ANTI CARIOGENIC ACTIVITY OF BETA ECDYSONE FROM ACHYRANTHES ASPERA LEAF EXTRACT-AN INVITRO STUDY

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Abstract

The objective of our study is to investigate the \textit{in vitro} anticariogenic activity of Beta Ecdysone from ethanolic leaf extract of \textit{Achyranthes aspera} against \textit{S.mutans}, \textit{L.acidophilus}. The inhibitory effect of the extract were tested against two oral pathogens commonly causes dental caries by using the Disc diffusion and Micro broth dilution method. The Beta Ecdysone from ethanolic leaf extract of \textit{Achyranthes aspera} did not showed anti cariogenic activity in any concentration ranging from using disc diffusion method, whereas in Micro broth dilution method, anti cariogenic activity is detected.

Key words: Beta Ecdysone, \textit{Achyranthes aspera}, \textit{S.mutans}, \textit{L.acidophilus}, Anti cariogenic activity.

Introduction

Dental caries is a bacterial disease and sugars are considered to be the most cariogenic substances because, apart from fermentation, they are also transformed into extracellular polysaccharide in dental plaque. The presence of these polysaccharides in biofilm also increases the dental plaque matrix porosity.\(^1\) The inorganic composition of dental plaque matrix is important in the development of dental caries.\(^2\) Dental caries is one of the most common chronic infectious diseases in the world\(^3,4\). \textit{Streptococcus mutans} is a major etiological agent of dental caries. Bacterial species of the genera \textit{Veillonella}, \textit{Lactobacillus}, \textit{Bifidobacterium}, and \textit{Propionibacterium}, low-pH non-\textit{S. mutans} streptococci, \textit{Actinomyces} spp., and \textit{Atopobium} spp., play an important role in caries progression.

\textit{Achyranthes aspera} is commonly known as \textit{Nayuruvi} (Tamil), \textit{Latjeera} (Hindi) & \textit{Rough Chaff tree} (English). It is an erect or procumbent, annual or perennial herb, 1-2m in height, often with a woody base, commonly found as a weed of waysides.\(^5,6\). Although it has many medicinal properties, it is particularly used as a spermicidal\(^8\) antipyretic\(^9\) and a cardiovascular agent\(^10\).
Achyranthes aspera contains triterpenoid saponins which possess oleanoic acid as the aglycone, ecdysone - an insect moulting hormone, and long chain alcohols. Ecdysone lead to anabolic activity on skeletal muscles, cell proliferation and growth leading to increased mass from vitamin-like effects, improved liver secretory function as they play a structural role in the mitochondrial membranes in cells, as well as immunomodulating effects. Some common names for ecdysonic acid include ecdisten, ecdysone, isoinokosterone, 20-hydroxyecdysone and β-ecdysterone. This study aims at evaluating the anti cariogenic property of beta ecdysone from Achyranthes aspera leaf extract against S. mutans and L. acidophilus.

Materials & methods

Plant material

Achyranthes aspera extract is obtained as a gift sample from Green Chem Herbal Extracts & Formulations, Bangalore.

Chemicals & reagents

Beta Ecdysone is purchased from Sigma, Acetonitrile HPLC grade solvents; all analytical grade solvents obtained from E-Merck Ltd. Mumbai, India.

Experimental section

All the chemicals and reagents used were from Sigma. Glass wares used were from Borosil. The media and broth used for microbial culture were from Hi-Media Pvt. Limited, Bombay, India.

Anti carcinogenic activity

Disc diffusion assay

The assay is carried out by disc diffusion method. The pooled extracts were concentrated and extracts were loaded into sterile readymade discs (Hi-media, MUMBAI) in different volumes of 15μl, 20μl and 25μl / disc respectively and allowed to dry for 24 hours at room temperature. Mueller Hinton agar plates were spread with 100μl of actively growing broth cultures of the respective bacteria and are allowed to dry for 10 min. The sterile readymade discs loaded with each extract individually (15μl and 25μl/ disc respectively) were imposed on the inoculated plates. The plates were then incubated at 37°C for 36 hours. The development of the zone of inhibition around the around the extract loaded disc was recorded.

Micro broth dilution method

Medium Used: Tryptic soy broth is used as a medium
Strains used

*Streptococcus mutans* ATCC 25175

*Lactobacillus acidophilus* ATCC 4356

Preparation of different concentrations of herbal extract

The herbal 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. 50μg/mL, 25μg/mL, 12.5μg/mL, 6.25μg/mL, 3.125μg/mL, 1.6 μg/mL and finally 0.8 μg/mL, respectively in to wells of micro plates. 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 Mac farland Standard. The different concentrations of extracts were taken in 100μl quantities in a U bottom micro culture plates. Streptomycin was used as control. 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.17

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of herbal extracts against tested micro organisms was determined by micro broth dilution method. A series of two- fold dilution of each extract (This was serially diluted to obtain the dilutions of 50μg/mL, 25μg/mL, 12.5μg/mL, 6.25μg/mL, 3.125μg/mL, 1.6 μg/mL and finally 0.8 μg/mL) 100 μl of 10⁵ CFU/ml of the microorganisms was inoculated in tubes with equal volume of nutrient broth and plant extracts as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1990).18,19 The control well received streptomycin antibiotic. 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.

Result and Discussion

Anticariogenic efficacy of beta ecdysone was tested against *S.mutans* and *L.acidophilus*, the two major oral pathogens that commonly causes dental caries in patients. disc diffusion assay was performed which revealed no zone of inhibition when tested the beta ecdysone against various concentrations. This shows that beta ecdysone has no anti
cariogenic activity.

But, keeping this in mind we tested the beta ecdysone against these oral pathogens adopting broth dilution method since this method is highly validated with regard to the herbal extracts, the results confirmed that when tested beta ecdysone at various concentrations it was observed that beta ecdysone showed activity (detected NO GROWTH) from the concentration ranging from 6.25 μg/ml to 50 μg/ml. But it failed to detect No growth against S. mutans.

**Conclusion**

Indian herbal extracts are used since ancient times to treat various disease ailments as these natural products exert broad-spectrum actions. L. acidophilus, S. mutans is a normal inhabitant of the oral cavity. The prevalence of L. acidophilus, S. mutans is increased in oral rinse samples from patients receiving dental treatment. Hence the finding suggest that Beta ecdysone from ethanolic leaf extract of Achyranthes aspera does not show any antibacterial activity and found to be less effective when tested at various concentrations against S. mutans. But found to be effective against L. acidophilus, hence Beta ecdysone from Achyranthes aspera leaf extract is suggested for further in vivo studies to prove its efficacy against eradicating L. acidophilus in patients undergoing dental treatment.

**Table 1: Anti cariogenic evaluation of herbal extracts against S. mutans, L. acidophilus-Disc Diffusion assay.**

<table>
<thead>
<tr>
<th>Beta ecdysone</th>
<th>Concentration of extracts (μg/ml)</th>
<th>Control (Streptomyces)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>S. mutans</strong></td>
<td>NZ</td>
<td>NZ</td>
</tr>
<tr>
<td><strong>L. acidophilus</strong></td>
<td>NZ</td>
<td>NZ</td>
</tr>
</tbody>
</table>

NZ- No Zone of Inhibition

**Table 2: Anti carcinogenic evaluation of herbal extracts against S. mutans, L. acidophilus-Broth dilution assay.**

<table>
<thead>
<tr>
<th>Beta ecdysone</th>
<th>Concentration of extracts (μg/ml)</th>
<th>Control (Streptomyces)</th>
<th>MIC/MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.8</td>
<td>1.6</td>
<td>3.125</td>
</tr>
<tr>
<td><strong>S. mutans</strong></td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>
| **L. acidophilus**| G | G | NG | NG | NG | G | 0.15 μg/ml |}

NG-NO GROWTH (INDICATES MIC/MBC) G=GROWTH (NO ACTIVITY)
Acknowledgment

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References


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