

# Anti Bacterial and Anti Inflammatory efficacy of *Zingiber officinale* and *Decalepis hamiltonii* – In Vitro Study

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## Abstract:

**Aim:** To evaluate the *in vitro* anti bacterial and anti inflammatory potential of *Zingiber Officinale* and *Decalepis Hamiltonii* against *E. faecalis*.

**Materials and Methods:** Ethanolic extract of *Zingiber* and *Decalepis* was subjected to microbiological assay to determine its Maximum zone of inhibition using Agar disk diffusion test, minimum inhibitory concentration using serial broth dilution method and anti inflammatory potential using protein denaturation assay against *E. faecalis*.

**Results:** Ethanolic extract of *Zingiber* and *Decalepis* showed: (a) Maximum zone of inhibition of 20 mm and 24 mm respectively, (b) MIC of 5% and 2.5% respectively, (C) Protein denaturation assay value (IC<sub>50</sub>) of 115 and 80 respectively.

**Conclusion:** Ethanolic extract of *Zingiber* and *Decalepis* was found to possess both anti bacterial and anti-inflammatory potential against *E. faecalis*.

**Keywords:** Anti bacterial, Anti-inflammatory, *Decalepis Hamiltonii*, *E. faecalis*, *Zingiber Officinale*.

## Introduction:

Medicinal plants are nature's hidden and to an extent-unexplored treasure. They have been used as a source of safe and effective medicine since time immemorial, as they are less toxic, cheap and suitable for use over a prolonged period<sup>(1)</sup>. As the Enterococcus stand alone in many cases of failed root canal treatment, it is the time to find out an effective means to minimize the failure rate and an alternate material to overcome the anti bacterial resistance and side effects of the current synthetic materials.

A rekindled interest in the pharmaceutical importance of plants has led to the discovery and adaptation of plant extract which were commonly used in traditional medicine as

alternative source of remedy<sup>(2)</sup>.

Ginger (*Zingiberofficinale*) one such medicinal plant is having antimicrobial property against various human pathogens; however, less data is available on its antimicrobial potential against oral pathogens.

*Decalepsis Hamiltonii*, popularly known as swallow root in English, belongs to the family Asclepiadaceae. Though many researchers establish the anti oxidant and anti ulcerogenic role of the root extract of *D. hamiltonii*, very few have explored the anti-inflammatory and anti bacterial potential.

Hence present study is an attempt to explore the Anti bacterial and Anti-inflammatory potentials of *ZingiberOfficinale* and *DecalepisHamiltonii* against

*E. faecalis*.

## Materials and Methods:

### (A). Test extract.

Ethanollic extracts of *Zingiber officinale* and *Decalepis hamiltonii* was obtained from "Green Chem" laboratory, Bangalore.

### (B). Microbiological Tests.

Various concentrations of ethanolic extracts of *Zingiber officinale* and *Decalepis hamiltonii* was subjected to microbiological tests namely Agar well diffusion test (to determine the maximum zone of inhibition), Serial broth dilution test (to determine minimum inhibitory concentration (MIC)) and protein denaturation assay (to determine the anti inflammatory potential (IC<sub>50</sub>)) against *E. faecalis*.

The standard strains of the organisms used in the study were *E. faecalis* (ATCC 35550).

### (C). Standardization of isolates:

A standard stock of the bacteria isolates was prepared by suspending a loop full of each microbial growth in about 10 mL of nutrient broth. After incubation at 37°C for 12 hours, the turbidity was adjusted to be visually comparable with a 0.5 McFarland's standard giving a bacterial load of about 1-2 × 10<sup>8</sup> cfu/mL.

### (D). Agar well Diffusion Test:

Lawn culture of *E. faecalis* was prepared on a TSA plate. Wells of 4mm depth were prepared, which were filled with 100 µl of various concentrations of the extracts. 0.2% chlorhexidine was used as the positive control. Plates were incubated at 37°C for 24 hours. Interpretation of diffusion results was carried out by noting the presence or absence of zone of inhibition around the wells.

### (E). Minimum Inhibitory Concentration:

Serial dilutions of 20mg, 10mg, 5mg, 2.5mg, 1.25 and 0.62mg per 1 ml were prepared in sterile test tubes containing 1 ml of *E. faecalis* suspension in TSB having 0.5 McFarland standard. Tubes were incubated at 37°C for 24 hours. After the incubation, the MIC values were determined by visual inspection of the tubes. In each series of tubes, the last tube with clear supernatant was considered to be without any growth and taken as MIC value. Turbidity in the MIC tube indicated growth of bacterial strain implying that the organisms were resistant to ethanolic extract.

### F). Protein denaturation Assay:

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the test extracts, so that final concentrations become 31.25, 62.5, 125, 250, 500, 1000 µg/ml. Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37±2°C in a BOD incubator for 15 minutes and then heated at 70°C for 5 minutes. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the final concentration of (78.125, 156.25, 312.5, 625, 1250, 2500 µg/ml) was used as reference drug and treated similarly for determination of absorbance.

The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ Inhibition} = 100 \times (V_t / V_c - 1)$$

Where, V<sub>t</sub> = absorbance of test sample, V<sub>c</sub> = absorbance of control.

## Results:

**Table 1:** Agar well diffusion Test

Extract	Conc – mg/ml	Zone – mm dmt.
Zingiber	15	20
	10	17
	5	10
Decalepis	15	24
	10	20
	5	12
chlorhexidine	2%	28

Table 1, shows the zone of inhibition of ethanolic extract of *Zingiber* and *Decalepis* against *E. faecalis*. For *Zingiber*, maximum zone of inhibition was 20 mm at 15mg/ml and minimum zone of inhibition was 10 mm at 5 mg/ml. For *Decalepis*, maximum zone of inhibition was 24 mm at 15mg/ml and minimum zone of inhibition was 12 mm at 5mg/ml when compared to positive control (0.2% Chlorhexidine), which had zone of inhibition of 28 mm.

**Table 2:** Minimum Inhibitory Concentration

Conc – mg/ml	20	10	5	2.5	1.25	0.62
Zingiber	NG	NG	NG	G	G	G
Decalepis	NG	NG	NG	NG	G	G

\* NG – No Growth, G – Growth

Table 2 shows MIC of ethanolic extract of *Zingiber* and *Decalepis* against *E. faecalis*. MIC of ethanolic *Zingiber* extract for was established at 5%, and *Decalepis* at 2.5%.

**Table 3:** Protein denaturation assay

Conc – ug/ml	1000	500	250	125	62.5	31.25
Zingiber	446	143	98	52	21	8
Decalepis	987	545	278	109	46	13
IC <sub>50</sub>	Diclo - 625	Zingiber - 115	Decalepis - 80			

Table 3, shows the protein denaturation assay of ethanolic extract of *Zingiber* and *Decalepis* against *E. faecalis*. IC<sub>50</sub> values of *Zingiber* and *Decalepis* were 115 and 80 respectively. When

compared to positive control (Diclofenac Sodium), which had an IC<sub>50</sub> value of 625.

## Discussion:

Members of the *Zingiberaceae* family are important components in traditional medicine for the treatment of many diseases. Ginger's pungent components offer powerful anti-inflammatory and antioxidant activities, making it useful in arthritis, Alzheimer's, cancer, and cardiovascular disease. The active compound responsible for this effect is zingibain, an enzyme that counteracts inflammation<sup>(3)</sup>. The active compounds contained in ginger are divided into two groups: volatile essential oils and fragrant or harsh phenol compounds<sup>(4)</sup>. Among these volatile essential components, which constitute gingerol and shagelol have been accounted for antimicrobial activity of ginger.

Although the roots of *D. hamiltonii* have been used for their alleged health benefits, scientific investigation in this regard need to be done. Earlier works have shown that the *D. hamiltonii* roots contain aldehydes, amyriols, lupeols and volatile flavour compounds such as 2-hydroxy-4-methoxybenzaldehyde, vanillin etc and essential oil like methylresorcyraldehyde, atlantone, terpinene, geraniol etc. A combinational molecule containing pectic polysaccharide with bound phenolics identified in the root of *D. hamiltonii* and their break down products have been known to have health beneficial properties. *Enterococcus faecalis* (causative agent for secondary root canal infection) have been considered very difficult to control as they have developed tolerance against various antimicrobial agents in routine use<sup>(3)</sup>. This calls for an urgent need to explore novel bioactive

compounds, which are safer and biodegradable. In this present study ethanolic extracts of *Zingiber officinale* and *Decalepis hamiltonii* were tested against *E. faecalis*.

In the present study, ethanolic extract of ginger showed antibacterial activity against *E. faecalis* exhibiting maximum zone of inhibition of 20 mm at 15mg/ml and minimum zone of inhibition was 10 mm at 5 mg/ml. Study conducted by Rahman *et al.* showed a zone of inhibition of 12 mm<sup>(4)</sup>. The difference observed could be attributed to variations in the quality of ginger used, differences in the microbiological techniques used, variation in temperature and solvent used to prepare ginger extract. MIC of ethanolic *Zingiber* extract for was established at 5%, and had an IC<sub>50</sub> value of 115.

In the present study, ethanolic extract of *Decalepis* showed antibacterial activity against *E. faecalis* exhibiting maximum zone of inhibition of 24 mm at 15mg/ml and minimum zone of inhibition was 11 mm at 5 mg/ml. Minimum Inhibitory Concentration of ethanolic *Decalepis* extract for was established at 2.5%, and had an IC<sub>50</sub> value of 115. Study done by Mohua *et al* showed that *Decalepis* possessed anti-inflammatory potential at a concentration of 250mg/kg body weight<sup>(1)</sup>.

In the present study, positive control was used in order to compare the antimicrobial and anti-inflammatory efficacy of ethanolic extracts of Zingiber and Decalepis. This study was first of its kind where ethanolic extracts of Zingiber and Decalepis along with positive control was used *E. faecalis* in order to compare their antibacterial and anti-inflammatory efficacy.

## Conclusion:

Within the limitations of this study it may be concluded that *Zingiber officinale* and *Decalepis hamiltonii* which are used as edible roots in Indian cuisine can be applied as an irrigant or intracanal medicament against *E. faecalis* in endodontic practice.

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