Antibacterial Potential of Gall Extract of *Quercus infectoria* against *Enterococcus faecalis*– an *in vitro* Study

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ABSTRACT

Background: Various herbal products are being tried to treat common ailments. Such a trend is witnessed in dentistry also. Gall extract of *Quercus infectoria* has been found to possess antibacterial properties against some common oral pathogens. **Aim:** To assess the antibacterial property and minimum inhibitory concentration (MIC) of gall extract of *Quercus infectoria* against *Enterococcus faecalis*. **Settings and Design:** *In vitro* experimental study-Laboratory setting. **Materials and Methods:** Dried galls of *Quercus infectoria* were crushed into small pieces and powdered in an electric grinder. About 250 g of the powder was packed into a Soxhlet apparatus and extracted using methanol as the solvent. The crude extract was dried and stored in sterile bottle. The antibacterial property of gall exract of *Quercus infectoria* against *Enterococcus faecalis* was assessed by the disc diffusion method. The zone of inhibition was measured using vernier calipers. The minimum inhibitory concentration of gall extract was assessed by a two-fold serial dilution method. Sodium hypochlorite (2%) and Chlorhexidine (2%) were used as positive controls and dimethyl sulphoxide was used as the negative control in the study. **Results:** The zone of inhibition of gall extract against *Enterococcus faecalis* was found to increase with increasing volumes of gall extract. The minimum inhibitory concentration (MIC) of the gall extract against *Enterococcus faecalis* was 16.6 µl/ml. **Conclusion:** Methanolic extract of galls of *Quercus infectoria* was found to possess antibacterial property against *Enterococcus faecalis*.

Key words: disc diffusion method, *Enterococcus faecalis,* methanolic gall extract, *Quercus infectoria,* tannins.

INTRODUCTION

Dental caries is a complex multi-factorial disease of ubiquitous distribution. It is a major public health problem because it has functional, esthetic, and economic consequences on the individual as well as to the community at large. If left untreated at the earlier stages, dental caries encroaches upon the dental pulp and in such cases endodontic therapy might be the only scope to save the tooth. Endodontic therapy is essentially a debridement procedure that aims at eradication of microbes and their byproducts from the root-canal system. Complex root canal anatomy demands the use of antimicrobial agent in the form of endodontic irrigant along with mechanical debridement to adequately prepare the root canal system.[1]

Microorganisms have been implicated in the development and protraction of pulpal and periapical pathoses of dental caries.[2] Bacterial interactions, poor nutrient availability, and low oxygen potential within root canals with necrotic pulp restrict the number of bacterial species present in endodontic infections.[3] These selective conditions favour the predominance of facultative and strictly anaerobic microorganisms. *Enterococcus faecalis* is one such microorganism known to survive and multiply in the hostile conditions prevailing within the root canal.[4] It is implicated in endodontic failure cases and is said to be more resistant to endodontic treatment.

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There is no single endodontic irrigant that alone sufficiently provides all the functions required for an ideal irrigant.[5] Though 5.25% sodium hypochlorite solution and 2% chlorhexidine are commonly used endodontic irrigants, their usage is not without disadvantages. Sodium hypochlorite is cytotoxic and can cause ulceration, allergic reaction, disagreeable odour and taste.[6] Chlorhexidine on the other hand, has no tissue dissolving property and its activity is reduced in the presence of organic matter.[5] Another area of growing concern is increase in the number of antibiotic resistant strains owing to the irrational usage of synthetic drugs. All these constraints led to a search for herbal alternatives as endodontic irrigants.

Quercus infectoria (commonly known as Gall oak) is a small shrub found in Greece, Asia Minor and Iran. The gall arising in the branches of the tree is called as '*majuphal*' in Sanskrit and *'machakai'* in Kannada (both are local languages in India). In India, the galls of *Quercus infectoria* are used since ages as a home remedy for sore throat and chronic diarrhea in both rural and urban areas. It is also used as an ingredient in Ayurvedic preparations.[7] Gall extract of *Quercus infectoria* has shown promising antibacterial potential against some common oral pathogens like *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis Staphylococcus aureus*, and *Lactobacillus acidophilus*.[8] An *in vitro* experimental study was designed to assess the antibacterial potential and MIC of gall extract of *Quercus infectoria* against *Enterococcus faecalis*.

MATERIALS AND METHODS

Preparation of methanolic extract of galls of Quercus infectoria

Dried galls of *Quercus infectoria* were purchased from the local market and identified by a botanist based on its physical characteristics. About 500 grams of the galls were crushed into small pieces in a mortar and pestle and then powdered in an electric grinder. Two hundred and fifty grams of the gall powder was accurately weighed in an electronic balance, packed into a Soxhlet apparatus and extracted exhaustively using methanol as the solvent. The obtained crude extract was evaporated at room temperature to get the dried residue of methanolic extract of galls of *Quercus infectoria*. It was stored in a sterile bottle and preserved in desiccator until further use. A stock solution was prepared by dissolving 10 grams of the extract in 50 ml of dimethyl sulphoxide (DMSO) to obtain a final concentration of 200 mg/ml. For 250 grams of gall powder dissolved in one liter of methanol, 135 grams of the extract (residue) was obtained and the yield was 54% w/w.

Microbiological procedures

The antibacterial potential of methanolic extract of galls of *Quercus infectoria* was assessed against *Enterococcus faecalis* (ATCC 35550) by agar diffusion method.[9] Brain heart infusion agar media was employed to culture *Enterococcus faecalis*. The concentration of the organism was adjusted to 1.5×10^8 colony forming units per ml by using 0.5 McFarland Standards and was applied on the surface of the plate. Five wells of 8 mm diameter each were cut in the agar plate and were filled with 75 µl, 50 µl, 25 µl, 10 µl, 5 µl of the gall extract respectively. The agar plates were incubated overnight at 37 °C. The antibacterial activity was interpreted from the size (diameter) of inhibition zone measured in millimeters observed as a clear zone surrounding each well on the agar plate. The zone of inhibition was measured using a a vernier caliper.

Two-fold serial dilution method was employed to find out the MIC of the methanolic extract of galls of *Quercus infectoria* starting from a concentration of 500 µl/ml to 1 µl/ml.[9] A standard amount (1.5×10^8 colony forming units/ml) of *Enterococus feacalis* culture was added to the diluted extract in sterile tubes and was incubated overnight at 37 °C. The result was interpreted as sensitive if the supernatant was clear and as resistant if the suspension was turbid. The last tube in the series showing clear supernatant was considered to be the MIC value of gall extract of *Quercus infectoria* against *Enterococcus faecalis*.

Control agents

Two percent sodium hypochlorite solution and 2% chlorhexidine solution were chosen as the positive control while dimethyl sulphoxide (DMSO), which was used as the solvent for dissolving the methanolic extract of galls of *Quercus infectoria*, was selected as the negative control. The antimicrobial potential of the control agents were tested against *Enterococcus faecalis* in a separate agar plate and were then compared with that of the test agent.

RESULTS

The data obtained was appraised observationally. Table 1 shows the zone of inhibition for different volumes of the gall extract of *Quercus infectoria* against *Enterococcus faecalis* as compared to that of the control agents. The zone of inhibition was 24 mm when 75 µl of gall extract was used while it decreased to 14 mm for 10 µl. Thus, zone of inhibition of gall extract of *Quercus infectoria* against *Enterococcus faecalis* was found to increase with an increase in the volume of the gall extract tested (Figure 1).

The two fold serial dilution method to assess the MIC showed that concentrations of 500 µl/ml, 250 µl/ml, 125 µl/ml, 62.5 µl/ml, 31.25 µl/ml and 16.6 µl/ml of gall extract of *Quercus infectoria* were found to inhibit bacterial growth while concentrations of 8.4 µl/ml, 4.2 µl/ml, 2.1 µl/ml

and 1.1 µl/ml were found to not inhibit *Enterococcus faecalis*. Thus, the minimum inhibitory concentration of methanolic extract of galls of *Quercus infectoria* against *Enterococcus faecalis* is 16.6 µl/ml (Figure 2).

Table 1: Zone of inhibition (in mm) for different volumes of gall extract of *Quercus infectoria* against *Enterococcus faecalis* compared to that of control agents

Particulars	Zone of inhibition (in mm)
Gall extract (µl) volume/well	
10	14
25	17
50	21
75	24
Control agents	
2% Chlorhexidine	21
2% Sodium hypochlorite	0
Dimethyl sulphoxide	0

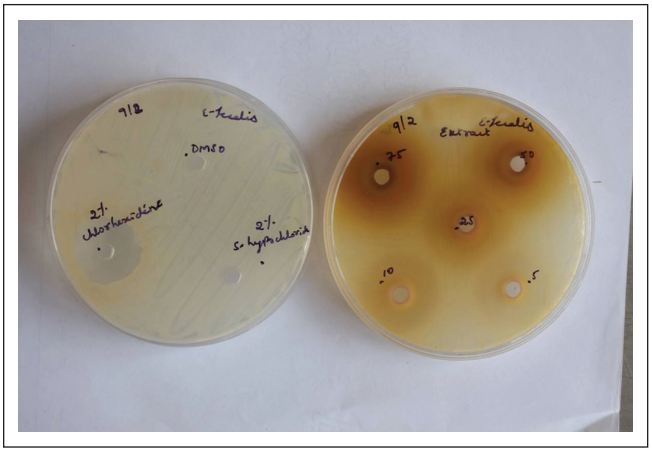

Figure 1: Zone of inhibition (in millimeters) for different volumes of test agent compared to positive and negative control against *Enterococcus faecalis*

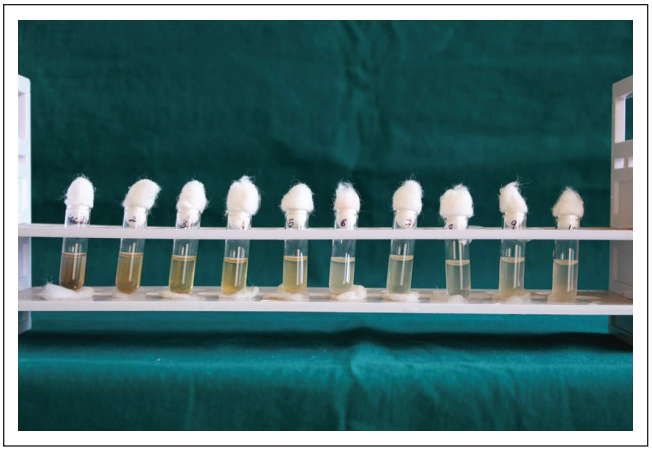

Figure 2: Minimum inhibitory concentration (MIC) of methanolic extract of galls of *Quercus infectoria* against *Enterococcus faecalis* by two fold serial dilution method

DISCUSSION

There is a growing interest to explore the potential of herbal products in treating common ailments. In the field of Endodontics, plant products such as *Morinda citrifolia,* green tea, Triphala, *Arctium lappa, Rosa damascena* have been assessed for its antimicrobial property against endodontic pathogens so that they can be used as an endodontic irrigant.[10-13] Gall extract of *Quercus infectoria* was tested against *Enterococcus faecalis* as it was already found to be effective against some common oral pathogens.[8] Moreover it is easily available, culturally acceptable and has relatively low tissue toxicity. The galls of *Quercus infectoria* have been pharmacologically documented to possess astringent, local anaesthetic, antiviral, antibacterial, antifungal, larvicidal and anti-inflammatory properties.[7,14]

The main constituents found in the galls of *Quercus infectoria* are tannins (50-70%) with small amounts of free gallic acid and ellagic acid.[7] Tannins are phenolic compounds that are known to possess antimicrobial property. The antibacterial property of gall extract of *Quercus infectoria* can thus be attributed to the high concentration of tannins present in it. The active agents in galls are soluble in a variety of solvents such as water, alcohol, petroleum ether and acetone. However, methanolic extract of galls was prepared in the present study based on the study conducted by ArchaVermani et al. which revealed that methanolic extract has more consistent antibacterial property against some common oral pathogens.[8] It is probably because various organic compounds present in galls of *Quercus infectoria* can leach more in methanol.

The zone of inhibition of gall extract of *Quercus infectoria* against *Enterococcus faecalis* was found to increase with an increasing volume and was even found to be greater than that of 2% chlorhexidine (positive control). It is also interesting to note that the other positive control, 2% sodium hypochlorite did not inhibit the growth of *Enterococcus faecalis*. This finding may be attributed to the very low concentration of sodium hypochlorite tested in the present study. Further, the antimicrobial property of a drug will be exhibited only when it diffuses through the solid agar medium. However, an *in vitro* study conducted by Gomes et al. revealed that concentrations as low as 0.5% sodium hypochlorite has antimicrobial property *in vitro*.[15] Dimethyl sulphoxide, which was used as a solvent for gall extract, served as the negative control in the present study. It did not inhibit the growth of *Enterococcus faecalis* suggesting that the zone of inhibition seen for gall extract was solely due to the active ingredients present in it and not because of the solvent used.

A direct comparison of the study results could not be made as there are no previous studies to assess the antibacterial property of the gall extract of *Quercus infectoria* against endodontic pathogens. Further *in vitro* studies need to be conducted in

biofilm models to ensure that the gall extract of *Quercus infectoria* has similar antibacterial potential against *Enterococcus faecalis* in biofilm. There exist no known agents that can mimic sodium hypochlorite in its tissue dissolving property. If proved to be effective then, gall extract of *Quercus infectoria* can be looked upon as an alternative to synthetic endodontic irrigants and it will substantially reduce the usage of sodium hypochlorite during cleaning and shaping of root canals.

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