

# Bactericidal activity of acetone extract of *Alpinia galanga* on multidrug resistant clinical isolates of *Enterococcus faecalis*

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## Abstract

**Introduction:** The objective of the study was to evaluate the inhibitory activity of five traditional Indian medicinal herbs against multidrug resistant clinical isolates of *E. faecalis*. **Methods:** Sixty five *E. faecalis* derived from clinical specimens were tested for their resistance pattern to 7 antibiotics by disc diffusion method. Extracts from 5 medicinal plant parts were obtained and tested for inhibitory activity against multi drug resistant clinical *E. faecalis* isolates. The minimum bactericidal concentration (MBC) for the active plant extract exhibiting highest activity towards the susceptible isolates was determined by macro broth dilution method followed by sub culturing on agar plate. **Results:** All the clinically derived *E. faecalis* isolates were resistant to two or more antibiotics. Acetone extract of *Alpinia galanga* inhibited 67.69% of *E. faecalis* and concentration of <5.0mg/ml was found to exert bactericidal activity against multi drug resistant isolates. **Conclusion:** Acetone extract of *A. galanga* exhibit bactericidal activity against multi-drug resistant clinical isolates of *E. faecalis* and hence could be potential phytotherapeutic candidate.

**Keywords:** *A. galanga*, drug resistance, *E. faecalis*, inhibition, medicinal plants

## Introduction

India is a land of rich heritage for employing medicinal plants in treating illness among its diverse indigenous cultures. Several of these folklore medicines, employing herbs and medicinal plants are validated through scientific research and made accessible to the global population. Therapy through plant products benefits its users by providing generalised immunostimulatory and immunomodulatory effects apart from abating the side effects of antibiotics and monetary expenditure for cure [1, 2].

Enterococci are one among the leading nosocomial pathogens commonly associated with urinary tract infections, blood stream infections and catheter related infections. Of more than a dozen species in the genus, *Enterococcus faecalis* is involved in 70 – 80% of clinical infections [3]. Infections by multidrug resistant *E. faecalis* encompassing even the last line drug of choice are emerging around the globe, accounting for increase in morbidity and mortality rates [4,5].

*E. faecalis* is an ideal candidate which deserves a new phytochemical therapeutic agent considering their significance in nosocomial infections and antibiotic resistance. This study unearths the inhibitory activity of five traditional Indian medicinal plants against clinical isolates of *E. faecalis*. The study is novel and innovative as there are no existing literatures proving the inhibitory potential of acetone extract of *Alpinia galanga* and its minimal bactericidal concentration (MIC) against drug resistant clinical isolates of *E. faecalis*.

## Materials and Methods

**Place of study and type of study:** The test-tube lab research study was carried in the Microbiology laboratory of Rajah Muthiah Institute of Health Science to demonstrate the inhibitory activity of traditional medicinal plants on multi-drug resistant *E. faecalis* obtained from clinical specimens.

**Isolation of *E. Faecalis*:** The clinical specimens– urine, blood and pus submitted for bacteriological investigation to the Microbiology laboratories at Rajah Muthiah Institute of Health Sciences were employed for

Manuscript received: 8<sup>th</sup> May 2018  
Reviewed: 18<sup>th</sup> May 2018  
Author Corrected: 24<sup>th</sup> May 2018  
Accepted for Publication: 27<sup>th</sup> May 2018

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the study. The specimens were inoculated on routine diagnostic culture media and Pfizer's selective enterococcus agar (Hi-Media Laboratories, India). Following aerobic incubation of the inoculated culture plates, colonies morphologically resembling *Enterococcus* were characterized and identified as *E. faecalis* by standard microbiological and biochemical procedures [6]. The isolates were stored at -20 °C in 20% glycerol Brain Heart Infusion broth (Sisco Research Laboratories Pvt. Ltd., India) until further use.

**Detection of antibiotic resistance for clinical *E. faecalis* isolates:** *E. faecalis* obtained from clinical specimens were studied for their antibiotic resistance pattern by disc diffusion test and the results were interpreted according to CLSI, 2012 [7]. The antibiotics (Hi-Media Laboratories, India) tested includes Penicillin (10 units), Ampicillin (10 µg), Gentamicin (high content) (120 µg), Streptomycin (high content) (300 µg), Ciprofloxacin (5 µg), Erythromycin (15 µg) and Vancomycin (30 µg) .

**Plant extracts employed for study:** The plants were chosen based on i) their documented use as antimicrobials in literature and ii) traditional use of these plants among the regional folklore against infections [8]. The plants employed in the study (Table 1) were harvested in Cuddalore district and taxonomically identified. Crude extracts from the plants were extracted with solvents mentioned in Table 1 as per the method described by Eloff [9]. The extracts were stored in the form of dried powder at -20 °C until it was used for further biological evaluation.

**Determination of inhibitory activity of the plant extracts by disk diffusion test:** The plant extracts were tested for their inhibitory activity against the 25 multidrug resistant *E. faecalis* isolates derived from clinical specimens. A single colony from overnight growth on blood agar plates were inoculated in Brain heart infusion broth and incubated at 37 °C for 4 h.

This bacterial suspension was adjusted to 0.5 McFarland Standard and used as inoculum to obtain a lawn culture on Mueller Hinton agar plates (Sisco Research Laboratories Pvt. Ltd., India). Discs (6 mm in diameter) obtained from Whatmann No.1 filter paper was soaked with the plant extracts reconstituted with 20% DMSO (to obtain a concentration of 5mg/ml) and placed on the inoculated plates. Suitable controls were included. Following incubation at 37 °C for overnight period, the plates were evaluated for the presence of zone of inhibition around the discs. The extracts were considered to exhibit anti-*E. faecalis* activity if a inhibition zone

>10mm was observed was found around the extract impregnated disc.

**Determination of the minimum bactericidal concentration (MBC) of *A. galanga* against *E. faecalis* isolates:** Macrobroth dilution test was employed to determine the minimum inhibitory concentration (MIC) of *A. galanga* susceptible multidrug resistant *E. faecalis* isolates according to the protocol of CLSI, 2012 [7]. Extract obtained from *A. galanga* was diluted with Mueller Hinton broth in test tubes to obtain a final concentration of 2.0, 2.5, 3.0, 3.5, 4.0, 4.5mg/ml. *E. faecalis* adjusted to  $1.5 \times 10^8$  CFU/ml was used as inoculum at a quantity of 1ml on the tubes containing *A. galanga* extract. Mueller Hinton broth containing *E. faecalis* suspension alone and *A. galanga* extract without the inoculum were included as controls. The minimum concentration of *A. galanga* extract which inhibited the visible growth of *E. faecalis* was considered as MIC value. The MBC of the extract was determined by spot inoculating 10 µl of the suspension from macrobroth dilution tubes lacking the growth of *E. faecalis*, on Mueller Hinton agar plates. Following incubation at 37 °C for overnight period the minimum concentration of the extract that produced no visible colonies was recorded as the MBC value for the strain tested.

## Results

**Isolation of *E. faecalis* from clinical specimens:** Sixty five isolates of *E. faecalis* was isolated from urine, pus, blood and ascitic fluid at the isolation rate of 44%, 28%, 16% and 12% respectively.

**Antibiotic resistance pattern of clinical *E. faecalis* isolates:** Antimicrobial resistance pattern for *E. faecalis* isolates showed 72% of isolates to be high-level gentamicin resistant and 68% of isolates demonstrated resistance to 2 antibiotics - erythromycin and ciprofloxacin. None of the isolates were found vancomycin resistant (fig. 1).

The clinical isolates of *E. faecalis* were found to be resistant to a combination of 2 or more antibiotics (fig. 2). Combined resistance to three antibiotics was the frequent observation and was seen among 35% of isolates. However, none of the isolates were found resistant to all the 7 antibiotics used in the study.

**Plant extracts used and their inhibitory activity against *E. faecalis*:** Of the 5 plant extracts studied for their inhibitory activity against 65 antibiotic resistant clinical isolates of *E. faecalis* by disc diffusion test, *A. galanga* was found to inhibit 67.69% of isolates

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followed by *M. fragrans* which inhibited 47.69% of isolates (fig.3). Since, *A. galanga* exhibited inhibitory activity on the majority of the *E. faecalis* isolates used in our study, this extract and the strains inhibited by the extract were chosen for further studies.

**Minimum bactericidal concentration (MBC) of *A. Galangal* extract for the test isolates:** The 44 *E. faecalis* isolates which were inhibited by *A. galanga* extract as determined by disc diffusion test was further studied for the MBC value of *A. galanga* extract required to inhibit them. A concentration of 3.0 mg/ml of *A. galanga* extract was found to be the MBC value for a majority of *E. faecalis* isolates (36.3%). MBC value for more than 50% of our isolates was  $\leq 3.0$  mg/ml (table 2).

## Discussion

In our study, *E. faecalis* was commonly isolated from urine, followed by pus and blood samples. The predominance of this pathogen in urinary tract infections and pyogenic infections are reported widely [10, 11]. The 1<sup>st</sup> line drug of choice for treating enterococcal infections is with a synergistic combination of beta lactam and aminoglycoside antibiotics [12]. In our study, 42% and 27% of isolates exhibited resistance to beta lactam class of antibiotics - penicillin and ampicillin respectively. Further, high-level resistance to aminoglycosides- gentamicin and streptomycin was noted with 72% and 46% of isolates respectively. With the prevalence of higher resistance rates towards the synergistic antibiotic combinations and the occurrence of multidrug resistance among our clinical isolates, the treatment options for infections demands the role of 2<sup>nd</sup> and last line drug of choice which are also noted for their adverse effects [10, 13].

The isolation of multidrug resistant *E. faecalis* from clinical specimens in our geographical locality makes the situation imperative to find an alternate treatment option. The phytochemicals of the extracts among the 5 plants selected in the study have proven antibacterial activity against common Gram positive and Gram negative pathogens [8, 14, 15]. In our study, the extracts from the five selected plants exhibited inhibitory activity on 13.84% - 67.69% of *E. faecalis* isolates with *A. galanga* demonstrating inhibition on a majority of isolates. *A. galanga* is widely cultivated medicinal herb

## Conclusion

As an attempt to elude from the repercussion of antibiotic usage for pathogens which exhibit multiple drug resistance, an extensive search is made towards herbal therapy. The study documents the inhibitory activity of acetone extract of *A. galanga* to be superior compared with other medicinal plants for their anti-*E. faecalis* activity. Further, bactericidal

in India and the traditional medicinal systems have used the rhizome for diuretic, expectorant, carminative purposes and in treating tubercular glands, bronchitis, kidney and heart diseases [16]. The chief chemical constituent of rhizome is flavonoid which exerts antibacterial activity through inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of the attachment and biofilm formation, inhibition of the porin on the cell membrane, alteration of the membrane permeability and attenuation of the pathogenicity [17, 18].

The inhibitory activity of *M. fragrans* succeeds *A. galanga* in our study, and was active on 47.69% of *E. faecalis*. Methanol extracts of *M. fragrans* was earlier reported to have strong antibacterial activity against multi-drug resistant *S. typhi* [19]. Extracts from *A. graveolens*, *B. serrate* and *H. indicus* inhibited 9%, 41.53% and 18.46% of our isolates. Literatures citing the inhibitory activity of these medicinal plants against several Gram positive, Gram negative bacterial pathogens, yeast and fungi are recorded earlier [20 - 22].

The acetone extract of *A. galanga* employed in our study proved to exhibit inhibitory activity on *E. faecalis*. However previous studies indicate ether, ethyl acetate, aqueous extract and essential oil of *A. galanga* for significant antibacterial activity against *S. aureus* and *S. pyogenes* [23]. In our study, the MBC of acetone extract of *A. galanga* for the clinical isolates of *E. faecalis* ranged between 2.0 - 4.5 mg/ml, and the concentration of 3.0 mg/ml of the extract inhibited 36.3% of isolates.

This concentration was found to be lesser when compared to the study of Okonogi *et al.* wherein 8mg/ml was the MBC of essential oil of *A. galanga* against *S. aureus* ATCC 25923 [24]. In another study involving human pathogens, an active component 1'-acetoxy chavicol acetate from the ethyl acetate extract of *A. galanga* demonstrated antibacterial activity against *P. acne* with the MIC and MBC values of 156 and 312  $\mu$ g/ml [25]. A study by Warit *et al.* showed the extract of *A. galanga* and its major component S-enantiomer of acetoxy chavicol acetate exhibited anti-tubercular activity on clinical isolates at 2.0  $\mu$ g/mL [26].

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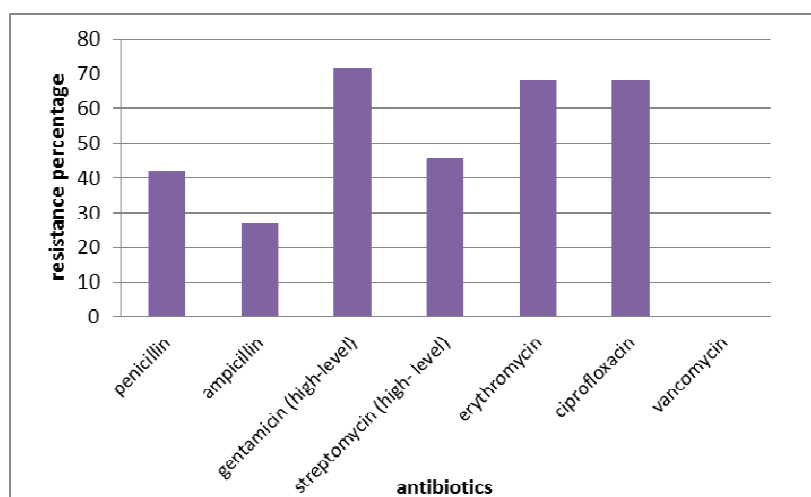
activity was demonstrated at concentration of <5.0mg/ml against all the multidrug resistant isolates used in our study. This substantiates the efficacy of *A.galanga* as an alternative therapeutic option to multiple drug resistant *E. faecalis*.

**Table-1: Plant extracts used for the study**

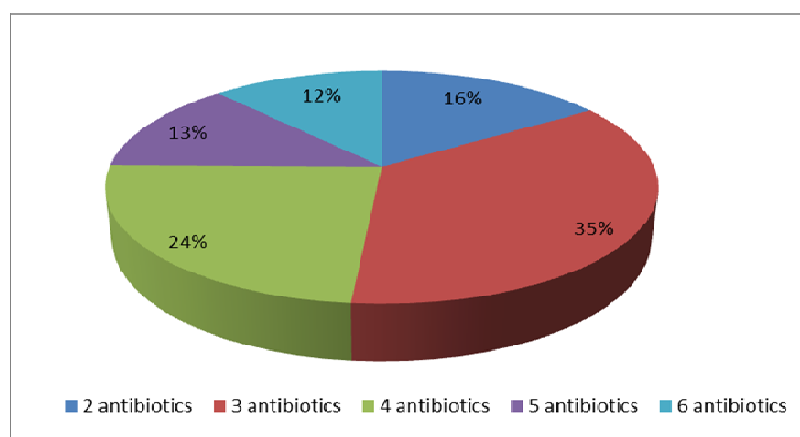
Plant(scientific name)	Family	Parts used	Solvent for extract preparation
<i>Anethumgraveolens</i>	Apiaceae	Fruits	Water
<i>Alpinia galanga</i>	Zingiberaceae	Rhizome	Acetone
<i>Boswellia serrate</i>	Burseraceae	Gum resin	Methanol
<i>Hemidesmus indicus</i>	Asclepiadaceae	Root	Chloroform
<i>Myristica fragrans</i>	Myristicaceae	Seeds	Methanol

**Table-2: MBC of *A. galanga* extract for *E. faecalis***

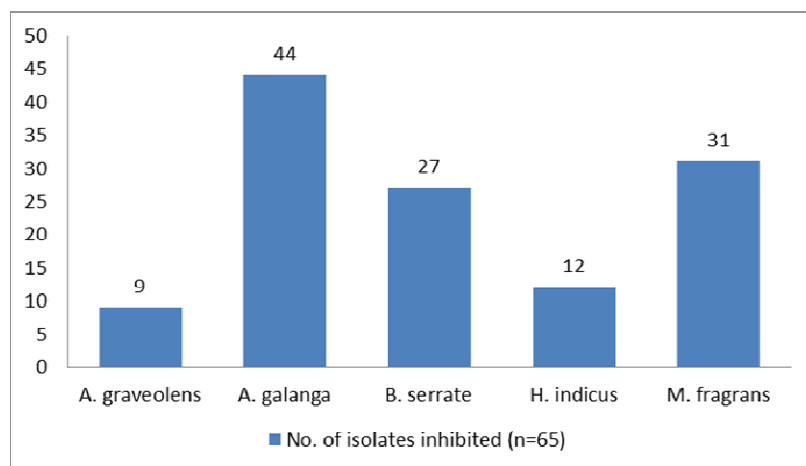
MBC values of <i>A. galanga</i> (mg/ml)	No. of <i>E. faecalis</i> isolates inhibited by the extract (Percentage of isolates) (n =44)
2.0	2 (4.5)
2.5	6 (13.6)
3.0	16 (36.3)
3.5	14 (31.8)
4.0	4 (9.1)
4.5	2 (4.5)



**Fig-1: Antibiotic resistance pattern of clinical *E. faecalis* isolates**



**Fig-2:Percentage of *E. faecalis* isolates demonstrating resistance to a combination of 2 or more antibiotics**



**Fig-3: Inhibitory activity of plant extracts on *E. faecalis* isolates**

**Acknowledgements:** The authors wish to acknowledge the technical assistance rendered by Mr. R. Venkatesan and the help for the laboratory work by Mr. Raman, Rajah Muthiah Dental College and Hospital, Annamalai University.

#### Authors Contributions

R. Arularasi Aberna and K.Prabhakar contributed to the conception and the design of the experiment.

R. Arularasi Aberna performed the experimental analysis, interpretation and manuscript writing.

K.Prabhakar critically evaluated the paper.

**Funding:** Nil, **Conflict of interest:** None initiated

**Permission from IRB:** Yes

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### How to cite this article?

Aberna R.A, Prabhakar K. Bactericidal activity of acetone extract of *Alpinia galanga* on multidrug resistant clinical isolates of *Enterococcus faecalis*. *Trop J Path Micro* 2018;4(2):201-206. doi: 10.17511/jopm.2018.i2.15

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