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Research Article

IN-VITRO ANTIBACTERIAL SCREENING OF THE PHYTOCHEMICAL EXTRACTS AGAINST *E. FAECALIS*

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ABSTRACT

Aim: The Objective of our study is to investigate the *in vitro* antibacterial activity of acetone bark and seed extract of *Acacia catechu willd* and *Aesculus hippocastanum*(Horse chest nut) against *Enterococcus faecalis*.

Materials and Methods: The inhibitory effect of acetone bark extract of *Acacia catechu willd* and *Aesculus hippocastanum* seed extract were tested against *E.faecalis* by using the Broth dilution method.

Results: The acetone bark extract of *Acacia catechu* exhibited antibacterial activity against *E.faecalis* with minimum bactericidal concentration of 10mg/ml whereas, *Aesculus hippocastum* acetone seed extract does not show any antibacterial activity at concentration ranging from 62µg/ml to 10mg/ml.

Conclusion: The acetone bark extract of *Acacia catechu willd* was found to be bactericidal in action against tested bacterial strain.

Keywords: *Acacia catechu willd, Aesculus hippocastanum,* Anti bacterial activity, *E. faecalis.*

INTRODUCTION

Natural products have been used for thousands of years in Dental practice, they were also believed to be the new source of antimicrobial agents.[1,2]

Acacia catechu Willd is widely used in Ayurveda for many diseases and mainly for skin diseases. Most of the people in Kerala use boiled Khadira water (karingali water) for drinking purpose. *Acacia catechu* is highly valuable for its powerful astringent and antioxidant activities. It is useful in dental, oral, throat infections and also as an astringent for reducing oozing from chronic ulcers and wounds. The main chemical constituents of *Acacia Catechu* are catechin, epicatechin, epigallocatechin, epicatechin gallate, phloroglucin, protocatechuic acid, quercetin, poriferasterol glucosides, lupenone, procyanidin, kaemferol, L-arabinose, D- galactose.D-rhamnose andaldobiuronic acid, afzelchin gum, mineral and taxifolin. [3, 4-8] The extracts of *Acacia catechu* exhibits various pharmacological effects like antipyretic, anti-inflammatory, anti diarrhoeal, hypoglycaemic, hepatoprotective, antioxidant and antimicrobial activities. [9-15] *Acacia catechu* is useful as a topical agent for sore gums and mouth ulcers.[16]

Aesculus hippocatnum (Horse chestnut), is believed to be derived from the brown conkers that look similar to chestnuts and because a horseshoe shaped mark .horse chest seed exract(HCSE), The primary active constituent found in horse chestnut seed extract is aescin[17]. Aescin is primarily an mixture of triterpene saponins present in two forms, which are distinguished by their water solubility and melting points. Other constituents include bioflavonoids (quercetin and kaempferol),proanthocyanidin A_2 (an antioxidant), and the coumarins fraxin and aesculin.[18]

Extract of horse chestnut bark (*Aesculus hippocastanum*) is one of the ingredients that gives Fortifying Mint Toothpaste, Sensitive Orange Tooth Gel for Children und Sage Mouthwash their fortifying effects. It contains aesculin, which firms the gums and has a harmonising influence on the formation and hardening processes within the body. These two opposing tendencies play an important role in the development of the teeth as the tooth grows and requires both forming and hardening [19].

Horse chest nut seed extract is found to be active against oral microbes like *streptococcus mutans, streptococcus salivarius, streptococcus mitis,streptococcus sanguis and Lactobacillus acidophillus.*[20]

Enterococci are gram positive cocci that can occur singly, in pairs, or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen. [21, 22] *Enterococci* survive in harsh environments including extreme alkaline pH (9.6) and salt concentrations. [23] *E. faecalis* is associated with different forms of periradicular disease including primary endodontic infections and persistent infections *E. faecalis* is found in 4 to 40% of primary endodontic infections [24]. Root canal treatment has been described as the disinfection of the root canal system, using endodontic instruments aided by an antimicrobial agent. [25,26]

The most effective method to eradicate *E faecalis* is the use of sodium hypochlorite and 2% chlorhexidine .[27] Sodium hypochlorite is extremely toxic to periapical tissues if injected beyond apex .[28] Presence of inflammatory exudate and killed micro organisms can inhibit the action of chlorhexidine in root canal .[29] Various Plant products have been reported to inhibit the growth of several oral microbes.Hence an attempt was taken to evaluate the *invitro* antibacterial activity of acetone bark extract of *Acacia catechu willd and* acetone seed extract of *Aesculus hippocastanum* against *E.faecalis* to prevent Root canal failure.

MATERIALS AND METHODS

Plant material

Acetone Bark extract of *Acacia catechu willd and* seed extract Of *Aesculus hippocastanum* was obtained from Green Chem. Herbal Extract & Formulations. Bangalore.

Test microorganisms

Bacterial strain used were *Enterococcus faecalis* (ATCC 29212) ,The organisms were obtained from Department of Microbiology , Saveetha Dental College & Hospitals, Chennai .

Methodology

The plant extract 200mg were weighed aseptically into a sterile tube and dissolved in 2ml of sterile Tryptic soy Broth (TSB).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 Antibactericial Activity

The tested organism was grown in (TSB) Tryptic soy broth medium [MHA-Hi media ,Mumbai] for 24hrs at 37°C and concentration was adjusted to 0.5 Macfarland standard.[30-32]

The above concentration of extracts was 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 duplicate to minimize the test error.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of herbal extracts against tested microorganism was determined by broth dilution method [33]. 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 10^6 organisms was made in Tryptic soy broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1990)[34].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/nogrowth is detected.then the MBC was 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, is noted as the MBC.

RESULT AND DISCUSSION

Various literature reveals the antibacterial efficacy of herbal extracts against *E.faecalis.*[35,36]

The extract at different concentrations exhibited antibacterial activity against the bacterial strain tested. The Acetone bark extract of *Acacia catechu* exhibited a high degree of activity against the organism tested . The Acetone bark extract showed no growth at a concentration of 10mg/ml. whereas the Acetone seed extract of *Aesculus hippocastanum* does not showed any activity against *E.faecalis.*

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 and 2.

Table 1: Antibacterial activity of phytochemical extracts against *E.Faecalis*

Herbal extract	62µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1mg/ml	5mg/ml	10mg/ml	Control
Acacia catechu Bark Acetone extract	++	++	++	++	++	++	--	++
Aesculus Hippocastanum seed Acetone extract	++	++	++	++	++	++	++	++

++ =Growth

-- =No Growth(Indicates MIC /MBC)

Table 2: Microbicidal Concentration (MBC)

Herbal Extracts	MBC Conc.Showing [No Growth]
Acacia catechu Bark Acetone extract V's *E.faecalis*	10mg/ml
Aesculus hippocastanum Seed Acetone extract V's *E.faecalis*	No activity

Fig. 1: *Acacia Catechu* bark acetone extract

CONCLUSION

Phytochemical extracts contain many chemical compounds which are biologically active within the human body. For centuries

humans have used plants and plant extracts to treat various disease conditions and more recently to produce new drugs.

Still most of the plants carry a large number of unidentified compounds which can be really useful of making new drugs and for the identification of lead compounds.

Hence Our finding suggest that the antibacterial activity of the acetone 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. However, further studies are necessary to isolate and reveal the active compound(s) contained in the refined extract of *Acacia catechu willd* and to establish the mechanism of action.

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