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

LAKSHMI.T*1 AND RAVISHANKAR.P2
1Assistant Professor, Department of Pharmacology, Saveetha Dental College, Chennai, 2Reader, Department of Conservative Dentistry, Thai moogambigai Dental College, Chennai. Email: lakshmi085@gmail.com

ABSTRACT

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

Materials and Methods: The inhibitory effect of acetone bark extract of Acacia catechu wild 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 was found to be bactericidal in action against tested bacterial strain.

Keywords: Acacia catechu wild, 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 Wild 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, quercitin, pariferasterol glucosides, lupenone, procyanidin, kaemferol, L-arabino, D-galactose, D-rhamnose and aluminiumic acid, afzelin gum, mineral and taxifolin.3-4,6 The extracts of Acacia catechu exhibits various pharmacological effects like antiinflammatory, anti-anitmicrobial activity, anti diarrheal, hypoglycemic, hepatoprotective, antioxidant and antimicrobial activities.5-15 Acacia catechu is useful as a topical agent for sore gums and mouth ulcers.16

Aesculus hippocastanum (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 extract (HCSE). The primary active constituent found in horse chestnut seed extract is aescin17. Aescin is primarily a mixture of triterpene saponins present in two forms, which are distinguished by their water solubility and melting points. Other constituents include bioflavonoids (quercitin and kaempferol), proanthocyanidin A2 (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 acidophilus.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.24,25 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 microorganisms 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 wild 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 wild and seed extract of Aesculus hippocastanum was obtained from Green Chem. Herbal Extract & Formulations. Bangalore.

Test microorganisms

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/100µl, 125 µg/100µl, 250 µg/100µl, 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 24 hrs.

**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.

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. 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). 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 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*. 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.

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

---

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

<table>
<thead>
<tr>
<th>Herbal extract</th>
<th>62µg/ml</th>
<th>125 µg/ml</th>
<th>250 µg/ml</th>
<th>500 µg/ml</th>
<th>1mg/ml</th>
<th>5mg/ml</th>
<th>10mg/ml</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia catechu</em> Bark Acetone extract</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>--</td>
<td>++</td>
</tr>
<tr>
<td><em>Aesculus Hippocastanum</em> seed Acetone extract</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

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

**Table 2: Microbicidal Concentration (MBC)**

<table>
<thead>
<tr>
<th>Herbal Extracts</th>
<th>MBC Conc. Showing [No Growth]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia catechu</em> Bark Acetone extract V's <em>E. faecalis</em></td>
<td>10mg/ml</td>
</tr>
<tr>
<td><em>Aesculus hippocastanum</em> Seed Acetone extract V's <em>E. faecalis</em></td>
<td>No activity</td>
</tr>
</tbody>
</table>

---

**Fig. 1: Acacia Catechu bark acetone extract**

**CONCLUSION**

Phytochemical extracts contain many chemical compounds which are biologically active within the human body.
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* wild, 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* wild and to establish the mechanism of action.

ACKNOWLEDGEMENT

Our Heartfelt thanks to Mr.Rajendran CEO Of Green Chem Herbarium,Chennai for providing the test organism for the study and we also thank Dr.Auxilia Hemamalini ,HOD of Microbiology , Saveetha Dental College & Hospitals,Chennai for providing the test organism for the study.

REFERENCES


