RESEARCH ARTICLE

# The Efficacy of *Cinnamomum Tamala* as a Potential Antimicrobial Substance against the Multi-Drug Resistant *Enterococcus Faecalis* from Clinical Isolates

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**The present study showcases the efficacy of *Cinnamomum tamala (C. tamala)* as a potential antimicrobial substance against the multi-drug resistant *Enterococcus faecalis (E. faecalis)* bacterium. The one of the most vulnerable bacteria to this spice was *E. faecalis* but due to lack of authentic evidences the treatment with this spice was diminished and used as a culinary spice till date to provide flavor without knowing its medicinal values which has given rise to the discovery of synthetic chemical antibiotics to treat these infections. This is an attempt to resurrect the ancient phyto-pharmaceutic techniques for combating the pathogenic bacteria as our time with the synthetic chemical antibiotic drug is draining out which gives rise to the multi-drug bacteria which becomes difficult to be treated. These types of natural resources is an alternate for the toxic synthetic chemicals with zero side effects. *Enterococcus faecalis* clinical isolate from the different samples showed the best susceptibility with the essential oil extract determined by the Kirby Bauer Disc Diffussion technique with satisfactory MIC and MBC results. The results obtained for the antibacterial properties of *Cinnamomum tamala* dried leaves essential oil extract recorded were excemplary from the performed standard antibiotic assay which determines that the efficacy of the natural essential oil proved to be an excellent alternative to treat infectious bacterial diseases.**

## Introduction

The main objective of this study is to determine the efficacy of *Cinnamomum tamala* as a potential antimicrobial substance against the multi-drug resistant *Enterococcus faecalis* bacterium [**1,2**]. *Cinnamomum tamala* is a traditional spice leaves used in the preparation of culinary to provide the taste and the aroma [**3,4**]. This spice is being used in recent times without the knowledge of its medicinal values. The ancestors has utilized this spices in their culinary for the proper bowel function and it also helped them to live healthy as it eradicated many pathogenic bacteria [**1,5,6**]. The one of the most vulnerable bacteria to this spice was *E. faecalis* but due to lack of authentic evidences the treatment with this spice was diminished and used as a culinary spice till date to provide flavor without knowing its medicinal values which has given rise to the discovery of synthetic chemical antibiotics to treat these infections [**1,3,7,8**]. The abuse of synthetic chemical antibiotics due to the abundant administration of drugs resultant in the mutant bacteria which are resistant to drugs [**1,5,9**]. These bacterium is called as the multi-drug organisms and one such bacterium of the present era is the *Enterococci* [**10**]. Many species of *Enterococci* has become drug resistant due to the constant ignorance of the WHO alerts [**2,7,8,10**]. *Enterococcus faecalis* is a nosocomial pathogen especially causes urinary tract infections and also found to be an important bacterium for dental caries [**2,8,9,10**]. The source of dental caries by this bacterium is still not evident and the research is underway [**7,8,11**]. Bacteria colonizing the root canal system play an essential role in the pathogenesis of periradicular lesions [**7,8**]. Contradictory to the primary endodontic infections, which are polymicrobial in state and inflenced by Gram negative anaerobic rods, the bacterium involved in secondary infections are composed of one or few bacterial species especially the Gram positive *Enterococci* [**7,8,10**]. The consistency of *Enterococcus faecalis* found in persistent

infections with periradicular lesions was found to be higher than in primary endodontic infection [**2,7**]. The concept of this work is to determine the antimicrobial activities of *Cinnamomum tamala* against the multi-drug resistant *Enterococcus faecalis* isolated from different clinical specimens by performing standard microbiological methods [**1,5,6,12,13**]. The chemical components composed of monoterpenes such as the trans-sabinene hydrate, (*Z*)-*β*-ocimene, myrcene, *α*-pinene and *β*-sabinene with sesquiterpenes such as germacrene A and *α*-gurjunene constitutes as a potential antimicrobial components of the spice [**3,4**]. This is an attempt to resurrect the ancient phyto-pharmaceutic techniques for combating the pathogenic bacteria as our time with the synthetic chemical antibiotic drug is draining out which gives rise to the multi-drug bacteria which becomes difficult to be treated [**1,5**]. These types of natural resources is an alternate for the toxic synthetic chemicals with zero side effects.

## Experimental

### *Materials*

Dried *Cinnamomum tamala* leaves, clinical samples *of Enterococcus faecalis,* thioglycolate broth, tryptic soy agar, De Man Rogosa, and Sharpe broth, Heart Infusion broth, Bile esculin agar, Columbia Agar, Mueller Hinton agar, Slidex-Strep Kit, API 20 Strep test kit, Dinitrogen and Sodium Sulfate, Gram's stain kit, Whatman No:1 filter paper, Standard antibiotic discs and Multi disc dispenser were used.

### *Methods*

The following standard techniques were employed for the determination antimicrobial properties of *Cinnamomum tamala* against the clinically isolates of multi-drug resistant *Enterococcus faecalis*.

### *Extraction process of Cinnamomum tamala essential oil*

The dried *Cinnamomum tamala* leaves were purchased from the local Indian spice market. The hydro-distilled extract of the leaves were obtained by preparing homogenized mixture of fine powder using a mechanical grinder and obtaining the concentration by filtering with the Whatman number one filter paper which was treated with a pure dinitrogen along with anhydrous sodium sulfate to store at 5ºC in an amber colored bottles. The moisture content of the leaves was around 5.24g/100g on a dry basis and essential oil yield in the raw material was approximately 1.216 mL essential oil/100g of dried *Cinnamomum tamala* leaves.

### *Isolation and purification of Enterococcus faecalis*

The clinical samples from the versatile infection sources of *Enterococcus faecalis* was collected by employing the standard microbiological sample collection methods and were processed in the microbiological laboratory by carefully following the aseptic techniques to isolate the *Enterococcus faecalis*. A loopful of sample was inoculated in to a sterile thioglycolate broth and incubated overnight incubation at 37ºC. A loopful of sample from the incubated broth was tested by performing Gram's test which showed Gram positive *cocci* in short chains and pairs. A loopful of the sample was inoculated on tryptic soy agar to observe the characteristic resistance towards vancomycin disc. The broth substrate test was applied to determine the enzymatic activity of pyrrolidonylarylamidase. The De Man-Rogosa, and Sharpe broth was used to check the gas production due to glucose fermentation while the Heart Infusion broth used for the carbohydrate determination test. A loopful of sample was inoculated on Bile esculin agar and after overnight incubation at 37ºC to observe the blackening of the media due to the hydrolysis of Entero*coccus faecalis.* The following standard microbiological confirmatory tests were done for the identification of *Enterococcus faecalis*. The sample was cultured on the Columbia Agar incorporated with 5% of sheep's blood. The presence of antigen-D in the Entero*coccus faecalis* was determined by Slidex-Strep Kit. The rapid slide test was performed to determine the ability of the organism to produce enzyme catalase by mixing a loopful of bacterium with 3% of hydrogen peroxide in a glass slide. The API 20 strep tests was also used as a confirmatory test for the identification of *Enterococcus faecalis*.

### *Determination of the antimicrobial activity of the dried Cinnamomum tamala leaves essential oil extract*

The standard microbiological techniques were employed to determine the anti-microbial activity of dried *Cinnamomum tamala* leaves essential oil extract against the isolated clinical specimens of *Enterococcus faecalis*.

## Kirby-Bauer disc diffusion method

The Kirby-Bauer disc diffusion methodis best suitable for routine testing in a clinical laboratory where a large number of isolates are tested for susceptibility to numerous antibiotics where the Mueller Hinton agar plate is uniformly inoculated with the test organism a paper disc impregnated with a fixed concentration of standard antibiotic along with the antibiotic from the extract was impregnate on the agar surface and incubated overnight at 37ºC. After incubation the growth of the organism and diffusion of the antibiotic results in the formation zone of inhibition in which the quantity of antibiotic surpasses inhibitory concentrations. The diameter of the inhibition zone is a function of the amount of drug in the disc and sensitivity of the bacterium. The test was repeated for all the isolates respectively.

### Tube dilution method - Minimum Inhibitory Concentration (MIC)

The MIC standard tube dilution method was employed [**14**] with separate set ups for all the isolates to determine the efficacy of dried *Cinnamomum tamala* leaves essential

oil extract inhibit the growth isolated clinical specimens of *Enterococcus faecalis*.

### Minimum Bactericidal Concentration method (MBC)

The MBC is the lowest concentration of an antibacterial agent required to kill a particular bacterium which be determined from broth dilution MIC tests by sub culturing to agar plates that do not contain the test agent. The standard MBC method was employed with separate set ups for all the isolates to determine the efficacy of dried *Cinnamomum tamala* leaves essential oil extract kill the growth isolated clinical specimens of *Enterococcus faecalis*.

## Results and discussion

The results obtained for the antibacterial activities of dried *Cinnamomum tamala* leaves essential oil extract against the clinical isolates of *Enterococcus faecalis* were exemplary when compared to that of standard prophylactic synthetic chemical antibiotics available commercially. **Table 1** indicates that, the *Enterococcus faecalis* clinical isolate from the urine sample showed the best susceptibility with the essential oil extract determined by the Kirby Bauer Disc Diffusion results of 27mm diameter zone formation sensitivity whereas the clinical isolate of the *Enterococcus faecalis* from the wound sample showed the least susceptibility with the essential oil extract determined by the Kirby Bauer Disc Diffusion results of 20mm diameter zone formation sensitivity. The clinical isolates of *Enterococcus faecalis* from the stool sample showed the second best susceptibility with the essential oil extract determined by the Kirby Bauer Disc Diffusion results of 25mm diameter zone formation sensitivity followed by the clinical isolates of the *E. faecalis* from catheter sample with 24mm diameter zone formation sensitivity and abscess sample with 22mm diameter zone formation sensitivity. All the clinical isolates of *Enterococcus faecalis* showed the best susceptibility towards the dried *Cinnamomum tamala* leaves essential oil extract when compared to that of standard synthetic chemical antibiotics available commercially as shown in **Table 2**. Many standard antibiotics shown susceptibility towards the urine sample and stool sample but the diameter of the zone was lesser when compared with that of the dried *Cinnamomum tamala* leaves essential oil extract. The clinical isolates isolates of the *Enterococcus faecalis* from the wound sample as well as abscess and catheter sample shown resistant towards some of the standard antibiotics. The clinical isolates of the *Enterococcus faecalis* from the dental carries sample showed mixed susceptibility with resistant and intermediates towards the standard synthetic chemical antibiotics available commercially where as shown a promising susceptibility towards dried *Cinnamomum tamala* leaves essential oil extract which is a major boost towards the better future for the dentistry industry in combating the infection which is considered one of the biggest concerns due to the non-availability of a single standard synthetic antibiotic rather depending on the broad spectrum drugs with the combination of antibiotics due to the unknown source of infection with the *Enterococcus faecalis* as the dental carries. The prolonged use of the broad spectrum drugs with the combination of antibiotics gives rise to the multi-drug resistant bacterium and the nature blessed dried *Cinnamomum tamala* leaves essential oil extract is a boon which can be utilized for the prophylaxis of the dental carries. Though this is not a new rather an ancient forgotten technique which needs to be dusted and resurrected. The bark of Cinnamomum species provides a good source of essential oil which serves as a proven antimicrobial activity against several pathogenic bacteria in vitro [**15,16**].

**Table 2.** Comparative enumeration of MIC and MBC values of *Cinnamomum tamala* leaves essential oil extract with the standard antibiotics against the clinical isolates of *Enterococcus faecalis.*

| Clinical isolate Specimens | MIC µ/ml | MBC µ/ml |
|---|---|---|
| Urine Sample | 0.25 | 0.5 |
| Stool Sample | 0.25 | 0.5 |
| Wound Sample | 0.50 | 0.75 |
| Abscess Sample | 0.75 | 1 |
| Catheter Sample | 1 | 1.25 |
| Dental Carries sample | 1 | 1.25 |

**Table 1.** Comparative study of Kirby Bauer Disc Diffusion results of *Cinnamomum tamala* leaves essential oil extract with the standard antibiotics against the clinical isolates of *Enterococcus faecalis*.

| Antiibiotic Discs | Urine Sample | Stool Sample | Wound Sample | Abscess Sample | Catheter Sample | Dental Carries sample |
|---|---|---|---|---|---|---|
| Essential Oil Extract | 27 mm Sensitive | 25 mm Sensitive | 20 mm Sensitive | 22 mm Sensitive | 24 mm Sensitive | 23 mm Sensitive |
| Ampicillin | 20 mm Sensitive | 22 mm Sensitive | 15 mm Intermediate | 5 mm Resistant | 5 mm Resistant | 13 mm Intermediate |
| Fosfomycin | 22 mm Sensitive | 5 mm Resistant | 5 mm Resistant | 20 mm Sensitive | 21 mm Sensitive | 18 mm Sensitive |
| Synercid | 14 mm Intermediate | 21 mm Sensitive | 3 mm Resistant | 1 mm Resistant | 5 mm Resistant | 15 mm Intermediate |
| Streptomycin | 19 mm Sensitive | 5 mm Resistant | 5 mm Resistant | 5 mm Resistant | 18 mm Sensitive | 1 mm Resistant |
| Linezolid | 5 mm Resistant | 15 mm Intermediate | 14 mm Intermediate | 1 mm Resistant | 1 mm Resistant | 5 mm Resistant |
| Gentamicin | 21 mm Sensitive | 5 mm Resistant | 20 mm Sensitive | 5 mm Resistant | 5 mm Resistant | 5 mm Resistant |
| Imipenem | 5 mm Resistant | 3 mm Resistant | 5 mm Resistant | 5 mm Resistant | 2 mm Resistant | 19 mm Sensitive |
| Penicillin | 24 mm Sensitive | 15 mm Intermediate | 19 mm Sensitive | 19 mm Sensitive | 18 mm Sensitive | 15 mm Intermediate |

The tube dilution method for the MIC shown excellent results with all the clinical isolates of the *Enterococcus faecalis* (**Table 2** and **Fig. 1**). The best value recorded was for the clinical isolates of the *Enterococcus faecalis* from the urine and stool samples with the values of 0.25µ/ml respectively followed by the wound sample 0.5 µ/ml and abscess sample 0.75 µ/ml whereas the catheter sample and dental carries sample shown the value of 0.1 µ/ml which is also acceptable.

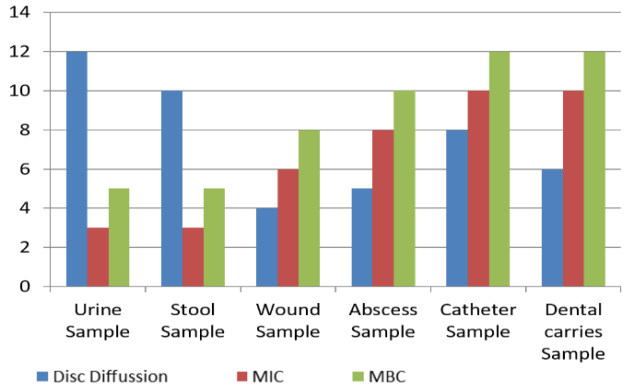


**Fig. 1.** Comparative chart of comparative antimicrobial activity of *Cinnamomum tamala* leaves essential oil extract with the standard antibiotics against the clinical isolates of *Enterococcus faecalis.*

## Conclusion

The antimicrobial activity results achieved from this work for the *Cinnamomum tamala* dried leaves essential oil extract against the multi-drug resistant clinical isolates of *Enterococcus faecalis* from different specimens suggests that the nature blessed substances were better remedy for the treatment of bacterial infections when compared with that of standard synthetic antibiotic chemicals. The WHO has alerted the abuse of antibiotics but the ignorance among the community given rise to the multidrug resident bacterial infections especially from the nosocomial infections which is believed to be difficult to treat. The standard chemical synthetic drugs not only treats but also causes serious side effects. This is an attempt to discover the alternate for these toxic substances by replacing it with the study of essential oil extract from the nature blessed spices which has better potential of antibacterial activities with zero side effects. In future more studies need to be conducted to find out the hidden treasure of the nature for the treatment of many dangerous diseases. This study can be stepping stone for the better future to replace the toxic synthetic chemical antibiotics by the natural essential oil extracts to save the mankind.

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### Author's contributions

All authors have made substantial contribution to the work and approved it for publication. No conflict of interest.

**Keywords**

Antimicrobial substance, *cinnamomum tamala*, bay leaf, multi-drug resistant, *enterococcus faecalis* bacterium.

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