

Research Report

### Antibacterial potential of *Ocimum sanctum* oils in relation to *Enterococcus faecalis* ATCC 29212

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#### ABSTRACT

**Background:** *Enterococcus faecalis* (*E. faecalis*) is a Gram positive cocci present in the root canal due to the failure of endodontic treatment and pulp tissue necrosis. The ideal root canal medicine offers biocompatible properties, ease of cleaning, absence of tooth staining and non-disruption of the root canal filling process. Basil (*Ocimum sanctum*) is one of the herbs widely used in salads which produces anti-bacterial, anti-fungal and anti-viral effects. The antibacterial effect of basil results from the eugenol which represents a main component demonstrating antibacterial properties. Basil essential oil has an antibacterial effect on both Gram positive and Gram negative bacteria. **Purpose:** This study aimed to determine whether the essential oils contained in basil leaves offer any antibacterial potential with regard to the growth of *E. faecalis* ATCC 29212. **Methods:** The research was experimental in nature incorporating a simple random sampling technique. In this study, groups of active substance compounds contained in basil leaves were extracted by distillation in order to obtain the essential oil. Preparation of the test solution involved essence of basil leaf oil at concentrations of 5,000 ppm, 10,000 ppm and 20,000 ppm in methanol solvent. A phytochemical test of basil was subsequently conducted in order to identify the content of the compound. The bacteria in this study was tested utilizing a disc diffusion method (Kirby and Bauer test) by measuring the diameter of the clear zone (clear zone) which is indicative of the bacterial growth inhibition response of antibacterial compounds in the extract. **Results:** The results of the research into the phytochemical test showed that basil contains phenolic flavonoids, triterpenoids, saponins, tannins which produce a negative result on steroids. The results of this study showed that the basil essential oil inhibition zone present in the *E. faecalis* growth had a diameter of 11.70 mm at a concentration of 20,000 ppm. This concentration therefore proved most effective in relation to *E. faecalis* than other concentrations. **Conclusion:** It can be concluded that essential oils of basil leaves demonstrate anti-bacterial inhibitory properties with regard to *E. faecalis*.

**Keywords:** *Ocimum sanctum*; anti-bacterial activities; *Enterococcus faecalis* ATCC 29212

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#### INTRODUCTION

The success of endodontic therapy depends on the number of infection-causing microorganisms that can be eradicated from the root canal. The reduction in the number of such microorganisms can be achieved by root canal preparation, irrigation, administration of sterilizing medication and filling material. The administering of irrigation agents and drugs to the root canal plays an important role in reducing the amount of infected tissue and eliminating microorganisms from the root canal.<sup>1</sup>

*Enterococcus faecalis* (*E. faecalis*) is a Gram positive cocci bacterium that appears in the root canal arising from the failure of endodontic treatment and is contained in the necrotic pulp tissue.<sup>2</sup> *E. faecalis* can tolerate a high alkaline atmosphere and is capable of entering the dentin tubules. This causes *E. faecalis* to become resistant and difficult to remove by means of root canal medications, such as calcium hydroxide.<sup>2</sup> Ideal root canal medications have biocompatible properties, are easy to clean, do not stain teeth and do not impede the root canal filling process.<sup>3</sup> Increased bacterial resistance to synthetic medications

induces researchers to resort to herbs, one being basil, as root canal medicines with antibacterial effects.

Basil (*Ocimum sanctum*) produces anti-bacterial, anti-fungal and anti-virus effects.<sup>3</sup> The antibacterial effect of basil results from its essential oil content,<sup>4</sup> a main component of which is eugenol that demonstrates antibacterial properties<sup>3</sup> and plays a major role in those of basil leaves.

Basil leaves containing essential oil are effective against both gram positive and Gram negative bacteria.<sup>3</sup> The essential oil is effective in inhibiting the growth of gram-positive cocci bacteria at a minimum inhibitory concentration (MIC) of 0.5 to 32 µl/ml and minimum bactericidal concentration of 8-32 µl/ml, with the exception of *E. faecalis*. *E. faecalis* can be inhibited by a concentration of >32 µl/ml.<sup>3</sup> Gram negative bacteria can be inhibited by basil essential oil of a lower concentration from 0.25 to 4 µl/ml and a higher minimum bactericidal concentration of 0.5-64 µl/ml.<sup>4</sup> Basil essential oil has an antibacterial effect on both gram positive and gram negative bacteria.

Research into the various stages of basil leaf development shows that, at the vegetative stage, basil leaves have an antibacterial effect on *E. faecalis* bacteria with a minimum antibacterial concentration (MBC) of > 64 µl/ml and MIC of 32 µl/ml. The tip of the leaf stage exhibits an antibacterial effect against *E. faecalis* with MBC of 8 µl/ml and MIC of 4 µl/ml. The full leaf stage shows an antibacterial effect against *E. faecalis* with MBC of 32 µl/ml and MIC of 16 µl/ml.<sup>4</sup> These results confirm the antibacterial effects of basil leaves essential oils at various leaf development stages against *E. faecalis*, with the minimum concentration being lowest for the tip of basil leaf.

The aim of this research was to determine the antibacterial effects of basil leaf essential oil on *E. faecalis*.

## MATERIAL AND METHODS

In this study, a group of active compound substances contained in the leaves of basil (*Ocimum sanctum*) was distilled to obtain basil leaf essential oil. A phytochemical test was subsequently performed to determine the content of the compound. An antibacterial activity test of basil leaf essential oil against *E. faecalis* was conducted by diffusion.

The basil leaves were obtained from a plantation in Lembang, West Bandung regency, West Java and later confirmed by the Biology Department of the Mathematics and Science Faculty, Universitas Padjadjaran Bandung. The bacteria studied were *E. faecalis* ATCC 29212 because this is the same bacteria as that present in the root canal.

The research methodology adopted constituted a laboratory experiment. A simple random sampling technique was used in selecting basil leaves on the assumption that each section of the population has an equal opportunity of being selected as part of the sample. In this study, a group of active compound substances contained in basil leaves

were extracted by distillation to obtain its essential oil which was subsequently dissolved with methanol to produce concentrations of 5,000 ppm, 10,000 ppm and 20,000 ppm. In order to detect antibacterial activity, a diffusion method was implemented which involved measuring the inhibition zone produced by essential basil leaves.

A phytochemical test was performed on the basil leaves at the Biology Department of the Mathematics and Science Faculty, Universitas Padjadjaran, Bandung to determine the compound content. The phytochemical tests include: phenolic tests, flavonoid tests, saponin tests, terpenoid tests, steroid tests and tannin tests. Their test procedures consisted of Phenolic Testing which involved a basil leaf sample being inserted into the test tube before 5% FeCl<sub>3</sub> reagent was added. If the result proved positive, the phenol would appear purple blue. The testing of flavonoids was carried out with basil leaf samples being inserted into the test tube before three drops of 2N H<sub>2</sub>SO<sub>4</sub> and ± 0.25 g of magnesium powder were added. Any changes were subsequently observed and recorded. When an orange solution had formed, the positive test sample contained a flavanoid compound to which 2N H<sub>2</sub>SO<sub>4</sub> reagent was added. If an orange-colored solution was formed, the positive test sample contained flavanoid compounds. When 10% NaOH reagent was added to the sample and an old orange solution had formed, the positive test sample contained flavanoid compounds.

The next step involved testing for steroids and triterpenoids. Basil leaf samples were placed on the drop plate before a Lieberman-Burchard test was conducted during which two drops of anhydrous acetic acid, one drop of concentrated H<sub>2</sub>SO<sub>4</sub> and 2 ml diethyl ether were added. If a change in color to brown-red occurred, the positive test samples contained terpenoid. Saponin Testing involved basil leaf samples being inserted into the test tube and the reagent HCl + H<sub>2</sub>O then added. If the foam was stable for 3-10 minutes, the positive sample contained saponins. Tannin testing constitutes another of the phytochemical testing procedures. Basil leaf samples were added to the tube followed by 1% FeCl<sub>3</sub> reagent. If the result was positive, the tannin would appear purple blue in colour.

Antibacterial properties were determined by means of disc diffusion method (Kirby Bauer test). Prior to the conduct of the test, the bacteria were rejuvenated by growing them in the Muller Hinton Broth liquid medium for 24-48 hours at an agitation rate of 150 rpm at 37°C. If turbidity in the liquid medium met the standard 0.5 Mc Farland, confirming that the turbidity concentration of the bacteria was equal to 108 CFU/ml, it could be tested. The bacteria growing in the liquid medium were extracted and transferred to a solid medium of blood agar and grown in an incubator in 5% CO<sub>2</sub> for 24-48 hours.

A disk diffusion method (Kirby and Bauer tests) was adopted to determine antibacterial agent activity. Discs containing antibacterial agents were placed on the agar medium that had been seeded with bacteria that would diffuse within it. The clear area indicated the inhibition

of bacterial growth by antibacterial agents on the surface of the agar medium. A disk containing basil leaf essential oil was placed in the media (Mueller Hinton Broth) within which *E. faecalis* bacteria had been grown. Two batches of media sample was produced for each concentration. The negative control was methanol, while the positive control consisted of chlorhexidine glucuronate 0.2%. A clear zone around the discs confirmed the antibacterial effect of basil leaf essential oil. The diameter of the clear zone around the discs was measured using a caliper.

The bacterial test comprised several stages: preparing the *E. faecalis*, sterilizing 50 ml of nutrient agar (Mueller Hinton) media in Erlenmeyer, adding the *E. faecalis* bacteria to the homogenized agar media, pouring it into a sterile petri dish and allowing it to solidify. In order to determine the antibacterial potential, a disc diffusion method was utilised in this study by measuring the diameter of the clear zone by means of a paper disc.

The paper disc was divided into five groups: three respectively containing 5,000 ppm, 10,000 ppm and 20,000 ppm of basil leaf essence, one containing 1,000 ppm of chlorhexidine as a positive control, and the final one containing methanol as a negative control which was added to first petri dish. The same procedure was followed with the second petri dish. Both petri dishes were incubated at 37°C for 24-48 hours, with the results interpreted and the diameter of each sample concentration measured. The effects of bacterial growth inhibition in an antibacterial compound is shown in the sample.

Data was obtained by measuring the diameter of the clear zone which appeared as a clear area around the disc

where substances promoting antibacterial activity had diffused.

## RESULTS

Phytochemical testing of basil leaves was carried out in this study to determine the content of the compounds contained within. In the phytochemical test, positive results for the phenolic group compound content such as flavonoids, triterpenoids, saponins, tannins and a negative result for steroids were obtained (Table 1).

Essential oil of basil leaves at concentrations of 5,000 ppm, 10,000 ppm and 20,000 ppm execute activities characterized by the formation of an inhibition zone against bacteria *E. faecalis* ATCC 29 212. A chlorhexidine concentration of 1,000 ppm generates antibacterial activity, while the solvent sample (methanol) creates an *E. faecalis* ATCC 29 212 bacteria inhibition zone (Figure 1).

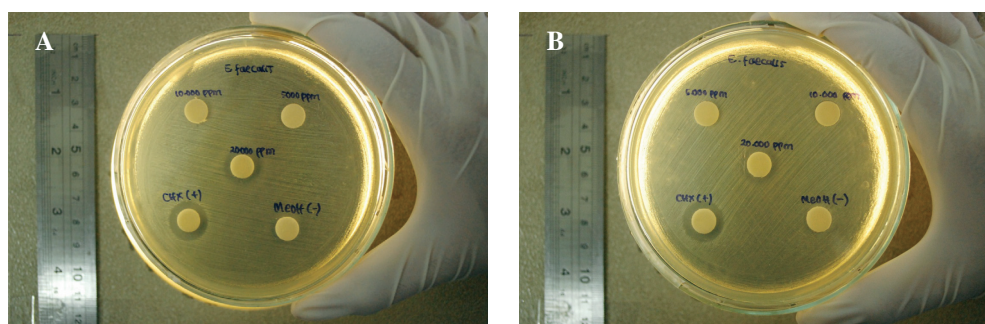
The three concentrations of active basil leaf essential oils can actively inhibit the growth of *E. faecalis* bacteria at concentrations of 20,000 ppm, 10,000 ppm and 5,000 ppm; while chlorhexidine gluconate, as an active positive control, is active at a concentration of 1,000 ppm. Methanol also acts as a negative solvent control (Table 2). The results of the basil leaf sample inhibition zone calculation confirmed the inhibition diameter at a concentration of 20,000 (11.70 mm) ppm which was greater than at concentrations of 5,000 (8.90 mm) and 10,000 (9.25 mm) ppm (Table 2).

**Table 1.** Phytochemical test

No.	Metabolit	Method	Result
1.	Phenolic	Reagent 5% FeCl <sub>3</sub>	+
2.	Flavonoid	a. Reagent HCl concentrated + Mg	+
		b. Reagent 2N H <sub>2</sub> SO <sub>4</sub>	+
		c. Reagent 10% NaOH	+
3.	Steroid	Reagent Lieberman-Burchard	-
4.	Triterpenoid		+
5.	Saponin	Reagent HCl + H <sub>2</sub> O	+
6.	Tanin	Reagent 1% FeCl <sub>3</sub>	+

**Table 2.** Potential antibacterial test essential oil *Ocimum sanctum* to *E. faecalis* ATCC 29212

No.	Sample & Concentrate (ppm)	Diameter of inhibition (mm)		Average (mm)	Result
1	Essential oil (20.000)	10.90	12.50	11.70	Active
2	Essential oil (10.000)	9.10	9.40	9.25	Active
3	Essential oil (5.000)	8.80	9.00	8.90	Active
4	Negatif control: Metanol	9.15	9.10	9.13	Active
	Positif control:				
5	Chlorhexidine (1.000)	13.80	15.10	14.45	Active



**Figure 1.** Potential antibacterial test essential oil *Ocimum sanctum* to *E. faecalis*, A) first test; B) second test.

## DISCUSSION

Basil is a herb known to have antibacterial effects against some strains of bacteria<sup>5</sup> which depends on the content of its leaves. The phytochemical test results produced within this study show that basil contains phenols, flavonoids, triterpenoids, saponins and tannins. These results are in accordance with those of phytochemical research which confirmed eugenol (methyl volatile), ursolic acid (triterpenoids) and rosmarinic acid (phenylpropanoid) to be active components contained in basil leaves. The phytochemical test conducted on its leaves by means of methanol extract indicated that basil contains steroids, alkaloids and tannins.<sup>6</sup>

The phytochemical content, consisting of steroids, alkaloids, flavonoids, tannins and phenols, appears to combat microorganisms.<sup>7</sup> The main content of basil leaves is eugenol whose phenols damage the cell wall of the plasma membrane and protein membranes of bacteria.<sup>8</sup> The hydrophobic properties of eugenol are paramount in producing the antibacterial effect of separating the fat tissue and mitochondria in the cell membrane of bacteria and altering structures to improve the penetration of eugenol into the cell membrane.<sup>8</sup>

The phytochemical test results of basil leaves in this study also confirmed the presence of tannin which, in medicinal plants, may indicate their antibacterial properties. Tannin forms an irreversible compound with proline protein that can inhibit bacterial cell protein synthesis.<sup>7</sup> The results of this study indicate that basil leaf essential oils have potentially inhibitive or antibacterial effects on *E. faecalis* bacteria, possibly due to their phenol content, specifically eugenol.<sup>3,8,9</sup>

*E. faecalis* constitutes Gram positive facultative cocci that are generally present in the root canal. The essential oils taken from several parts of the basil plant can produce an anti-bacterial effect against Gram positive bacteria cocci in almost all parts of the plant - the leaves, stem and bud.<sup>4</sup> Other research has been conducted to distinguish the essential oil of basil leaves from that of other plants.<sup>4</sup> The results of these studies confirmed the antibacterial effects of essential oils of basil leaves on *E. faecalis*, while proposing that the antibacterial effect of the essential oils of basil leaves is due to their *linoleic acid*, *linolenic acid* and eugenol content. The working mechanism of essential oils is thought to inhibit the replication of DNA, thereby causing the eradication of the bacteria. Research findings can be enhanced when the inhibitory effect on *E. faecalis* bacteria due to the use of essential oils of basil leaves is evident.

The manufacture of essential oils in this study used a methanol solution, while methanol was also used as a negative control. However, this investigation confirmed the

use of methanol to have an antibacterial effect on *E. faecalis*, a fact which, in turn, probably enhances the antibacterial effect of basil leaf essential oil on *E. faecalis*.

The positive control consisted of chlorhexidine which is an antiseptic widely used to sterilize the root canal. The antibacterial effect of chlorhexidine shows reasonably effective inhibition of *E. faecalis* because it can penetrate the dentine tubules.<sup>2</sup>

From the results above, *Ocimum sanctum* can be seen to have chemical properties that produce an antibacterial effect. This research proved that *Ocimum sanctum* has antibacterial potential with regard to *E. faecalis*, although it is less than that of chlorhexidine. Nevertheless, *Ocimum sanctum* can be used as an alternative material for root canal sterilization, although further research in this area is necessary. It can be concluded that the essential oils of basil leaves have an anti-bacterial inhibitory effect on *E. faecalis*.

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