“PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF TERMINALIA CHEBULA AGAINST BACTERIA ISOLATED FROM TEETH”

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Abstract

Dental caries is a common oral bacterial pathological process of Disruption of tooth structure by microorganism also known as Tooth decay and Commonly called cavities.

It is mainly associated with *Streptococcus mutants* and *Lactobacillus* spp. In these study antimicrobial activities of Terminalia chebula extract against Bacterial strains Isolated from Tooth surface is investigated. The aim of these studies is to evaluate the possible Antimicrobial potential of T. Chebula fruit extract by using Acetone and Ethanol against different dental caries causing microorganism. The Ethanol and Acetone extract of T. Chebula show zone of inhibition ranging from 100 mm at conc. of 10 mg/ml. The extracts of Terminalia chebula fruit are known to have antibacterial properties.

Phytochemical studies of T. Chebula reviled the presence of Antimicrobial activity in the tested Plant material exhibited by its Bioactive compounds and surving them as an alternative Antibacterial Agent against Dental caries causing microorganism. The present study deals with the fruit extracts of *Terminalia chebula* contained different types of phytochemicals such as glycosides, alkaloids, flavonoids, phenolic compounds, saponin, steroids, quinine and tannin.

Keywords: - Dental caries, Antimicrobial activity, Agar Disc Diffusion, Inhibition zone.
Introduction

Dental caries is the common Bacterial pathological process of destruction of tooth structure by microorganism also known as Tooth decay and commonly called cavities or Caries. Dental plaque which leads to caries is the oral flora which adhere to Teeth and cementum. It is the disease that has been associated with streptococcus spp. mainly streptococcus mutants and lactobacillus spp. Tooth brushing can reduce the caries by reducing the number of cariogenic organism and removing the substrate from tooth surfaces. According to Berge’s manual of systematic Bacteriology S.mutant are Gram positive cocci. They are 0.5-0.75mm in diameter occurring in pairs short medium length chains without capsule. They show different properties first they colonize on Tooth surface. Second they synthesized insoluble polysaccharides to form sucrose. Third they ferment sucrose to form lactic acid. Another mutant lactobacillus is Gram positive non-spore forming rod shape, compared to S.mutant, lactobacillus species are not important in the initiation of caries. Herbal remedies have long history of use for gum and Tooth problems. In many traditional cultures, there are no plastic-bristle brushes, rather the use of herbal chewing sticks for relieving dental problems in common. World wide approximately 2.43 billion people [36% of the population] have the dental caries in their permanent Teeth, in baby teeth it affect about 620 million people or 9% of the population. These disease is common in latin American countries in the middle east and south Africa and least prevalent to china. In United states dental caries is the most common chronic childhood disease bring at least five time more common than asthma. Dental caries is still prevalent in most African countries, Nigeria inclusive.

T.chebula is king of medicine it always listed at the top of list in ayurvedic plants due to its extraordinary power of healing. T.chebula is flowering evergreen tree attaining a height up to 30 cm.
the round tree is crowned and branches spread out with diameter of 1.5-2.5 meters. In India the tree can be found in forest of Madhyapradesh, Bihar, Assam and Maharashtra.

The dried ripe fruits have traditionally been use in the treatment of Asthma, sore throat, vomiting, high cough, bleeding piles, gout, Heart and bladder disease.

Antimicrobial activity of \textit{T}.chebula extract against several bacterial strains have been reported. It is effective in inhibiting \textit{Helicobacter pylori} \textit{Xanthomonas campestris} \textit{pv. Citri} and \textit{salmonella typhi}. In view of this reported medicinal value the present work was carried out to examine the antimicrobial potential of five different extract of \textit{T}.chebula fruit against Dental caries causing microorganism.

Materials and Methods

\textbf{Collection of Plant materials :-}

The fresh matured fruits of \textit{T}.chebula where collected from Botanical garden of Milind collage of science Aurangabad.

\textbf{Methodology}

1. \textbf{Isolation of microorganism from dental surfaces}

Bacterial sample was collected from oral cavity by swabbing across the gingival and subgingival region as well as from the roof and floor of the buccal cavity. Representative samples were collected from three persons and the samples were inoculated in Nutrient broth. The overnight broth culture was serially diluted with autoclaved distilled water upto 10^-6 dilution and 100 μl of each dilution was spreaded on to Nutrient agar plates. Incubated overnight at 37°C. After incubation period of 12 -18 hours the number of viable colonies were counted using total viable plate count method.

2. \textbf{Identification of Microorganism}

2.1. \textbf{Cultural Characteristics of microorganism}

Cultural characteristics of isolated bacteria such as size, shape, elevation and margin of colony were recorded by culturing them on nutrient medium and incubated for 24 - 48 hours at 37°C ± 1°C. The colonies were observed under transmitted and reflected light condition to understand their optical properties.

2.2. \textbf{Morphological Characteristics}
The bacteria were gram stained and observed under light microscope (100×). The shapes of bacterial cells were coccid, bacilli, single, paired, chain and dense clusters.

3. Preparation of plant extract

The fruit extract thoroughly washed with distilled water and then dried under shade condition. The dried fruit were powdered and stored in air sealed plastic container at room temperature until the time of extraction. The fruit powders were subjected to extraction using organic solvents. 5g of powered plant material was soaked in 10ml of solvent such as acetone and ethanol for 72 hours, with stirring every 24 hours. At the end of extraction period, it was centrifuged and supernatant was filtered through Whatman No.1 paper. This extraction was repeated three times. Filtrates were pooled and evaporated to air dry and stored at 20°C for further use.

4. Phytochemical analysis

Phytochemical analysis for major phytoconstituents of the plant extracts undertaken using standard qualitative methods for alkaloids, carbohydrates, saponins, phytosterols, phenol, tannins, flavonoids and proteins plant extracts is screened for the presence of biologically active compounds.

Antibacterial activity

The antibacterial test was carried out against gram positive bacteria isolated from dental surfaces. The antibacterial activity of leaf and fruit extracts were tested against bacteria by disc diffusion method. The extraction with acetone, ethanol is used for the screening of 100μl, 150μl and 200μl concentration

Extract loaded disc were placed on the surface of the agar medium by pressing with sterile forceps in an aseptic condition. Standard antibiotic Ampicillin (5mg/ml) used as control. The inoculated and treated plates were incubated at 37°C for 24 hours. After the incubation, the diameter of zone was measured. The respective control was also run simultaneously using different solvents to compare the effect of plant extracts. After overnight incubation, the diameter of each zone of inhibition is measured. In all measurements, the zones of inhibition are measured from the edges of the last
visible colony-forming growth. The results are recorded in millimeters (mm) and interpretation of susceptibility is obtained by comparing the results to the standard zone.

Observation

1. **Enumeration of Total viable cell count:**
   Total viable count was determined from selected plates having 30 to 300 colonies.
   Total viable count was calculated from the formula
   \[
   THB = \text{No. of colonies} \times \text{Dilution factor} / \text{Inoculum size in CFU/ml}
   \]
   
   **Table No.-1**
<table>
<thead>
<tr>
<th>Sr no</th>
<th>Number of bacterial colonies</th>
<th>Dilution factor</th>
<th>THB (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>280</td>
<td>10-2</td>
<td>2.8×10^5</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>10-3</td>
<td>2.0×10^5</td>
</tr>
<tr>
<td>3</td>
<td>160</td>
<td>10-4</td>
<td>1.6×10^5</td>
</tr>
</tbody>
</table>

   Three bacterial strains with observable difference in colony morphology is randomly selected from initial spread plate.

   Spread plate culture of dental plaque bacteria

2. **Identification of microorganisms**

   **Table No. 2**
### 3. Photochemical analysis of T. Chebula

**Table No. 3**

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Constituent</th>
<th>Acetone</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Phytosterols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Protein/AA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Diterpenes</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- = not present  + = present in varying degrees  Tr = Trace

### 4. Antibacterial activity

**Table No. 4**  (Zone of inhibition)

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Sample name</th>
<th>Acetone</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>100 mm</td>
<td>100 mm</td>
</tr>
<tr>
<td>2</td>
<td>T. Chebula</td>
<td>50 mm</td>
<td>3 mm</td>
</tr>
</tbody>
</table>
Result and Discussion

The present study showed that the T. chebula contained phytochemicals such as alkaloids, flavonoids, saponin, phenolic compounds, steroids, carboxylic acid, tannin and glycoside. Presence of phytochemical differed in different types of solvent in the fruit extracts. Agar paper disc method relived that all the extract of T. chebula showed antimicrobial activity against dental caries causing bacteria. Highest mean diameter of inhibition zone was produced by the acetonic extract.

Conclusion:
Since all the tested extracts of T. chebula were highly effective against the tested dental caries causing bacteria, purification and toxicological studies of the plant and in vivo trials should be carried out so that it can be used as a potential source for the development of a phytomedicine to act against dental caries causing bacteria. The antimicrobial activities can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs from T. chebula should be emphasized for the control of dental caries.

References


