Evaluation of antimicrobial activity of siddha formulation against dental pathogens

Thirunarayanan Gopalakrishnan, Tamizharasan Seetharaman, Saravanan Ganesan, Balakrishnan Thathagounder Raja, Tamilselvan Ganapathi , Sampathkumar B, Karthik Loganathan, Shreedevi MS*

Department of Gunapadam and Microbiology, Sivaraj Siddha Medical College, Salem, TN, India
*For correspondence: shreemd@gmail.com

ABSTRACT

The present study was carried out to evaluate the antimicrobial activity of poly herbal siddha formulation. The formulation contain Syzygium aromaticum, Piper cubeba, Acacia nilotica and latex of Jatropha curcas. The drugs used in the formulation was subjected to antimicrobial activity both in combination and in isolation against four different dental pathogens such as Staphylococcus sp., Streptococcus sp., Lactobacillus and Pseudomonas sp., isolated from Dental plaque patients. Aqueous extract of siddha formulation was subjected to antimicrobial studies by Agar well diffusion method. The extract showed significant inhibitory activity against Staphylococcus sp., Streptococcus sp, Lactobacillus sp and Pseudomonas sp..

Keywords: Dental pathogen, Syzygium aromaticum, Piper cubeba, Acacia nilotica and Jatropha curcas

1. INTRODUCTION

Medicinal plants indigenous to the Indian sub-continent have been widely used for various therapeutic applications and for various diseases. The study evaluates the use of herbal extracts against oral pathogens commonly found in patients with dental caries. In Worldwide, approximately 2.43 billion people (36% of the population) have dental caries in their permanent teeth [1] The World Health Organizations estimates that nearly all adults have dental caries at some point in time [2] In baby teeth it affects about 620 million people or 9% of the population [1] They have become more common in both children and adults in recent years [3] Hence we tried a natural solution with Syzygium aromaticum, Acacia nilotica, Piper cubeba and Jatropha curcas.

Syzygium aromaticum

Syzygium aromaticum (Clove) is a perineal herb belong to Myrtaceae family, which contains 72 – 90% of essential oils like acetyl eugenol, beta-carophyllone, vanillin, gallic acid, methyl salicylic acid etc. [4]. The drugs also used as a pain killer and had been traditionally used as an instant relief in tooth pains [5].
**Piper cubeba**

*Piper cubeba* (Valmilagu) which belong to Piperaceae family has a stimulant effect and in Siddha medicine for the same. The dried seeds contains monoterpenes, sequisteptines. The Siddha system uses this herb for dental diseases, halitosis, fever and cough [6].

**Acacia nilotica**

*Acacia nilotica* (karuvelam pattai) from Fabaceae family is a perinial herbused in various siddha formulation to combat dental disease and has an astringent effect [7].

**Jatropha curcas**

*Jatropha curcas* (Kattamanaku) belong to Euphorbiaceae family has an astringent, effect. According to siddha system of medicine. The oil and series are made up of protein [8].

The aforementioned four herbs were selected based on siddha medicine principles and their wide spread uses in the traditional siddha medicine and as a home remedy in various house hold of South India. The Focus of this Present work is to evaluate the antibacterial activity herbal extracts used in siddha formulation both in combination and in isolation.

**2. MATERIALS AND METHODS**

**2.1. Collection of samples**

25 Samples were collected from clinically suspected cases of Dental caries in various hospitals at Salem city. The samples were collected by using sterile cotton swab. The collected samples were transported by using sterile container to laboratory for microbial examination [9].

**2.2. Isolation of Bacteria**

Collected samples were inoculated separately in tubes contains 5ml Nutrient broth and incubated at 37°C for 24 hours. Freshly grown cultures were diluted (1ml culture in 10 ml distilled water) and the diluted samples were spread over Nutrient agar plates by using pour plate technique. The inoculated plates were incubated at 37°C for 24 hours. After incubation period, the colonies were picked for identification [10].

<table>
<thead>
<tr>
<th>S.No</th>
<th>Local name</th>
<th>Botanical name</th>
<th>Parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Elavangam</td>
<td><em>Syzygium aromaticm</em>,</td>
<td>Flower</td>
</tr>
<tr>
<td>2</td>
<td>Vaalmilagu</td>
<td><em>Piper cubeba</em>,</td>
<td>Seeds</td>
</tr>
<tr>
<td>3</td>
<td>Karuvel</td>
<td><em>Acacia nilotica</em></td>
<td>Barks</td>
</tr>
<tr>
<td>4</td>
<td>Kattamanaku</td>
<td><em>Jatropha curcas</em></td>
<td>Latex</td>
</tr>
</tbody>
</table>

Seeds of *Piper cubeba* and flowers of *Syzygium aromaticm* were collected from Kolli hills in Namakkal district and barks of *Acacia nilotica* were collected around Salem district.

**2.3. Identification of Bacteria**

The isolated bacteria were subjected to common biochemical tests such as Gram Staining, Motility, Iodole, MR, VP and Citrate utilization test. Based on biochemical and Gram staining reaction the isolates were identified as *Staphylococcus sp*, *Streptococcus sp*, *Lactobacillus sp*, *Pseudomonas sp* [10].

**2.4. Collection of Plant materials**

**2.5. Preparation of Siddha formulations**

Seeds of *Piper cubeba*, flowers of *Syzygium aromaticm* and barks of *Acacia nilotica* were powdered separately and sieved using filters. Then 10 grams of each powder was taken and mixed thoroughly. This mixture was grained with the latex of *Jatropha curcas* in kalvam for 1hour and it is shadow dried and stored in air tight container.

**2.6. Preparation of extract**

1 gram of prepared medicine and 1 gram of each drug used in this formulation were diluted in 1ml of distilled water separately in test tubes. Then the extracts were subjected to anti-microbial assay [2]

**2.7. Anti-microbial screening**

The prepared herbal extracts were tested against identified bacterial genus such as *Staphylococcus sp*, *Streptococcus sp*, *Lactobacillus sp*, *Pseudomonas sp*. Anti-microbial screening is done by agar well diffusion method. Muller Hinton agar was prepared and poured in petri dishes and the test organisms were inoculated from nutrient broth by cotton swab. Then the test extracts were added to 6mm wells in the petri plates. The plates were incubated at 37°C for 24 hour. Anti-microbial activity is assayed by measuring the zone of inhibition formed around the wells (in triplicates) [2].
3. RESULTS
The activity of Siddha formulation showed significant inhibition of bacterial growth in the culture plates. In this maximum zone of inhibition is formed against Staphylococcus and latex of Jatropha curcas showed maximum inhibition against all pathogens. The inhibition by the Siddha formulation were represented in Table 1 and by individual drugs in Table 2 on the microbes were tabulated.

Table 1. Zone of inhibition formed by siddha formulation

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of organism</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staphylococcus</td>
<td>31±0.5mm</td>
</tr>
<tr>
<td>2</td>
<td>Streptococcus</td>
<td>27±0.7mm</td>
</tr>
<tr>
<td>3</td>
<td>Lactobacillus</td>
<td>26±0.6mm</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas</td>
<td>29±0.4mm</td>
</tr>
</tbody>
</table>

Table 2. Antimicrobial activity of drugs in isolation

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the organism</th>
<th>Zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Syzygium aromaticum</td>
<td>Acatia nilotica</td>
</tr>
<tr>
<td>1</td>
<td>Staphylococcus</td>
<td>28±0.4mm</td>
</tr>
<tr>
<td>2</td>
<td>Streptococcus</td>
<td>26±0.6mm</td>
</tr>
<tr>
<td>3</td>
<td>Lactobacillus</td>
<td>25±0.3mm</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas</td>
<td>27±0.7mm</td>
</tr>
</tbody>
</table>

4. DISCUSSION
The plants used in this formulation have already reported to cure various infectious diseases. So, we sought to evaluate scientifically. The drugs used in the formulation was selected based their properties given in the Siddha texts [8]. Syzygium aromaticum gives instant relief in tooth pains [8], Acacia nilotica has astringent (substance is a chemical compound that tends to shrink or constrict body tissues) property [8], Jatropha curcas has an astringent, styptic (anithemorrhagic) [8] and Piper cubeba has antiseptic property and it reduces halitosis [6]. Hence we made these drugs into a formulation. From the results obtained the medicinal plants used has a direct anti-microbial activity against oral pathogens such as Staphylococcus sp, Streptococcus sp, Lactobacillus sp and Pseudomonas sp. Further studies needs to be done for isolation of active principle of the above mentioned compound.

5. CONCLUSION
This study suggests that, this siddha formulation will be an excellent remedy for dental problems. In future by making in the form of tooth paste, tooth power and mouth wash will be useful as a preventive measure for dental problems.

Conflict of Interest
The authors declare that they have no conflicts of interest.

Acknowledgement
We greatly acknowledge Mr.C.Sakthivel, Sivaraj group of institutions, Salem, for his moral support during the course of this work and we acknowledge the college management and Principal for their constant support. Also we thank DR A.P.Uma MD (siddha) for her constant help throughout this work.

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