Evaluation of the Antimicrobial and Antibiofilm activity of ethanol and acetone extracts of Neem and Guava on oral pathogens

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Abstract- The research is based on the evidence of plant extracts; neem and guava against bacteria.

Neem and guava leaves have enormous medicinal wealth having anti-inflammatory, antimicrobial, antioxidant, antidiarrheal, antimutagenic properties as has been reported in various studies. This research was done to assess the antimicrobial and antibiofilm efficacy of a combination of these plant extracts in the form of leaves against dental caries and periodontal pathogens. The ethanol and acetone extracts of the guava and neem powder (obtained from their leaves) were used to check the anti microbial and anti-biofilm activity against common oral pathogens.

Index Terms- Acetone, Ethanol, Extracts, Guava, Neem, Oral pathogens etc.

I. INTRODUCTION

Herbal extracts have recently gained high significance in the field of alternative medicine for curing various ailments and conditions. Most of these extracts are prepared from natural sources such as trees and plants and their components like stem, root, leaves and other components. These components are found to be rich in the phyto-constituents like flavonoids, tannins, glycosides and other factors which provides them resistance against stress conditions which may occur due to climate, temperature fluctuations as well as protects them against infectious agents like bacteria and fungi. In other words, it can be simply said that these components also provide anti-microbial property to the plants to protect themselves from the microbial infection.

Fruit bearing plants like Guava is one of the best examples to show antimicrobial activity against a diverse group of pathogens. Leaves of Guava (Psidium guajava) are rich in flavonoids which have toxic action against numerous bacteria present in the mouth. Apart from flavonoids, the other vital constituents present in them includes guaijaverin and tannins obtained from the barks of guava plant which has antibacterial properties. The other vital constituent present in them is Vitamin C or Ascorbic acid which is well known for its antioxidant properties. According to various studies Guava extracts have been shown to possess some inhibitory properties against growth of many organisms.

Neem, a well known medicinal plant is also cited for its strong antimicrobial activity on oral pathogens. Its twigs are used by most of the people in rural India for brushing purposes. Phytochemically it possesses several constituents like Limonene, nimbolin, nimbolide and azadirachtin, making it a potent eliminator of oral pathogens like Porphyromonas gingivalis and Fusobacterium nucleatum. Apart from these properties, they also possess anti-inflammatory activities which minimise the inflammation caused in gums by these organisms.

The necessity of these extracts is utmost during these days due to the prevalence of drug resistant microorganisms on which several groups of antibiotics have failed to show any result (as recorded by CrossRef and RSC journals- 12 citations). One of the prime reasons for becoming resistant to antibiotics is the unique property of biofilm formation. These biofilms are developed by multiple groups of bacteria and obstruct the penetration of the drug, thus interfering with the bactericidal and bacteriostatic properties of various drugs. Thus, in order to eliminate these groups of bacteria, an alternative approach is opted in order to eliminate drug resistant pathogens.

The oral cavity is the natural environment for various species of microorganisms which form a compound society that can adhere to tooth surface forming biofilm. Surface adhesion of bacteria and fungi is an essential step in dental plaque formation. When acidogenic oral bacteria are present in sufficient numbers due to fermentation of sucrose, it might result in development of dental caries. The most common species of bacteria in the oral cavity which have a cariogenic potential are Streptococcus sanguis, S. mutans, S. mitis, S. sobrinus and other lactobacilli spp. A well exploitation of these herbal extracts by various methods can prove out to be beneficial and can also aid in various innovative methods in the medical and dental field.

Equipments/Materials used during investigation:

1. Acetone
2. Ethanol
3. Filter paper
4. Conical flask
5. Aluminium foil
6. Test tubes
7. Petri dish
8. Eppendorf tubes
9. Muller Hilton Agar medium
10. Micropipettes
11. Cotton plugs

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12. Funnel
13. Tripod stand etc.

II. RESULTS

The antibacterial activity of the ethanolic and acetone extract of neem and guava was performed by Well Diffusion method against these oral pathogens; E. faecalis, Candida albicans, S. aureus, E. coli and Klebsiella. The zone of inhibition of acetone extract of neem against E. faecalis was found to be the maximum.

The results have been tabulated as:

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Neem Ethanolic Extract(mm)</th>
<th>Guava Ethanolic Extract(mm)</th>
<th>Neem Acetone Extract(mm)</th>
<th>Guava Acetone Extract(mm)</th>
<th>Control(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella spp</td>
<td>7</td>
<td>20</td>
<td>16</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>S. aureus</td>
<td>27</td>
<td>20</td>
<td>12</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>26</td>
<td>18</td>
<td>29</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>E. coli</td>
<td>11</td>
<td>11</td>
<td>13</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>C. albicans</td>
<td>17</td>
<td>16</td>
<td>13</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>
Comparative analysis of Antimicrobial activity of ethanolic and acetone extracts of neem and guava on oral pathogens.

ACKNOWLEDGMENT

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REFERENCES/REVIEW OF LITERATURE

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Authors</th>
<th>Year</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Heyman et al.</td>
<td>2017</td>
<td>• Neem leaf extract, containing polyphenols that provides long-lasting antibacterial effect. Thus, it might be especially effective in periodontal diseases.</td>
</tr>
</tbody>
</table>
2. GM Prashant et al., 2007  
   - Neem extract produced the maximum zone of inhibition on *Streptococcus mutans* at 50% concentration.

3. Khan and Khan, 2018  
   - Herbal antimicrobial mediators may inhibit the biofilm and oral infections. Therefore, the antimicrobial mediators need to have the capability of eliminating the oral microbes and preventing many oral infections.

4. Kankariya et al., 2016  
   - This study on natural product may open the possibilities of finding a new clinical and effective herbal remedy for prevention of dental caries.

5. Lekshmi et al., 2012  
   - The results demonstrate that the chloroform extracts of neem has a strong antimicrobial activity and suggest that it can be useful in the treatment of dental caries.

6. Shetty et al., 2018  
   - Guava extract may be a potential therapeutic agent for periodontitis as it shows significant activity against both P. gingivalis and A. actinomycetemcomitans.

7. Saraya et al., 2008  
   - It can be formulated as oral cavity consumer herbal products such as chewing gums, toothpastes, mouthwashes and dental floss.

8. Gurnani et al., 2016  
   - Only the extracts prepared with ethanol and water were found to have antibacterial effect against L.acidophilus and 20% ethanolic extract of guava was found as efficacious as 0.2% chlorhexidine.

9. Rani et al., 2013  
   - This study has shown the importance of guajava and indicated that the active compounds present in these two varieties could serve as a lead compound in the formulation of a new antibacterial herbal drug to cure dental caries.

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