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Antimicrobial activity of Glycyrrhiza glabra against *Streptococcus mutans, Lactobacillus acidophilus* and *Candida albicans*-An invitro study

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Abstract

Herbal products have been found beneficial for the prevention of dental caries. This study aims to compare the antimicrobial activity, of Glycyrrhiza glabra against *Streptococcus mutans, Lactobacillus acidophilus* and *Candida albicans* against oral pathogens. The study sample was prepared by collecting Glycyrrhiza glabra roots and dried under shade for a week, then they were grounded and stored for further analysis. Using Soxhlet apparatus twenty five (25) grams of powdered sample were extracted with 500ml of ethanol solvent for ten hours .the extract were condensed using rotary evaporator in their respective solvents to obtain a stock of 100mg/ml and were stored in are refrigerator and used for antimicrobial analysis against *Lactobacillus acidophilus, Streptococcus mutans , Candida albicans,* Amphotericin was used as standard for fungal strains, Ampicillin was used as standard for bacterial strains. The extract showed significant activity against all the organisms. Among them *Streptococcus mutans* exhibited high antibacterial activity with the inhibition zone in range of 12mm to 14mm the zone of inhibition observed for *Lactobacillus acidophilus* was 8mm to 12 m and for *Candida albicans* it was found to be 10mm to 12mm**.** In this study it is showed that ethanol extract has significant activity against all the tested oral pathogens. Hence it can be used as a mouthwash replacing chemical plaque control measures which has side effect.

Keywords: *Streptococcus mutans*; Mouthwashes; *Lactobacillus acidophilus; Candida albicans*; Dental Caries; Dental Caries Susceptibility

Full-length article **Corresponding Author*, e-mail: *pcnanu@gmail.com*

1. Introduction

The oral cavity cultivates numerous microorganisms which comprise of bacteria, viruses, fungi, and protozoa. The oral cavity is a complex of hard and soft tissues which favors the colonization of microbes. Dental caries is a global pandemic multifactorial disease. Oral microorganisms like *Streptococcus mutans, Lactobacillus acidophilus*, etc play a major causative role in occlusal and root dental caries [1,2]. Oral candidiasis is a commonly occurring fungal infection caused by *Candida albicans* [3]. Plaque control measures, even if followed properly, are usually insignificant in removing plaque causing microorganisms. Chemical plaque control measures are found to have a significant effect, but still, it has its own side effects in the long run. Hence, herbal products have gained importance in dental markets today.

Glycyrrhiza glabra commonly known as Adhimathuram in Tamil is most used in treating intestinal diseases, sore throat, tonsillitis, flatulence, fever, skin diseases etc., because of its rich bioactive compounds [4-6]. This study was undertaken to check the effect of Glycyrrhiza glabra against *Streptococcus mutans, Lactobacillus acidophilus* and *Candida albicans*.

2. Materials and Methods

The Medicinal plant sample Glycyrrhiza glabra roots were purchased from a local market in Chennai, Tamil Nadu. The samples were collected in sterile containers and transported to the laboratory. Adherent particles were removed if any and dried under shade for a week of time. After drying, the samples were grounded and stored for further analysis. Using Soxhlet apparatus, 25 grams of powdered samples were extracted with 500 ml of ethanol solvent using soxhlet apparatus for ten hours of time. The extracts were condensed using a rotary evaporator. After condensation, the samples were reconstituted in their respective solvents to obtain a stock of 100 mg/ml and were stored in a refrigerator. The extract was then used for the antimicrobial activity assay.

2.1. Antimicrobial Activity Assay

The test organisms were obtained from the Lifeteck Research centre, Chennai, Tamil Nadu and inoculated into a fresh broth and incubated at 37ºC. Young cultures (8 hrs of incubation) were taken for antimicrobial study.

Antibacterial activity of the extracts was determined by disc diffusion method using Muller Hinton agar (MHA) plates [7,8,9]. The test organisms were swabbed on the plates. Samples were diluted for 3 different concentrations viz., 1000

µg/ml, 750 µg/ml and 500 µg/ml. Ampicillin was used as the standard for bacterial strains and Amphotericin was used as the standard for fungal strain. 20 µl of diluted samples and positive control were added in sterile discs and placed on MHA plates. The plates were incubated for 24 hours at 37ºC. Diameter of inhibition zone was measured to determine the antibacterial activity.

Table 1: Antimicrobial activity of Glycyrrhiza glabra against oral microorganisms

Organisms	Zone of Inhibition (mm) Concentration(µg/ml)			Antibiotic (1mg/ml)
	1000	750	500	
Streptococcus mutans	14	13	12	17
Candida albicans	11	11	10	18
Lactobacillus acidophilus	12	11	8	18

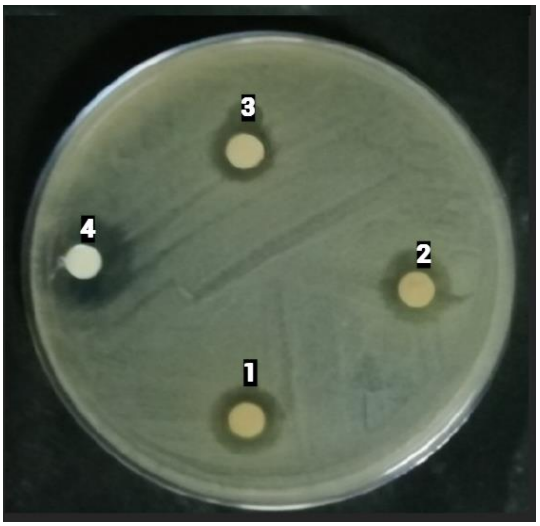

Figure 1: Antibacterial effect of the Glycyrrhiza glabra ethanol extract against *S.mutans*

1= 500µg/mg dilution, 2=750µg/mg dilution, 3=1000µg/mg dilution 4=Antibiotic (1mg/ml)

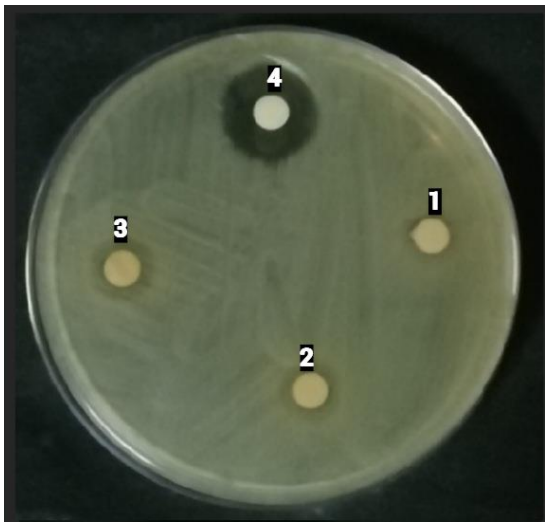

Figure 2: Antibacterial effect of the Glycyrrhiza glabra ethanol extract against *L.acidophilus*

1= 500µg/mg dilution, 2=750µg/mg dilution, 3=1000µg/mg dilution 4=Antibiotic (1mg/ml)

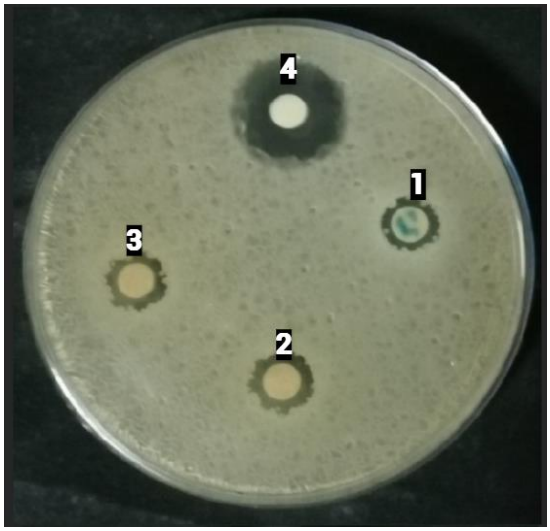

Figure 3: Antibacterial effect of the Glycyrrhiza glabra ethanol extract against *C.albicans*

1= 500µg/mg dilution, 2=750µg/mg dilution, 3=1000µg/mg dilution 4=Antibiotic (1mg/ml)

3. Results and discussions

The antibacterial analysis of the Glycyrrhiza glabra ethanol extract is shown in Table.1 and figures1,2, and 3.The extract showed significant activity against all the organisms. Among the organisms tested *Streptococcus mutans* exhibited high antibacterial activity, with the inhibition zones in the range of 12mm to 14mm. The zone of inhibition observed for *Lactobacillus acidophilus* was 8mm to 12mm, and for *Candida albicans* it was found to be 10mm to 12mm.

The present study showed that Glycyrrhiza glabra has potential antimicrobial effect against oral microorganisms, for use in toothpaste, mouth wash etc., for preventing and treating oral infections. Gauniyal et al., 2014[10] studied the antimicrobial activities of 12 different medicinal plant extracts against five oral pathogens. Glycyrrhiza glabra ethanol extract had an inhibition zone of 17mm against *Candida albicans*. Comparatively, the results obtained in our study were similar to the above study the zone of inhibition was observed to be 11mm, in two concentrations. The roots of Glycyrrhiza glabra have an active constituent called Glabridin, that is found to have high fungal activity [11,12].

A 14 mm of inhibition zone were observed against *S. mutans*, thus suggesting that the ethanolic extract has activity against Streptococcus. In a study done by Sedighinia et al., (2011) [13] the inhibition zone for *Streptococcus mutans* was observed to be 26.5±0.8 and MIC was found to be 12.5 mg/ml, in case of calculated zone the study was found to be in agreement with the results of our study. 18mm zone was observed against *Lactobacillus* in the study conducted by Guniyal et al., (2014) [10] in our study at highest concentration, the zone formed is measured to be 12 mm the extract has significant activity against the bacteria.

Our study showed that Glycyrrhiza glabra has a significant zone of inhibition at crude concentrations against common oral pathogens such as *C.albicans*, *S.mutans* and *L.acidophilus*. Although Ampicillin that was used as control for bacterial strains and Amphotericin was used as the standard for fungal strain, had a higher zone of inhibition than Glycyrrhiza glabra it can be exempted considering the fact that the chemical drugs has its own complications and also, these are not commonly recommended for treating dental diseases.

Chlorhexidine is a commonly recommended oral mouthwash against oral pathogens. Various studies have been conducted to prove the antimicrobial activity of chlorhexidine [14,15]. Failure to use it as positive control is the major drawback of this study. However, chlorhexidine alters the sensation of taste, cause a brownish discoloration of teeth and erodes the oral soft tissues [16]. Hence, introducing mouth washes made of herbal products like Glycyrrhiza glabra will be beneficial for long term use.

4. Conclusions

Dentistry has gained its importance today by inducting modern technologies to clinical practice. New economical treatment modalities with fewer side effects are being developed to treat oral infections at an early stage. The main objective of the study was to find the antimicrobial activity Glycyrrhiza glabra. We showed that the ethanolic extract showed significant activity against all the tested oral pathogens. Future clinical research can be carried out to use this extract in early treatment of dental diseases.

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