expression and secretion.

5. Vu Dang La et al (2011) [11] studied human derived macrophages treated with non-toxic concentrations of Licoricidin and Licorisoflavan-A before being stimulated with lipolysaccaride of *A. actinomycetemcomitans*. They provided evidence that licorice-derived Licoricidin and Licorisoflavan-A possesses interesting therapeutic properties for the treatment of periodontal disease because of their ability to inhibit the secretion of inflammatory cytokines and MMPs by host cells. The authors believe Licoridin and Licorisoflavan have development potential of unique strategies for host-modulation for the remedy of cytokine and/or MMP-mediated disorders such as periodontitis.

6. Bodet et al (2008) [12] carried out a study to investigate the response of licorice on periopathogen-induced inflammatory response. In their study, macrophages derived from monocytes were treated with varying concentrations of the licorice extract before being subjected to stimulation with lipopolysaccharide (LPS) of *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*) and *Porphyromonas gingivalis*. A laboratory model of whole blood which was stimulated with *P. gingivalis* lipopolysaccharide also demonstrated that licorice extract had the ability to moderate the inflammatory response. The authors observed that licorice extract inhibited the periopathogen LPS-induced IL-6, IL-8, IL-1β and TNF-α response of macrophages that were pretreated with *P. gingivalis* and *A. actinomycetemcomitans* lipopolysaccharide, displaying significant anti-inflammatory activity.

MATERIALS AND METHODS

The present in vitro project was undertaken to assess and compare the antimicrobial activity of the licorice gel and chlorhexidine gel on *Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia*.

This study was conducted in KLE's Dr.Prabhakar Kore Basic Science Research Centre, Belagavi.

METHOD OF COLLECTION OF DATA:

Methodology of extract preparation:

The entire root of the *G. glabra* plant were 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 300g 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 300mg 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 BHI broth. Standard bacterial colonies of the same morphological type of *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Tannerella 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 hours 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 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 Fildes' anaerobic Jar and Resazurin reagent was added after 48 hours and observed after 4 hours for probable colour change. Any colour change from blue/violet to slight pink/pink/magenta was recorded as MIC of emulsion. The results were recorded by taking quality photographs.

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

Sl No.	Ingredients	Quantity	Uses
1.	Licorice extract	300 mg	Antibacterial agent
2.	Carbopol 934	1 gm	Gel forming agent
3.	Triethanolamine	1 drop	Neutralizing agent (adjusts pH to 7)
4.	Potassium sorbate	10 mg	Preservative
5.	Propyl paraben	0.5 ml	Bactericidal agent

Gel preparation

1 gm carbopol was dispersed in 50ml 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, 0.5ml of propyl paraben was added and 5ml of this preparation was further diluted to 1%. Then to this solution, 300 mg of licorice extract was added to get 60mg/ml concentration of drug. In another beaker, 10 mg potassium sorbate was mixed with 5 ml of hot water for 30 minutes using a magnetic stirrer. To this mixture, 5 ml of the carbopol-extract mix was added and the mixture was stirred for 5-10 minutes 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 *Glycyrrhiza glabra* in the prepared gel was 30mg/ml.

Agar well diffusion assay

The agar well diffusion assay was performed on bacteriological agar plates prepared. The Brain Heart Infusion 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 *Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans* and *Tannerella 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 minutes at room temperature. Then, using a cork borer of 8mm size, wells were made in the agar plates and sample reagents [100 µl chlorhexidine, 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 [Dessicator jar] for 24-72 hours of incubation.

The plates were observed for growth pattern and results were noted against Chlorhexidine as standard.

Fig 1. Whole dried Licorice Roots

Fig 2. Powdered licorice roots undergoing maceration

Fig 3. Crude extract filtration using Whatman No 1 filter paper

Fig 4: Evaporation of crude extract using rotary evaporater

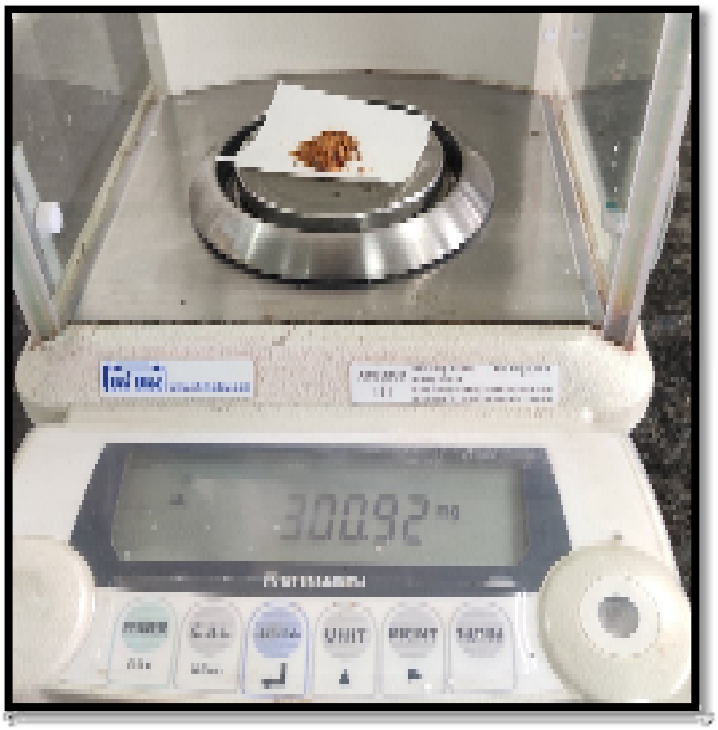

Fig 5. 300mg of licorice crude extract used to prepare stock solution

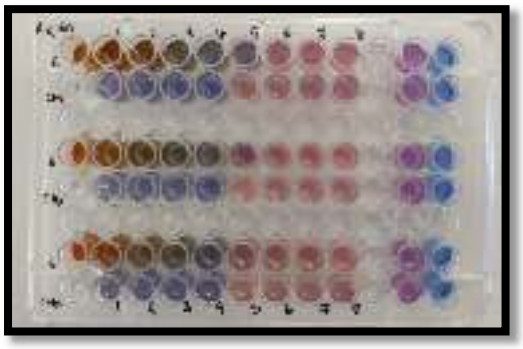

Fig 6. Broth dilution method with resazurin test showing MIC of *Aggregatibacter actinomycetamcomitans.*

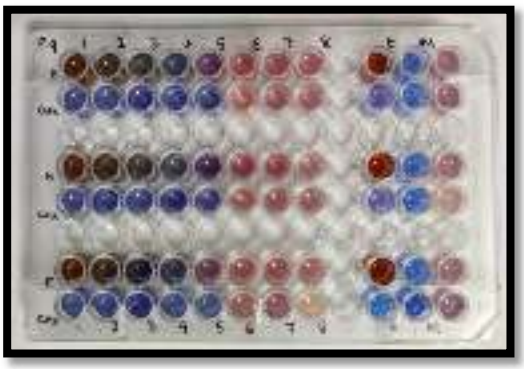

Fig 7. Broth dilution method with resazurin test showing MIC of *Porphyromonas gingivalis*

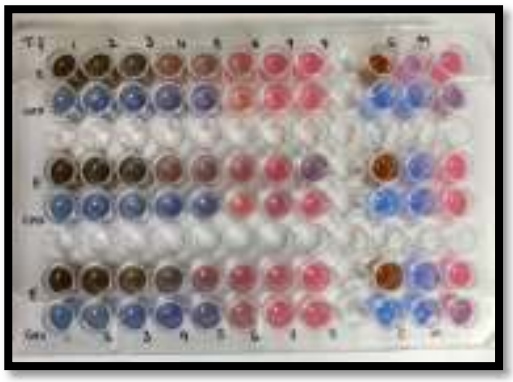

Fig 8. Broth dilution method with resazurin test showing MIC of *Tannerella forsythia.*

Fig 9. Prepared licorice gel

Fig 10. Agar well diffusion test for prepared licorice gel and commercially available chlorhexidine gel showing MBC of *Aggregatibacter actinomycetamcomitans.*

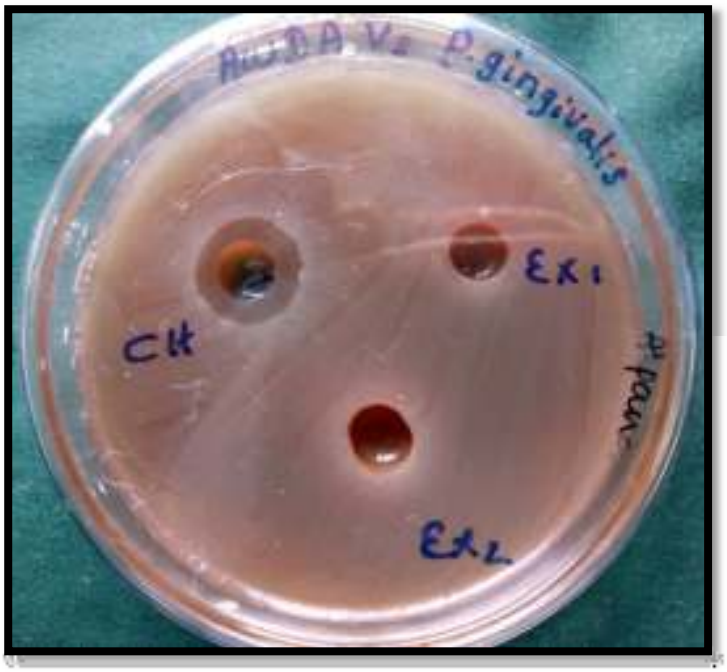

Fig 11. Agar well diffusion test for prepared licorice gel and commercially available chlorhexidine gel showing MBC of *Porphyromonas gingivalis*

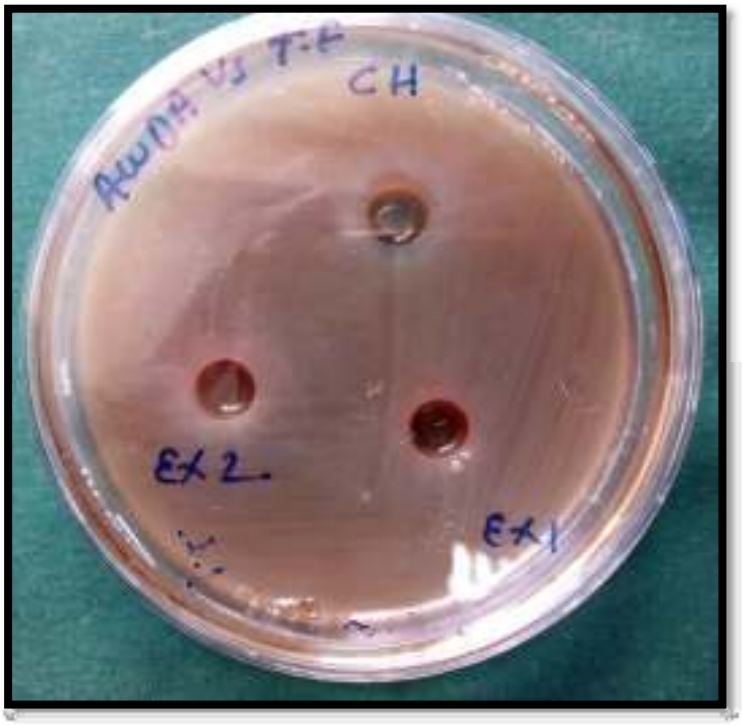

Fig 12. Agar well diffusion test for prepared licorice gel and commercially available chlorhexidine gel showing MBC of *Tannerella forsythia.*

RESULTS AND OBSERVATIONS

Antibacterial susceptibility tests

The antibacterial effects of *Glycyrrhiza glabra L*. extract, were evaluated using broth dilution assay (Resazurin) for its minimum inhibitory concentration (MIC) (Table 1) and agar plate assay for minimum bactericidal concentration (MBC) (Table 2) were conducted against three anaerobic bacteria *Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia*.

The control group for all the tests was Chlorhexidine extract/gel, a known gold standard antimicrobial agent used to treat periodontal infections. The test group for the MIC and MBC test was Glycyrrhiza 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.

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

Sr. No.	**Samples**	**Minimum inhibitory concentration (MIC) in milligram (mg)**					
		A.comitans	**Average**	***P.gingivalis***	**Average**	***T. forsythia***	**Average**
1	*Glycyrrhiza glabra L.* (extract)	15	15 mg	7.5	7.5 mg	15	15 mg
2		15		7.5		15	
3		15		7.5		15	

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

Sr. No.	Samples	Minimum bactericidal concentration (MBC) in milligram (mg)					
		A.comitans	**Average**	***P.gingivalis***	**Average**	***T. forsythia***	**Average**
1	*Glycyrrhiza glabra L.*	30	30mg	30	30mg	30	30mg
2		30		30		30	
3		30		30		30	

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.

Table 3. Agar well diffusion assay of licorice gel and Chlorhexidine gel against A.comitans, P.gingivalis, T.forsythia

Samples	*A. comitans*	*P. gingivalis*	*T. forsythia*
Chlorhexidine	11mm	14mm	10mm
Extract (50 µl)	10mm	10 mm	10 mm
Extract (100 µl)	12mm	11mm	11mm

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

Table 4. Inter- and intragroup comparison of Chlorhexidine group and licorice extract groups

	t-test	p-value
Chlorhexidine v/v Extract 50 µl	1.4	0.3
Chlorhexidine v/s Extract 100 µl	0.3	0.826
Extract 50 µl v/s Extract 100 µl	4.0	0.008*

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 multi-factorial poly-microbial 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. Most common causative pathogens in the disease causing plaque are Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans, Tannerella forsythia.[13]

Mulethi (*Glycyrrhiza 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. The term *Glycyrrhiza* is derived from the ancient Greek words; glycos meaning sweet and rhiza meaning root. The root of *G.glabra* is commonly called Licorice (England) or Liquorice (North America). Anethole is the aromatic and unsaturated ether compound, additionally found in other varieties of herbal plants, ("trans"-1-methoxy-4-propenylbenzene) that contributes to the sweet flavor of licorice. [6] Licorice is also sweetened by glycyrrhizic acid (an antiviral compound that is significantly sweeter than sugar). Licorice is composed of more than 20 triterpenoids and nearly 300 flavanoids. 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 *Glycyrrhiza 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. [14]

Almaz et al (2016) studied the effectiveness of an herbal preparation of a lollipop containing extract of licorice root on salivary *Streptococcus mutans* in children who were caries-free and at a high risk for caries development. The results of the study showed a significant decrease in *S. mutans* counts in high-risk children. [15] This result was similar to a study conducted by Peters et al (2010) where it was found that participants of the study who ingested licorice lollipops twice a day for 3 weeks showed a marked decline in the number of S. mutans. These numbers remained lessened for 22 days after the last lollipop was consumed and then rose again.[16] Several in-vitro studies that have been conducted to ascertain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration of alcoholic extracts of Licorice against *Streptococcus mutans, Actinomyces viscosus, Enterococcus faecalis, Lactobacillus acidophilus*. It has been confirmed that Licorice extract shows positive inhibitory activity against these bacteria. [14,17,18] To our knowledge, this is the first in-vitro study determining the MIC and MBC of hydroalcoholic extract of Licorice against perio-pathogens *Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans, Tannerella forsythia*.

Ajagannanavar et al (2014) conducted an in-vitro study to assess the efficacy of aqueous and alcoholic forms of extract of licorice root against *S. mutans* and *L.*

acidophilus in contrast with Chlorhexidine. They were able to clearly show that alcoholic form of licorice root extract inhibited the growth of *S. mutans* and *L. acidophilus* better when compared to the aqueous form of licorice extract and Chlorhexidine. [5] Wittschier et al. (2006) observed that polysaccharides derived from licorice roots reduced ability of bacteria to bind to host cells after being pre-treated with *P. gingivalis*. The first step of periodontal infection is the adhesion of periopathogens to the host cell. This study found that the polysaccharides of *G. glabra* are potent agents that can prevent bacterial adhesion to host cells. Therefore, they could potentially serve as prophylactic tools as a part of alternate treatment regimens against periodontal infection. [18]

In our study, a hydroalcoholic extract of *Glycyrrhiza glabra* (Licorice) was prepared which was then evaluated for its antibacterial activity. MIC of licorice extract (determined using broth dilution assay) against *Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia* was found to be 15 mg while for *Porphyromonas 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 *Glycyrrhiza glabra* show anti-bacterial 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 *Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia* in comparison to the control group (Chlorhexidine) (Table 3) but the results were not significant statistically (Table 4). For *Porphyromonas 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 prepared Glycyrrhiza glabra extract gel showed better antibacterial effect in great quantities. Hence, Glycyrrhiza glabra can be a suitable antibacterial alternative to Chlorhexidine to treat periodontal disease.

Shivaprasad DM et al (2017) compared scaling and root planing 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 local drug delivery agent for treatment of periodontal pockets. (8)

SUMMARY AND CONCLUSION

The aim of the study was to assess and compare the antimicrobial activity of licorice gel and chlorhexidine gel on *Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans* and *Tannerella forsythia*. The present study supports the hypothesis that Glycyrrhiza glabra root extract is a useful antibacterial agent against oral pathogens to fight periodontal disease. The findings of the current study conclude that Glycyrrhiza glabra can discourage the growth of *Porphyromonas gingivalis, Aggregatibacter actinomycetamcomitans,* and *Tannerella forsythia*. The plant extracts exhibit potential to be used as a local drug delivery agent.

Further research is needed to develop and formulate a licorice extract local drug delivery 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.

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ANNEXURE – I – ETHICAL CLEARANCE LETTER

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362 Web: http://www.kledental-bgm.edu.in
FAX: 0831-2470640 E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1310

CERTIFICATE

This is to Certify that the synopsis titled

TO ASSESS AND COMPARE THE ANTIMICROBIAL ACTIVITY OF LICORICE GEL AND CHLORHEXIDINE GEL AGAINST PORPHYROMONAS GINGIVALIS, AGGREGATIBACTER ACTINOMYCETACOMITANS & TANERELLA FORSYTHIA - IN VITRO.

Submitted by Dr. ALPANA ANDREWS *P. G. Student / Staff, Guided by* DR. NEELAMMA SHETTI *from Department of* PERIODONTICS *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date :

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Research & Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI.

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLE VK Institute of Dental Sciences

ANNEXURE – II – DRUG AUTHENICATION CERTIFICATE

SHRI B.M.K. AYURVEDA MAHAVIDYALAYA
A constituent unit KLE Academy of Higher Education & Research
Deemed-to-be-University
Central Research Facility
DRUG AUTHENTICATION REPORT

Submitted By: Dr. Alpana Andrews
Submitted Date : 17/7/19

Date of Issue: 17/07/2019

Sl. No	Sample Name	Scientific Name	Family	Part submitted	CRF Code	Authenticated as				Remarks
						Ayurvedic Name	Scientific Name	Family	Part Authenticated	
1.	Yashtimadhu	*Glycyrrhiza glabra* L.	Fabaceae	*Stolon*	CRF/Auth /2019/102	Yashtimadhu	*Glycyrrhiza glabra* L.	Fabaceae	*Stolon*	

Signature:
Authentication Expert Name: Mr. Ajit Lingayat
Date:17/07/2019

CRF

Signature of Coordinator
ASU Drug Testing Laboratory

ANNEXURE – III – MIC RESULTS

Dr. Prabhakar Kore Basic Science Research Centre, KLE Academy of Higher Education and Research

Report

Name of Student: Dr. Alpana Andrews

Name of Guide: Dr. Neelamma Shetti

Strains tested: *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, tenerella forsythia.*

Objectives:

To evaluate the Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *Glycyrrhiza glabra* for *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *tenerella forsythia.*

Results: MIC and MBC of extract, *Glycyrrhiza glabra L.*

Experimental methodology

Antibacterial susceptibility tests

The antibacterial effects of *Glycyrrhiza glabra L.* extract, were assessed using broth dilution assay (Resazurin) for minimum inhibitory concentration (MIC) and agar plate assay for minimum bactericidal concentration (MBC) were conducted against each bacteria.

Media: Brain Heart Infusion (BHI) agar and Muller Hinton Agar and broth (MHA and MHB)

Test organisms: Three bacteria were selected for the study i.e. *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Tenerella forsythia.*

Inoculum preparation: Inoculum preparation was carried out in BHI broth. The standard colonies of the same morphological type were selected from an agar culture plate further, each colony was scooped with a sterile loop, and the grown bacteria were transferred into a tube containing 4–5 mL of BHI broth. The broth culture was incubated at 37°C for 8–14 h until it achieved the turbidity of the 0.5 McFarland standards. The turbidity of actively growing bacterial culture was adjusted with broth to obtain a final turbidity of 0.5 McFarland standards.

Broth dilution method [Resazurin]. 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 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 Fildes' anaerobic Jar and resazurin reagent was added after 48 hours and observed after 4 hours for possible colour change. Any colour change from blue/violet to slight pink/pink/magenta was recorded as MIC of emulsion. The results were recorded by taking quality photographs.

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

Results:

MIC of *Glycyrrhiza glabra L.* extract

Determination of MIC of *Glycyrrhiza glabra L.* extract

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

Sr.No.	Samples	Minimum inhibitory concentration (MIC) in mg					
		A.a. comitans	*Average*	*P.gingivalis*	*Average*	*T. forsythia*	*Average*
1	extract	15		7.5		15	
2	*Glycyrrhiza*	15	15 mg	7.5	7.5 mg	15	15 mg
3	*glabra L.*	15		7.5		15	

Table. 2. Minimum bactericidal concentration of *Glycyrrhiza glabra* emulsion

Sr.No.	Samples	Minimum bactericidal concentration (MBC) in milligram					
		A.a. comitans	*Average*	*P.gingivalis*	*Average*	*T. forsythia*	*Average*
1	*Glycyrrhiza*	30		30		30	
2	*glabra L.*	30	30mg	30	30mg	30	30
3		30		30		30	

Dr. Suneel Dodamani
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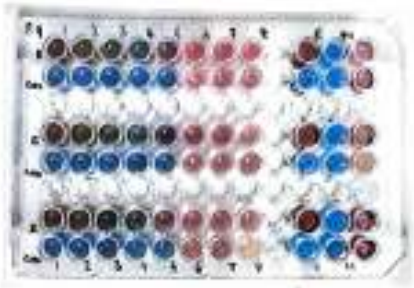

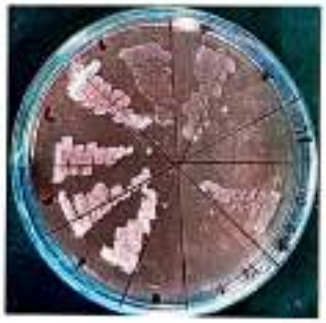

ANNEXURE – IV – MBC RESULTS

Dr. Prabhakar Kore Basic Science Research Centre, KLE Academy of Higher Education and Research

Report

Name of Student: Dr. Alpana Andrews

Name of Guide: Dr. Neelamma Shetti

Strains tested: *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia.*

Objectives: To evaluate agar well diffusion assay of *Glycyrrhiza glabra* gel and chlorhexidine gel for *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Tannerella forsythia.*

Results: Agar well diffusion assay of *Glycyrrhiza glabra* gel and chlorhexidine gel for *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Tannerella forsythia.*

Experimental Methodology :

Agar well diffusion assay

The agar well diffusion assay was performed on bacteriological agar plates prepared. The Brain heart infusion agar with blood [sterilized] was prepared. and kept at room temperature for 10-15 min before solidifying. The bacterial broth cultures of A.a, P.g and T.f were taken [0.5 McFarland's] and spreaded on agar plates [100 µl] with sterile cotton spreader, and kept at room temperature for 15min. After this step, wells were made with cork borer of 8mm size and sample reagents [100 µl chlorhexidine, extract and 50 µl extracts] were added into the respective wells and kept for diffusion and afterword's the plates were placed in 37°C, CO_2 incubator [Jar of desiccators] for 24-72 hours of incubation and observed for growth pattern and results were noted against chlorhexidine as standard.

Agar well Diffusion Assay

Sample	*A.comitans*	*P.gingivalis*	*T. forsthia*
Chlorhexidine	11mm	14mm	10mm
Extract 50 µl	10mm	10 mm	10 mm
Extract 100 µl	12mm	11mm	11mm

Interpretation of Results

The plant extract was taken in lower and higher concentration and tested against three anaerobic bacteria *A A commitans, P.Gingivalis, T. forsythia*. The plant extract showed inhibitory activity against *A A commitans at* 50 µl 10mm and at 100 µl 12 mm, showing stronger inhibitory activity as compared to standard Chlorhexidine (11mm). The plant extract showed inhibitory activity against *p.gingivalis at* 50 µl 10mm and at 100 µl 11 mm, showing stronger inhibitory activity as compared to standard Chlorhexidine (14mm). The plant extract showed inhibitory activity against *T. forsthia at* 50 µl 10mm and at 100 µl 11 mm, showing stronger inhibitory activity as compared to standard Chlorhexidine (10mm).

A. comitans

T. forsythia

P. gingivalis

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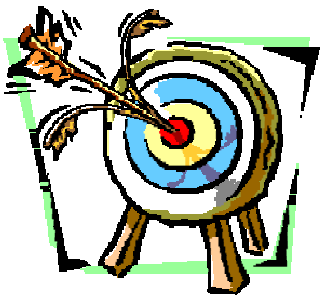
Introduction



Objectives

Review of Literature

Methodology

Results

Discussion

Conclusion & Summary

Bibliography

Annexures