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Research Article

ANTIBACTERIAL EFFICACY OF *BETA VULGARIS* AND *CINNAMOMUM ZEYLANICUM* AGAINST *ENTEROCOCCUS FAECALIS*: *IN VITRO* STUDY

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ABSTRACT

Objective: The objective was to evaluate the *in vitro* antibacterial potential of *Beta vulgaris* and *Cinnamomum zeylanicum* against *Enterococcus faecalis*.

Methods: Ethanolic extract of *B. vulgaris* and *C. zeylanicum* was subjected to microbiological assay to determine its maximum zone of inhibition using Agar disk diffusion test against *E. faecalis.*

Results: *B. vulgaris* did not show any antibacterial potential against *E. faecalis*, whereas *C. zeylanicum* showed a marked and significant efficacy against *E. faecalis* when applied alone and also in combination with calcium hydroxide.

Conclusion: *C. zeylanicum* can be used as an ICM, or it can be combined with $CaOH_2$ for effective removal of bacterial pathogens inside the root canal.

Keywords: Antibacterial efficacy, *Beta vulgaris, Cinnamomum zeylanicum, Enterococcus faecalis*.

INTRODUCTION

Plants are the primary source of medicines, fiber, food shelters, and other items in everyday use by humans with roots, stems, leaves, flowers, fruits, and seeds providing food for humans [1]. Plants serve as an indispensable constituent of the human diet supplying the body with minerals salts, vitamins, and certain hormone precursors, in addition to protein and energy [2].

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 antibacterial resistance and side effects of the currents 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 an alternative source of remedy [3].

Beta vulgaris is best known in its numerous cultivated varieties, the best known of which is the purple root vegetable known as the beetroot or garden beet. Phytochemical analysis indicated that the root of *B. vulgaris* is rich in phytochemicals responsible for both pharmacological and toxic activities [4].

The oil extract of *Cinnamomum zeylanicum* has been reported to have cidal or inhibitory activity on various bacterial, fungal and viral agents. Cinnamaldehyde was also shown to inhibit the growth of antibiotics resistant bacteria [5].

Hence, the present study is an attempt to explore the antibacterial efficacy of *B. vulgaris* and *C. zeylanicum* against *Enterococcus faecalis*.

METHODS

Test extract

Ethanolic extracts of *B. vulgaris* and *C. zeylanicum* was obtained from "Green Chem" Laboratory, Bangalore.

Microbiological tests

Various concentrations of ethanolic extracts of *B. vulgaris* and *C. zeylanicum* were subjected to microbiological test namely agar well diffusion test (to determine the maximum zone of inhibition) against *E. faecalis.*

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

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 hrs, the turbidity was adjusted to be visually comparable with a 0.5 McFarland's standard giving a bacterial load of about 1-2 × 08 cfu/mL.

Agar well diffusion test

Lawn culture of *E. faecalis* was prepared on a TSA plate. Wells of 4 mm depth were prepared, which were filled with 100 µl of various concentrations of *B vulgaris*. Combinations of varying concentrations of *C. zeylanicum* and calcium hydroxide were also used. About 0.2% chlorxedine and calcium hydroxide were used as a positive control. Plates were incubated at 37°C for 24 hrs. Interpretation of diffusion results was carried out by noting the presence or absence of the zone of inhibition around the wells.

RESULTS

Table 1 shows the zone of inhibition of ethanolic extract of *B. vulgaris* against *E. faecalis*. No detectable zone of inhibition was seen for the test extract when compared to the positive control (0.2% chlorhexidine), which had zone of inhibition of 24 mm.

Table 2 shows the zone of inhibition of ethanolic extract of *C. zeylanicum* against *E. faecalis*. For *C. zeylanicum,* maximum zone of inhibition was 28 mm, when compared to positive control (calcium hydroxide), which had zone of inhibition of 12 mm. Inhibition zones for combination

Table 1: Zone of inhibition for *Beta vulgaris*

Groups	Conc – mg/ml	Zone – mm dmt
Beta vulgaris	15	0
	10	0
	5	0
Chlorhexidine	2%	24

Table 2: Zone of inhibition for *Cınnamomum zeylanicum*

Groups	Conc – Per 100 µl	Zone – mm dmt
Cınnamomum zeylanicum	100%	28
Calcium hydroxide	100%	12
C. zeylanicum+calcium hydroxide	10+90 µl	16
	20+80 µl	18
	30+70 µl	19
	40+60 µl	21

of *C. zeylanicum* and calcium hydroxide showed a marked rise with increase in concentration of *C. zeylanicum*. Showed a minimum zone of inhibition was 16 mm (10 + 90 µl) and a maximum zone of inhibition of 21 mm (40 + 60 µl).

DISCUSSION

Phytochemical analysis indicated that the root of *B. vulgaris* is rich in phytochemicals such as alkaloids, flavonoids, tannins, saponins, terpenoids, cyanogenetic glycosides, steroids, and reducing sugars. The presence of these secondary metabolites has contributed to its medicinal value as well as physiological activity. For instance, flavonoids have been shown to have antibacterial, anti-inflammatory, antiallergic, antiviral, antineoplastic properties [6].

The antimicrobial activity of Cinnamon oil is attributed to the presence of cinnamaldehyde which is the predominant active component found in Cinnamon oil [7]. Ooi *et al.* have found both cinnamon oil and cinnamaldehyde equally active on Gram-positive and Gram-negative bacteria and unicellular fungi [8]. Cinnamaldehyde was also shown to inhibit the growth of antibiotics resistant and sensitive *Helicobacter pylori* [9].

E. faecalis (causative agent for secondary root canal infection) has been considered very difficult to control as they have developed tolerance against various antimicrobial agents in routine use [10]. This calls for an urgent need to explore novel bioactive compounds, which are safer and biodegradable. In this present study, ethanolic extracts of *B. vulgaris* and *C. zeylanicum* were tested against *E. faecalis*.

In the present study, ethanolic extract of *B. vulgaris* did not show any antibacterial activity against *E. faecalis* exhibiting no zone of inhibition when compared to positive control (0.2% chlorhexidine), which had zone of inhibition of 24 mm. Study done by Aleksandra *et al.* also showed no detectable zone of inhibition with lesser concentrations of the extract [11]. Which shows that *B. vulgaris* is not active against *E. faecalis* at any concentrations.

In the present study, ethanolic extract of *C. zeylanicum* showed a significant antibacterial activity against *E. faecalis* exhibiting maximum zone of inhibition of 28 mm when compared to positive control (calcium hydroxide), which had zone of inhibition of 12 mm. Combination of *C. zeylanicum* and calcium hydroxide was shown to have an marked rise in antibacterial activity with increase in concentration of *C. zeylanicum*. Study done by Gupta *et al.* showed that *C. zeylanicum* possessed marked antibacterial activity against *E. faecalis* [12], which supports the results of the current study.

In the present study, positive control was used in order to compare the antimicrobial efficacy of ethanolic extracts of *B. vulgaris* and *C. zeylanicum*. This study was first of its kind where ethanolic extracts of *B. vulgaris* and combination of *C. zeylanicum* and calcium hydroxide along with positive control was used against *E. faecalis* in order to compare their antibacterial efficacy.

CONCLUSION

Within the limitations of this study, it may be concluded that *B. vulgaris* did not possess any antibacterial efficacy against *E. faecalis* whereas, *C. zeylanicum* can be used as an ICM or it can be combined with $CaOH_2$ for effective removal of bacterial pathogens inside the root canal.

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