Research Article

Antibacterial activity of rosemary oleoresin against *Enterococcus faecalis* – An *in vitro* study

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ABSTRACT

Introduction: Essential oils have been long recognized for their antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties. They are widely used in medicine and in the food industry for these purposes. Oleoresins are combination of resin with essential oil. *Enterococcus faecalis* is one of the infectious organism leading to morbidity and mortality. Recently, *Enterococcus* has become the third most common nosocomial pathogens. **Aim:** The aim of the study was to evaluate the antibacterial activity of rosemary oleoresin against *E. faecalis*. **Materials and Methods:** Different concentrations of rosemary oleoresin were evaluated for its antibacterial activity against. *E. faecalis* using agar well diffusion method. **Results:** Rosemary oleoresin was effective against *E. faecalis* with good zone of inhibition in all tested concentrations. In fact, it showed a dose-dependent zone of inhibition with a maximum of 26 mm at the maximum concentration tested (100 µL). **Conclusion:** The study reveals that rosemary oleoresin has very good antibacterial activity against *E. faecalis*. Hence, it may be utilized for the management of infections with *E. faecalis,* especially in root canal failures.

KEY WORDS: *Enterococcus faecalis*, Rosemary oleoresin, Zone of inhibition and antibacterial activity

INTRODUCTION

Plants produce a high diversity of secondary metabolites with a prominent function of protecting plants against predators and microbial pathogens due to their biocidal properties against microbes or repellence to herbivores. These metabolites are involved in defense mechanisms against abiotic stress and are most important in the interaction of plants with other organisms.[1,2] Essential oils or aromatic plant essences are volatile and fragrant substances with an oily consistency produced by plants. They can be liquid at room temperature though a few of them are solid or resinous and showing different colors ranging from pale yellow to emerald green and from blue to dark brownish-red.[3] They are synthesized by all plant organs, i.e., buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood, or bark. Several techniques can be used to extract essential oils from different parts of the aromatic plant, including water or steam distillation, solvent extraction, expression under pressure, supercritical fluid, and subcritical water extractions.[4]

Plant oils and extracts have been used for variety of purposes for >1000 years.[5,6] They are used in perfumery, flavoring drinks and for preservation of food crops.[7] Many of these plants have antimicrobial activity and, hence, got many application including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies.[8] Medicinal plants are used in developing countries as alternative treatments choice for health problems.[9,10] Many studies have shown that aromatic plants traditionally used in folk medicine exert inhibitory effects on bacteria, fungi, and yeasts.[11]

Rosmarinus officinalis is an edible evergreen shrub usually seen in Mediterranean area, which is used around the world for culinary and alternative medicines. It is an aromatic plant come under the family of Lamiaceae.[12] Rosemary is a unique spice commercially used as antioxidants and extracts have been used for Alzheimer's disease. They have been

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also used in food preservation because they prevent oxidation and microbial contamination. Rosemary is a rich source of antioxidants and anti-inflammatory compounds, which help to boost the immune system and improve blood circulation.[13]

Enterococcus faecalis is a Gram-positive, commensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals. *E. faecalis* is found in healthy humans but can cause life-threatening infections, especially in the nosocomial environment, where the naturally high levels of antibiotic resistance found in *E. faecalis* contribute to its pathogenicity.[14] *E. faecalis* has been frequently found in reinfected, root canal-treated teeth in prevalence values ranging from 30% to 90% of the cases.[15] *E. faecalis*, the predominant human *Enterococcus*, has been also related to oral diseases such as caries, endodontic infections, periodontitis, and peri-implantitis. It has been frequently implicated in failure of endodontic treatment, due to high resistance to endodontic medicaments, and the ability to form recalcitrant biofilms both in treated and untreated root canals.[16] In the present study, the antibacterial activity of rosemary oleoresin was tested against *E. faecalis*.

MATERIALS AND METHODS

Rosemary oleoresin was obtained from Synthite Industries Limited, Kerala, as a gift sample. Rosemary oleoresin extract was used to evaluate the antibacterial activity against *E. faecalis* using well diffusion method. *E. faecalis* culture was obtained from the Department of Microbiology, Saveetha Dental College, Chennai.

Agar Well Diffusion Method

Agar well diffusion method is widely used to evaluate the antibacterial activity of plants or microbial extracts.[17,18] The agar plates were inoculated with *E. faecalis*. Then, wells punched aseptically with a sterile cork borer and were loaded with different concentrations of rosemary oleoresin (20–100 µL). Then, agar plates were incubated at 37°C and zone of inhibition was measured [Figure 1]. The experiment was carried out in triplicate to avoid manual error.

RESULTS

From the study, it was clear that rosemary oleoresin has antibacterial activity against *E. faecalis*. Rosemary oleoresin has produced a zone of inhibition of about 22 mm at the concentration level of 25 µL. At the concentration level of 50 µL, rosemary oleoresin has zone of inhibition of about 25 mm. At the concentration level of 100 µL, rosemary oleoresin has zone of inhibition about 26 mm; chlorhexidine showed zone of inhibition of about 24 mm which acted as control group [Table 1, Figure 2 and 3]. When the concentration level increases, rosemary oleoresin increases zone of inhibition against *E. faecalis* [Table 1].

DISCUSSION

This study clearly shows that rosemary oleoresin has antibacterial activity against *E. faecalis* and showed good zone of inhibition at the maximum concentration level used. The antibacterial activity of rosemary has been determined in various assay types based on either minimal inhibitory concentration or minimal bactericidal concentration.[19] The antibacterial activities of plants such as basil (*Ocimum basilicum* L.) and rosemary

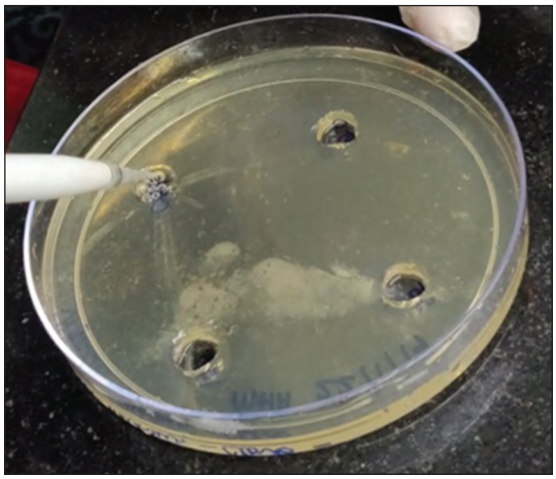

Figure 1: Agar plates with Prepared wells

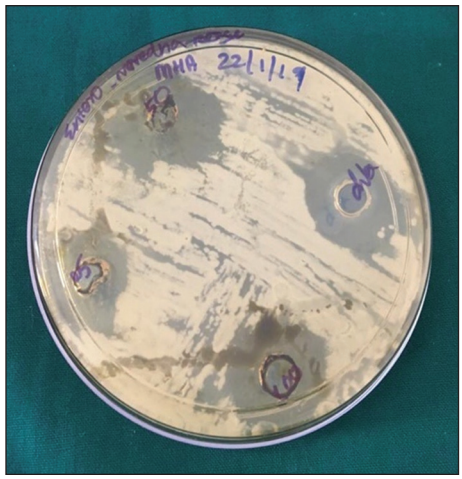

Figure 2: Zone of Inhibition with Rosemary oleoresin against *E .faecalis*

Table 1: Antibacterial activity of Rosemary oleoresin against *E. faecalis*

Concentration (µL) of rosemary oleoresin	Zone of inhibition (mm)
25	22
50	25
100	26
Chlorhexidine	24

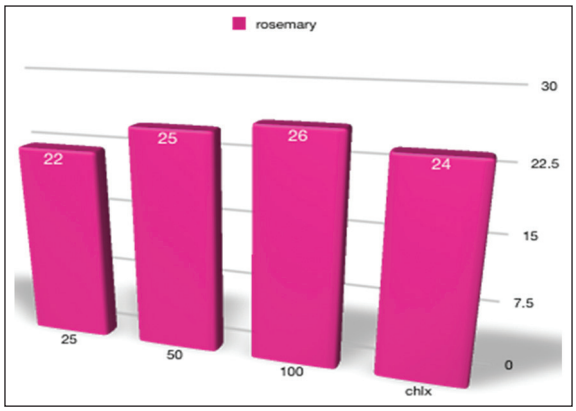

Figure 3: Graph showing the antibacterial activity of Rosemary oleoresin against *E.faecalis*

(*Rosmarinus officinalis* L.) were also reported. The inhibitory effect of rosemary is the result of the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol, and isorosmanol. They interact with the cell membrane, causing changes in genetic material and nutrients, altering the transport of electrons, leakage of cellular components, and production changes in fatty acid.

CONCLUSION

The present study reveals that rosemary oleoresin has good antibacterial activity against *E. faecalis*. Hence, rosemary oleoresin may be used to control infections with *E.faecalis*.

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