Antiadherence and antimicrobial property of herbal extracts (*Glycyrrhiza glabra* and *Terminalia chebula*) on *Streptococcus mutans*: An in vitro experimental study

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Abstract

**Background:** Herbal agents are used for treating different forms of diseases since decades. In the current study, the antiadhesive property of herbal extracts has been evaluated using *Glycyrrhiza glabra* (GG) and *Terminalia chebula* (TC) herbal extracts on *Streptococcus mutans*.

**Materials and Methods:** The plant extracts (GG and TC) were powdered in mechanical grinder. Ten gram of each plant extract in powder form was placed in porous bag or thimble. The extract was placed in a round-bottom flask and was transferred into clean preweighed universal tubes. The yield strength of the extract was calculated.

The antiadherence property of the herbal extract was evaluated using glass surface adherence test.

**Statistical Analysis:** The statistical analysis was done using one-way analysis of variance followed by *post hoc* Tukey’s test.

**Results:** Both herbal extracts have significant antiadhesive and antimicrobial activity against *S. mutans*, however, high antiadherence property was seen with TC than GG.

**Conclusion:** Both the plant extracts exhibit inhibitory activity against *S. mutans*. However, TC had more clinically significant results than GG, but it was found statistically insignificant.

**Keywords:** Antiadherence activity, antimicrobial activity, *Glycyrrhiza glabra*, herbal agent, *Terminalia chebula*

INTRODUCTION

Dental caries is the most common oral disease that results in demineralization of tooth, pain and discomfort. The dental caries process starts with microbial adherence on tooth surfaces, after which dental plaque is formed which leads to localized demineralization of tooth enamel by acids of bacterial origin produced from the fermentation of dietary carbohydrates.[1] There are numerous bacteria which cause initiation and progression of dental caries; however, *Streptococcus mutans* and *Lactobacillus* species are considered as the principal microorganism for causing dental caries.[2] There are numerous agents which are used...
for the treatment and prevention of dental caries, of which herbal agents are considered as one of the options.

Herbal agents are used since ancient times for the treatment of various diseases. These herbal agents are used in the form of there extracts and essential oils, which have numerous biological properties that are confirmed by in vitro and in vivo studies. It has antibacterial, antiseptic, antifungal, antiviral, antiprotozoal and anthelmintic property. It was found to have anti-inflammatory, analgesic, anti-allergic, antitumor, antioxidative, anticonvulsant, antidepressant, contraceptive, antimutagenic and diuretic properties.[3,4] Considering these properties, herbal agents have been used in the field of conservative dentistry and endodontics.[5]

*Glycyrrhiza glabra* (GG) also known as licorice or sweet root or mulethi. It belongs to the pea and bean family. It is extensively used in foods and is also used as traditional and herbal medicine. It is very sweet, moist and soothing herb. It is commonly used as a flavoring agent in Kampo medicines.[6] It is stated that the roots of GG contain high concentration of triterpene saponin glycyrrhizin (about 2.5%–9%). He compound glycyrrhizin is an diglucuronide of glycyrrhetinic acid, which is responsible for the positive properties,[1] and has anti-inflammatory (because of glycyrrhetinic acid), antiviral and anticarcinogenic properties.[6]

*Terminalia chebula* (TC) is an herbal plant having medicinal values and is known as Kadukka in Tamil Nadu, India. It contains hydrolysable tannins constituting gallic acid, chebulic acid, chebulagic acid and corilagin in a concentration of 13%. Because of these acids, it can be used for the treatment of oral diseases such as gingivitis and stomatitis and was found to have antibacterial activity.[7]

Considering the above properties, this experimental in vitro research has been undertaken to evaluate the effect of herbal extracts (GG and TC) against commonly found organism causing dental caries, i.e., *S. mutans*.

**MATERIALS AND METHODS**

**Preparation of extract**
The roots of GG were dried and powdered using mechanical grinder. Similarly, the dried ripe fruits of TC were taken and powdered using mechanical grinder. Soxhlet apparatus was used to prepare the extract. This apparatus has three sections, namely a percolator which circulates the solvent; a thimble made of thick filter paper, which retains the solid to be extracted and a siphon mechanism that empties the thimble. Ten gram of each plant extract in powder form was placed in thimble. It was then fitted with appropriate size round-bottom flask with 100 ml water, and the upper part was fitted with condenser. The solvent was recycled by providing constant heat (50º–60ºC) using Mantox heater. After complete extraction, the extract in round-bottom flask was transferred into clean and preweighed universal tubes.

**Percentage of herbal extract**
The percentage yield was calculated using the formula given below.

\[
\text{Percentage yield} = \frac{\text{N g of extract}}{10 \text{ g of extract}} \times 100
\]

Where N indicates the final weight of extract.

Using the above formula, the yield obtained was about 30% and 36% for GG and TC, respectively.

**Antiadhesive property**
This property was determined by glass surface adherence test as described by Hamada *et al.*[8] The pure strains of *S. mutans* (ATCC 890) were obtained from Microbial Type Culture Collection, Chandigarh. These bacteria were grown for 24 h at 37ºC at an angle of 30º in a glass tube with 10 ml of BHI broth (HiMedia Laboratories Pvt. Ltd., Mumbai, Maharashtra, India). The readings were obtained from spectrophotometer. The total free and adherent bacterial cells were determined.

**Percentage of bacterial adherence**

\[
\text{Percentage adherence} = \frac{\text{Optical Density (OD) of adherent cell}}{\text{OD of supernatant cells + OD of adherent cells}} \times 100
\]

**Antimicrobial activity**
It was determined by agar well diffusion method. Whole experiment was repeated six times for each isolate, and the mean zone of inhibition was then calculated.

**RESULTS**

**Antiadherence property**
Antiadherence property was seen with both the herbal agents (GG and TC). However, TC showed higher antiadherence property of 59% at a concentration of 01:16, and lower percentage (37.46%) was seen at a...
concentration of 01:01. Similarly, higher antiadherence property of 49.90% at a concentration of 01:16 and lower percentage (40.00%) was seen at a concentration of 01:01 with GG. Hence, when compared in percentage, TC showed higher antiadherence property than GG [Graph 1].

Antimicrobial activity
The mean zone of inhibition was found to be maximum at a concentration of 01:02 (22.73) for GG; however, no zone of inhibition was seen at concentration of 01:16 (0.00). Similarly, for TC, maximum zone of inhibition was seen at a concentration of 01:01 (26.47) and minimum at a concentration of 01:16 (13.60) [Table 1].

When GG and TC were compared using unpaired t-test, significantly higher inhibitory effect (zone of inhibition) of TC extract on S. mutans than GG extract was seen at a concentration of 01:01, 01:04, 01:08 and 01:16, respectively. At 01:02 concentration, inhibitory effect of GG was significantly higher than TC extract [Table 2].

One-way analysis of variance showed a significant difference in inhibitory effect (zone of inhibition) of GG and TC extract on S. mutans at different concentrations [Table 3].

For GG, when post hoc Tukey’s test was applied for pairwise comparison, it showed that at 01:01 and 01:02 concentrations, inhibitory effect was significantly higher than other concentrations, whereas for TC, 01:01 concentration showed highest inhibitory effect. However, minimum zone of inhibition was seen at 01:16 concentration for both GG and TC. Further, inhibitory effect was not significant between 01:01 and 01:02 concentrations and between 01:02 and 01:04 concentration for GG and TC, respectively [Table 4].

DISCUSSION
Dental caries is considered to be one of the major oral health problems and has resulted in increased use of mouthwash. To overcome the side effects of major component of mouthwashes i.e. antisepsics such as chlorhexidine, various studies have investigated

Graph 1: *Streptococcus mutans* adherence

Table 1: Descriptive statistics calculating the mean and standard deviation of inhibitory effect (zone of inhibition in mm) of Glycyrrhiza glabra and Terminalia chebula extract on Streptococcus mutans at different concentrations

| Extracts | Concentrations | Mean | SE  | SD  |
|----------|----------------|------|-----|-----|
| GG       | 01:01          | 22.63| 0.25| 0.00|
| TC       | 01:01          | 26.47| 0.35| 0.21|

GG: Glycyrrhiza glabra, TC: Terminalia chebula, SD: Standard deviation

Table 2: Comparison of inhibitory effect (zone of inhibition in mm) of Glycyrrhiza glabra and Terminalia chebula extract on Streptococcus mutans at different concentrations (using unpaired t-test)

| Concentrations | Mean difference | SE  | P     | Mean difference | SE  | P     |
|----------------|-----------------|-----|-------|-----------------|-----|-------|
| 01:01          | −0.100          | 0.305| >0.05 | 4.800          | 0.281| <0.001|
| 01:01          | 3.033           | 0.350| <0.001| 5.700          | 0.281| <0.001|
| 01:08          | 7.100           | 0.350| <0.001| 7.800          | 0.281| <0.001|
| 01:16          | 22.633          | 0.350| <0.001| 12.867         | 0.281| <0.001|

* DF: Degree of freedom, *P<0.05 is considered to be significant

Table 3: Comparison of inhibitory effect of Glycyrrhiza glabra and Terminalia chebula extract at different concentrations using one-way analysis of variance

| Name of extracts | Sum of squares | Df | Mean square | F     | P     |
|------------------|----------------|----|-------------|-------|-------|
| GG               | 1075.400       | 4  | 268.500     | 1920.357| <0.001*|
| TC               | 262.947        | 4  | 65.737      | 553.961| <0.001*|

* *P<0.05 is considered to be significant. GG: Glycyrrhiza glabra, TC: Terminalia chebula

Table 4: Tukey’s post hoc test for pairwise comparison of inhibitory effect of Glycyrrhiza glabra and Terminalia chebula extract on Streptococcus mutans at different concentrations

| Groups | Mean difference | SE  | P     | Mean difference | SE  | P     |
|--------|-----------------|-----|-------|-----------------|-----|-------|
| 01:01 and 01:02 | −0.100          | 0.305| >0.05 | 4.800          | 0.281| <0.001|
| 01:01 and 01:04 | 3.033           | 0.350| <0.001| 5.700          | 0.281| <0.001|
| 01:01 and 01:08 | 7.100           | 0.350| <0.001| 7.800          | 0.281| <0.001|
| 01:08 and 01:16 | 22.633          | 0.350| <0.001| 12.867         | 0.281| <0.001|

* SE: Standard error, GG: Glycyrrhiza glabra, TC: Terminalia chebula

Discussion
Dental caries is considered to be one of the major oral health problems and has resulted in increased use of mouthwash. To overcome the side effects of major component of mouthwashes i.e. antisepsics such as chlorhexidine, various studies have investigated
Root of licorice plant has several useful pharmacological properties such as anti-inflammatory, antiviral, antimicrobial and anticancer activities in addition to immunomodulatory, hepatoprotective and cardioprotective effects. Sedighinia et al. stated that GG can be used for the treatment of various oral diseases (periodontal, endodontic and dental caries infection). In the current study, minimal inhibitory concentration (MIC) of GG was 15% at 1:2 dilution, which was in consonance with other study. Wittscher et al. found that licorice has strong antiadhesive effects against Porphyromonas gingivalis, prevents production of matrix metalloproteinase by host cells and is as useful as antibiotic like doxycycline. Ahn et al., in their study, found that when glucose or sucrose concentration of 4 µg/ml and 16 µg/ml, respectively, was used, then the antibacterial activity (S. mutans) was strongest with licorice root extract. The results of the present study were in accordance with other study where they found that licorice extracts inhibit the growth of cariogenic pathogens and have good antibacterial activity.

Parts of TC have been used for the prevention and cure of diseases. Tannic acid, gallic acid and chebulic acid are the major constituents of the ripe fruit of TC. The amount of tannic acid in the aqueous extract of TC is 13%. Tannic acid is bacteriostatic or bactericidal to some Gram-positive and Gram-negative pathogens. The extract of TC prevents plaque formation on the surface of tooth by inhibiting sucrose-induced adherence and glucan-induced aggregation, two processes which foster the colonization of S. mutans on the surface of tooth. Tannic acid can be well adsorbed to the hydroxyapatite of the tooth or to the salivary mucus; alternatively, it can bind to the anionic groups on the surface of bacterial cells, which resulted in protein denaturation and ultimately bacterial cell death.

In this study, both the extracts inhibited the adherence of bacteria on smooth glass surface. It was also found that as the concentration of extract increased, the adherence of bacteria on glass surface decreased. The antiadherence property of both the extracts can be attributed to the presence of phytochemicals in plants such as alkaloids, tannins, essential oils and flavonoids. It also exhibits anticariogenic property through various modes of action, involving bactericidal effects on oral bacteria, prevention of adherence of bacteria to the tooth surfaces, inhibition of glucan production and inhibition of amylases. Glabridine, one of the important substances in GG, is active against Gram-positive strains then Gram-negative strains. In addition, the presence of glycyrrhizin, glycyrrhetinic acid, flavonoids, asparagine, isoflavonoids and chalcones in GG further improves its antiadhesive and antimicrobial properties.

According to the NCCLS 2000 standards, the antimicrobial efficacy of any agent can be evaluated by broth dilution method, agar dilution method, disc diffusion method, agar well diffusion method and ditch plate method. The present study employed agar well diffusion method because of its reliability and acceptability. The ethanolic extracts were used because the antimicrobial efficacy can be enhanced if the phytoconstituents of these plant extracts are purified using different solvents such as ethanol, methanol and acetone. In the present study, MIC of TC came about 9% at 1:4 dilution which correlated with a study by Carounanidy et al. in which 10% aqueous extracts of TC were used. In an in vivo study by Jagtap and Karkera, found that TC extract successfully inhibits glycolysis of salivary bacteria for up to 90 min after rinsing.

S. mutans has the ability to adhere to a smooth surface, providing suitable conditions for it and for other bacteria to aggregate at the tooth surface. The present results showed that both the extracts strongly inhibited the adherence ability of S. mutans on a smooth glass surface. However, few studies are documented on the adherence inhibition of S. mutans by GG. The mechanism of adherence inhibition should be studied further. Hence, this plant should be studied further for their active compounds and further developed into products for the prevention of dental caries.

This study showed promising results of both the extracts against S. mutans. However, the evaluation of these extracts on secondary and tertiary plaque colonizers should also be evaluated such that it inhibits both caries and plaque microorganisms.

CONCLUSION

The development of new therapies for the treatment of oral diseases is of great importance. From this study, we found that both GG and TC showed significant inhibitory
activity against S. mutans at different concentrations. Thus, from this in vitro study, we can propose that both GG and TC can help in the reduction of dental caries. However, further studies can be done by incorporation of GG and TC in mouth rinse and their effect on S. mutans and other caries causing microorganisms.

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Conflicts of interest
There are no conflicts of interest.

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