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Research Article

ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF *QUERCUS INFECTORIA* GALLS ON *ROTHIA DENTOCARIOSA* ISOLATED FROM DENTAL CARIES

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ABSTRACT

Objective: The present study aimed to study *Quercus infectoria* gall extract for phytochemical analysis, antibacterial, and antibiofilm activity against *Rothia dentocariosa* isolated from dental caries.

Methods: *R. dentocariosa* was isolated, characterized, and identified by 16S rRNA sequence and also checked for biofilm formation ability. Phytochemical analysis of *Q. infectoria* aqueous gall extracts was carried out. Antibacterial and antibiofilm activity was performed using agar well diffusion method and microtiter plate assay, respectively.

Results: Bacterial isolate from dental caries was identified as *R. dentocariosa* by 16s rRNA sequencing technique with accession number MH824681 obtained from NCBI. Phytochemical analysis of *Q. infectoria* aqueous gall extract revealed the presence of alkaloids, phenol, tannin, glycosides, phenolic compound, and flavonoids. Significant antibacterial activity was observed with 19.00 (±7.07) mm diameter zone of inhibition. The biofilm inhibition assay was performed by microtiter plate method indicated 92.89% inhibition of bacteria at the concentration of 100 mg/mL of aqueous extract.

Conclusion: The results indicated the efficacy of *Q. infectoria* gall extracts that could be explored as an alternative to current treatment.

Keywords: *Rothia dentocariosa*, Anti-biofilm activity, Phytochemical screening, *Quercus infectoria*.

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INTRODUCTION

Dental caries and periodontal diseases are biofilm mediated and have a major public health concern globally. Imbalance of bacteria can cause dental biofilm. A biofilm is a microbial community attached to a solid surface and surrounded by the extracellular polysaccharides and proteins [1]. Formation of biofilm is due to the production of organic acid by bacterial fermentation, resulting in tooth decay or caries.

Rothia dentocariosa is Gram-positive bacteria acts as an opportunistic pathogen present in the oral cavity. *R. dentocariosa* and other *Rothia* sp. are involved in the formation of biofilm [2]. It can cause other serious infections such as infective endocarditis, fistula infection, pilonidal abscess, pneumonia, and endophthalmitis [3].

Biofilm protects the bacteria from the surrounding environment, including attacks from antibiotics, antiseptics, and chemotherapeutic agents. These agents have difficulty to reach and affect the pathogenic microorganisms due to the formation of exopolysaccharides [4]. At present, biofilm-mediated antibiotic resistance has a major cause of concern for many clinically associated infections [5]. The increase in resistance and adverse effects has lead researchers to explore novel anti-biofilm compound, which could be used for the effective treatment of oral diseases.

Nature has been a source of biologically important compounds which is used for the treatment of pathogenic infections. In recent days, this natural resource has also received attention worldwide for alternative treatment [6]. Biofilm inhibition is considered a major target for the treatment of dental caries infections. Plant species contain many reported phytochemicals that interfere with biofilm formation which control bacterial infections [7].

The *Quercus infectoria* (Fagaceae) is a small tree or shrub found in Greece, Asia, and Iran. The gall arise on branches of this tree, resulting from the deposition of eggs by gall wasp [8]. In Indian traditional medicine, the gall have been used to treat diarrhea, dysentery, internal hemorrhages, gonorrhea, impetigo, tonsillitis, and menorrhagia [9]. The present study was to determine phytochemical analysis of *Q. infectoria* aqueous extracts and determine their antibacterial activity and biofilm inhibition activity against *R. dentocarisa.*

METHODS

Preparation of plant aqueous extracts

The gall of *Q. infectoria* were washed with distilled water, cut into small pieces, and dried at room temperature, before grounding into a fine powder. Aqueous plant extract was prepared from air-dried *Q. infectoria* gall plant powder. *Q. infectoria* gall plant powder (1 g) was added into 25 mL sterile distilled water and heated on for 10–15 min and filtered through Whatman filter paper.

Isolation and characterization of bacterial isolate

Isolated bacterial culture from dental caries was identified using 16s rRNA technique. Sequences were submitted to Gen bank for accession number. Accession numbers for the isolated *R. dentocariosa* were obtained from NCBI Gen Bank. Biofilm formation ability of isolated bacteria was studied by microtiter plate assay.

Bacterial culture was maintained on brain heart infusion (BHI) agar with 2% sucrose. Freshly prepared cultures were obtained by inoculation of a loopful of each strain into separated 15 mL BHI broth and incubated for 24 h at 37°C.

Screening for phytochemical compounds

Screening of *Q. infectoria* plant extract was carried out for phytochemicals compounds, i.e., alkaloids, phenolic compounds, tannins, proteins,

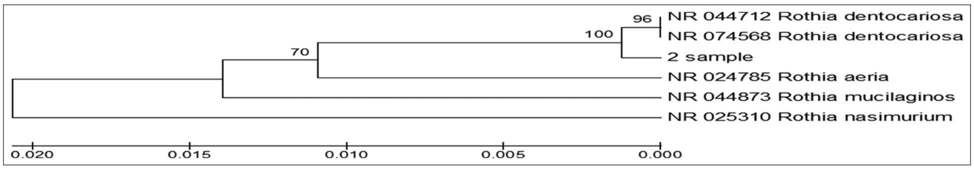

Fig. 1: Phylogenetic tree showing homology to *Rothia dentocariosa* strain

amino acids, reducing sugar, glycosides, flavonoids, phenols, coumarins, resins, and steroids/terpenoids. These phytochemicals were analyzed as per the standard method [10,11].

Antimicrobial activity

The antimicrobial activity of *Q. infectoria* plant gall aqueous extracts was performed by modified agar well diffusion assay. Briefly, 100 µL of fresh *R. dentocariosa* bacterial culture (approximately 10^6 CFU/mL) was spread uniformly to Mueller-Hinton Agar. Aqueous extracts of *Q. infectoria* galls (100 µL) were added to the agar wells. Chlorhexidine was used as a control. Plates were incubated at 37°C for 24 h. Antibacterial activity was checked by the presence of a zone of inhibition [12,13].

Antibiofilm activity

The biofilm inhibition effect of gall aqueous extracts on *R. dentocariosa* was checked using 96 well microtiter polystyrene plate. Different concentration of extract (10–100 µg/mL) was added into each well of a microtiter plate in the presence of different concentrations (10, 25, 50, 75, and 100 µg/mL). Briefly, 100 µL of bacterial cell suspension (final concentration 10^6CFU/mL) was added into each well. The plate was then incubated at 37°C for 24 h. After incubation, the supernatant was carefully removed without disturbing the adhering cells and washed with sterile distilled water to remove free-floating cells. The plates were air-dried for 15min and 100 µL ethanol was added to separate the cells adhered to the surface and to remove loosely attached cells. 1% crystal violet solution was added to observe for the adhered cells. The excess of stain was removed washing with sterile distilled water. Finally, the dye bound to the cells was solubilized by adding 200 µL of 95% glacial acetic acid. Moreover, after 15min of incubation, absorbance was measured using microplate reader at a wavelength of 570nm. Biofilm determination was calculated using this formula SBF = (AB–CW)/G, where SBF is the specific biofilm formation, AB is the OD570nm of the attached and stained bacteria, CW is the OD570nm of the stained control wells containing an only bacteria-free medium, and G is cell growth in broth [14].

The data of anti-biofilm assays were analyzed using the SPSS software. The ANOVA test was used to check the differences in mean scores among the groups.

RESULTS

Characterization of isolated bacteria

Bacterial isolate from dental caries was identified by 16s rRNA sequencing technique and was found homology to *R. dentocariosa*. Phylogenetic analysis is given in Fig. 1. Sequences were submitted to Genbank NCBI, and accession number (MH824681) was obtained from NCBI. Isolated bacteria were studied for biofilm formation indicated the significant biofilm-forming ability (OD 2.4575 at 570 nm) by microtiter plate method.

Phytochemical analysis

Phytochemical analysis of *Q. infectoria* gall aqueous extracts indicated the presence of secondary metabolites such as alkaloids, phenolic compound, tannin, glycosides, and flavonoids (Table 1).

Antibacterial activity

Aqueous extracts of a plant *Q. infectoria* gall were tested for their antibacterial activity against the oral bacteria *R. dentocariosa.*

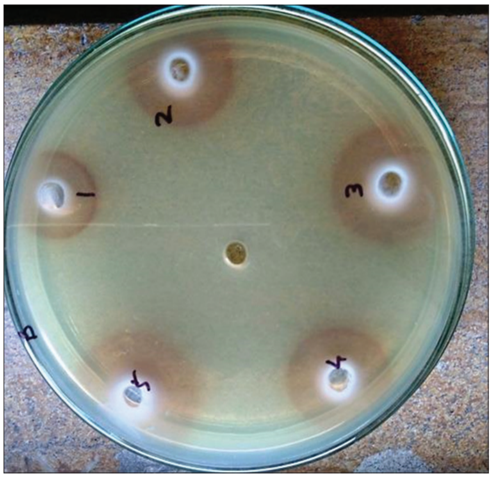

Fig. 2: Antibacterial activity of *Quercus infectoria* gall extracts against *Rothia dentocariosa*

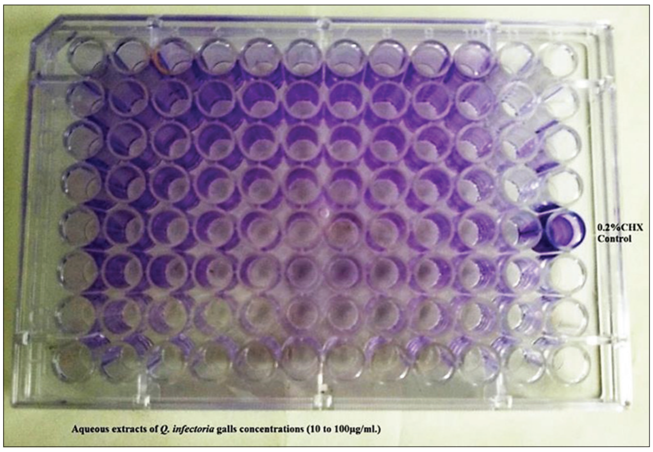

Fig. 3: Antibiofilm assay *Quercus infectoria* gall extracts against *Rothia dentocariosa*

Table 1: Phytochemical characterization of plant extract

Photochemical test	*Quercus infectoria* gall aqueous extracts
Detection of alkaloids (Hager's test)	+
Phenolic compounds (Lead acetate test)	+
Tannin (Gelatin test)	+
Protein (Nitric acid test)	−
Reducing sugar (Benedict's test)	−
Glycosides	+
Flavonoids	+
Phenols (Ferric chloride test)	−
Coumarins	−
Resins	−

+: Indicate the presence of phytochemical compound, −: Indicate the absence of compound

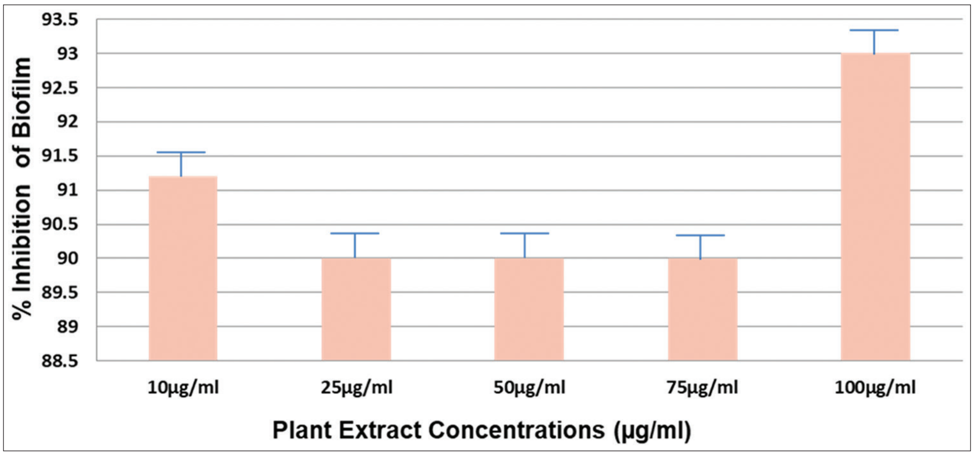

Fig. 4: Quantitative determination of antibiofilm efficacy of *Quercus infectoria* gall extract against *Rothia dentocariosa* (a) biofilm reduction assay by microtiter plate. (b) concentration-dependent percentage inhibition of biofilm

Aqueous extracts of *Q. infectoria* gall showed the potent antibacterial activity against *R. dentocariosa* studied by agar well diffusion assay. Chlorhexidine (0.2%) having maximum antibacterial efficacy taken as a positive control showing the zone of inhibition 15 mm against *R. dentocariosa* (Fig. 2). The diameter of zone of inhibition (19.00) ±7.07 mm was observed at the concentration of 100 µg/mL of extract.

Biofilm inhibition assay

Biofilm inhibition activity of different concentration aqueous extracts of *Q. infectoria* gall was evaluated by crystal violet staining method. The aqueous extracts of *Q. infectoria* gall showed strong inhibitory effects against *R. dentocariosa* ($p<0.05$) (Fig. 3).

Q. infectoria gall has shown anti-biofilm activity at all the concentrations ranging from 10 to 100 µg/mL. Chlorhexidine (0.2%) has taken as a positive control. Maximum anti-biofilm activity (92.89%) against R. dentocariosa was observed with the extract from 100 µg/mL (Fig. 4).

The values are expressed as percent biofilm inhibition by plant extract, compared with the control (no plant extract) and expressed as means ± standard deviation of triplicate assays.

DISCUSSION

R. dentocariosa is an opportunistic pathogen of the oral cavity and is responsible for causing dental plaque and dental caries through biofilm formation [15]. In our study, similar results were obtained with significant biofilm formation by the isolated *R. dentocariosa.*

In the present study, screening for photochemical compounds of *Q.* infectoria gall plant aqueous extracts was carried out and observed the presence of secondary metabolites such as alkaloids, phenolic compounds, tannin, glycosides, and flavonoids in the *Q.* infectoria gall plant aqueous extracts. Similar reports were reported by Basri *et al.* 2012 [8].

The presence of the secondary metabolites in the gall of Q. infectoria could be responsible for antibacterial activity against various pathogens. The high content of tannin in the gall of Q. infectoria is responsible for the antibacterial activity [16,17].

The antibacterial activity of methanol and acetone extracts from galls of *Q. infectoria* against oral bacteria. They observed inhibitory zones showed by methanol and acetone extracts are 22.67±0.33 and 21.33±0.33 mm, respectively, against *S. mutans* [8].

In the present study, we found the significant antibacterial activity (19.00±7.07 mm) by galls of Q. infectoria in aqueous extracts against *R. dentocariosa* which make it a potentially good source of the antimicrobial compound [18]. Earlier reports indicated percent biomass inhibition from 30.6% to 87.0% against *S. mutans* using various concentrations of plant extract [19]. Therefore, Q. infectoria gall extracts and their bioactive compound have shown very good antibacterial, anti-biofilm activity against biofilm-forming *R. dentocariosa* causing dental caries.

CONCLUSION

Based on this study, *Q. infectoria* gall aqueous extracts possess a wide range of phytochemicals which demonstrated antimicrobial activities. This study has proved the potential ability of antibacterial (19.00±7.07 mm) and antibiofilm (92%) of *Q. infectoria* gall aqueous extract to resist the biofilm growth of *R. dentocariosa.* It could be used as an alternative approach for the prevention of biofilm-forming bacteria. However, further research is needed to study in detail the effect of the gall extracts on different activities involved in dental caries formation and understand the pathogenesis of periodontitis and endodontics.

AUTHOR'S CONTRIBUTIONS

The author declares that this work was done by the author named in this article.

CONFLICTS OF INTEREST

The author declares that he has no conflicts of interest.

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