

Research Article

ANTIBACTERIAL ACTIVITY OF ACACIA CATECHU LEAF AND BARK EXTRACT AGAINST *E.FAECALIS*Lakshmi.T^{*1}, Ravishankar.P², Anitha Magesh³¹Assistant Professor, Department of Pharmacology, Saveetha Dental College & Hospitals, Chennai, India.²Reader, Department of conservative dentistry, Thai moogambigai Dental College, Chennai, India.³Senior lecturer, Department of Biochemistry, Saveetha Dental College & Hospitals, Chennai, India.*Corresponding author's E-mail: lakshmi085@gmail.com

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ABSTRACT

The Objective of our study is to investigate the *in vitro* anti bacterial activity of ethanolic, aqueous and acetone leaf and bark extract of *Acacia catechu willd* against *Enterococcus faecalis*. The inhibitory effect of *Acacia catechu willd* was tested against *E.faecalis* at different concentrations by using the Broth dilution method. The ethanolic, aqueous, acetone leaf and bark extract of *Acacia catechu* exhibited antibacterial activity against *E.faecalis* tested at various concentrations. Leaf and bark extract of *Acacia catechu willd* was found to be bactericidal in action against tested bacterial strain.

Keywords: *Acacia catechu willd*, Anti bacterial activity, MBC, *Enterococcus faecalis*.

INTRODUCTION

Herbal products have been used since ancient times in folk medicine for treatment of various disease conditions. *Acacia catechu Willd* (Family: Fabaceae and subfamily: Mimosoideae.) is a potent medicinal plant. The extracts of *Acacia catechu* exhibits various pharmacological effects like antipyretic, anti-inflammatory, antidiarrhoeal, hypoglycemic, hepatoprotective, antioxidant and antimicrobial activities.^{1, 2-6}

Enterococci, a Gram positive commensal, coccoid bacteria inhibiting the alimentary canals of humans and animals, are now proved to be organisms that are capable of causing life-threatening infections in humans, especially in the hospital environment. *Enterococci* occur singly, in pairs or short chains, the cells are ovoid. They are present in the colon of all humans in numbers as high as colony-forming units per gram of faeces and are recognized as facultative anaerobes. *Enterococci* grows at temperatures ranging from 10-45°C, at pH 9.6 and in 6.5 % NaCl.⁷⁻⁹

The major cause of endodontic failure is the survival of *E.faecalis* in the apical portion of root filled teeth. *E.faecalis* can adhere to the root canal walls, accumulate, and form communities organized in biofilm, which helps it resist destruction by enabling the bacteria to become more resistant to phagocytosis, antibodies, and antimicrobials than non-biofilm producing organisms.¹⁰ In dental practice herbs have been used for its anti-inflammatory, antibiotic, analgesic and sedative properties. In endodontics to eliminate *E.faecalis* NaOCl and chlorhexidine are commercially used but due to few disadvantages of these root canal irrigants the trend of recent medicine attends to use biologic medication extracted from natural products.¹¹

Hence an attempt was taken to find out the Antibacterial potency of various solvent form of *Acacia catechu willd* against *E.faecalis*.

MATERIALS AND METHODS

Plant material

Leaf and Bark extract of *Acacia catechu willd* were obtained from Green Chem. Herbal Extract & Formulations, Bangalore, India.

Test microorganism

Bacterial strain used in the study was *Enterococcus faecalis* (ATCC 29212). The organism has obtained from Department of Microbiology, Saveetha Dental College & Hospitals, Chennai.

Methodology

The herbal extract 200mg were weighed aseptically into a sterile tube and dissolved in 2ml of sterile Brain heart infusion agar media (BHA). From the stock solution various concentrations were prepared, viz., 62µg, 125 µg, 250 µg, 500 µg/100µl, 1mg, 5mg, 10mg/100µl respectively in to wells of micro plates. 100µl of these concentration were taken and the plates were incubated at 37°C for 24hrs.

Screening of antibacterial activity

The tested organism was grown in Brain heart infusion agar medium (Hi media, Mumbai) for 24hrs at 37°C and concentration was adjusted to 0.5 Macfarland standard.¹²⁻¹⁴ The above concentration of extracts were taken in 100µl quantities in a U bottom micro culture plates. 100µl of the bacterial suspension was added to each well. Control well received plain broth without plant extract. The plates were kept in sealed covers and incubated at 37°C overnight and growth/no growth was detected. All the tests were done in triplicate to minimize the test error.



Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of herbal extracts against tested microorganism was determined by broth dilution method.¹⁵ A series of two-fold dilution of each extract (62 µg/100µl to 10mg/100µl) was made in to which 100µl of the standardized bacterial suspension containing 10⁶ organisms was made in Tryptic soy broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1990).¹⁶ The control well received plain broth without herbal extract. The plates were incubated at 37°C for 24 hours and observed for visible growth. As the extracts were colored, MIC could not be read directly by visual methods. Hence subcultures from all the wells were made and growth/no growth is detected, then the MBC were obtained.

Minimum bactericidal concentration (MBC)

The MBCs were determined by selecting wells that showed no growth. The least concentration, at which no growth was observed, was noted as the MBC.

RESULTS AND DISCUSSION

Enterococci, an facultative anaerobe is recognized as an opportunistic pathogen.¹⁷ It is a major cause for urinary tract infections.¹⁸ among *Enterococci*, the most clinically abundant species is *Enterococcus faecalis*, which accounts for approximately 5%–8% of nosocomial associated bacteremia.¹⁹

Various literatures reveal the antibacterial efficacy of herbal extracts against *E.faecalis*. Studies have shown that 3% to 5 % sodium hypochlorite (NaOCl) is effective for all presentations of *E.faecalis* including its existence as a biofilm. EDTA when used along with NaOCl is effective in removing the inorganic portion of the dentin. Smear layer removal provides access of irrigants to the dentinal tubules 2-min rinse of 2% chlorhexidine liquid can be used to remove *E.faecalis* from the superficial layers of dentinal tubules up to 100 µm. 2% chlorhexidine gel is effective at completely eliminating *E.faecalis* from dentinal tubules for 15 days.²⁰

In the *in vitro* study it was revealed that the extract at different concentrations exhibited antibacterial activity against the bacterial strain tested. The ethanolic leaf and bark extract of *Acacia catechu* exhibited a high degree of activity against the organism tested when compared to aqueous and acetone extract. *Acacia catechu* ethanolic leaf and bark extract showed no growth at a concentration of 1mg/ml, 5mg/ml and 10mg/ml whereas, the acetone bark, leaf and aqueous leaf extract of *Acacia catechu* showed no growth at a concentration of 5mg/ml and 10mg/ml, the aqueous bark extract of *Acacia catechu* exhibited no growth at a concentration of 1mg/ml, 5mg/ml and 10mg/ml respectively.

The presence of No growth is an indication of high effectiveness of the extract whereas presence of Growth indicates the less effectiveness of the extract, which was represented in Table 1.

Table 1: Antibacterial activity of *acacia catechu willd* against *e.faecalis*

	62µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1mg/ml	5mg/ml	10mg/ml	Control	MBC
<i>Acacia catechu</i> leaf									
Ethanolic	++	++	++	++	--	--	--	++	1mg/ml
Aqueous	++	++	++	++	++	--	--	++	5mg/ml
Acetone	++	++	++	++	++	--	--	++	5mg/ml
<i>Acacia catechu</i> Bark									
Ethanolic	++	++	++	++	--	--	--	++	1mg/ml
Aqueous	++	++	++	++	--	--	--	++	1mg/ml
Acetone	++	++	++	++	++	--	--	++	5mg/ml

++ = Growth -- = No Growth (Indicates the MIC/MBC)

CONCLUSION

The data suggest that the antibacterial activity of the leaf and bark extract of *Acacia catechu willd* is an indication of its broad spectrum antibacterial potential which may be helpful in eradicating *E.faecalis* for the management of Root canal failure that occurs frequently during Endodontic procedure.

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