Evaluation of tulasi extract mouthwash in the management of oral candidiasis

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Abstract

Aim: The aim of this study is to evaluate the efficacy of mouthwash containing tulasi (Ocimum sanctum) extract on oral candidiasis.

Methodology: Minimum inhibitory concentration of tulasi extract was determined, and Tulasi mouthwash was prepared using 4% of the extract. A total of 90 patients with clinically diagnosed oral candidiasis were randomly allocated to chlorhexidine and tulasi groups. Pre- and post-oral symptoms such as burning sensation and altered taste were recorded using a VAS scale of 0–10. Patients were asked to rinse 10 mL of mouthwash thrice daily for 2–3 min for 7 days after food. Smear from oral candidal lesions before and after the treatment was taken, and colony-forming units (CFUs) were calculated.

Results: The patients with oral candidiasis showed significant reduction in burning sensation and improvement in taste perception. CFU showed a significant reduction in both groups pre- and post-treatment. Tulasi showed statistically significant improvements over chlorhexidine.

Conclusion: As tulasi was found to be more effective against oral candidiasis, it can be considered as viable substitute for synthetic drugs.

Keywords: Chlorhexidine, mouth wash, oral candidiasis, tulasi

Introduction

A common problem among immune compromised, elderly, and chronically ill patients is oral candidiasis. Precipitation of oral candidiasis causes burning sensation and altered taste which further lowers the quality of life.

The commonly used antifungal drugs are azoles such as fluconazole and polyenes such as amphotericin B. They are known to produce adverse effects and should therefore be used with precaution in patients with liver and renal diseases. The emergence of multidrug-resistant strains of Candida has further complicated the management of oral candidiasis.[1] To overcome such problems, the WHO has advised researchers to investigate the possible use of natural products in the management of infections.

One such plant is tulasi which means “incomparable one.” It is considered as an elixir of life due to its great therapeutic potential. It consists of phytochemicals whose synergistic actions result in vital biological effects.[2] Eugenol, linalool, and methyl chavicol present in tulasi have been proven to possess potent antifungal properties as they target ergosterol synthesis in the fungi.[3]
Bapuji Hospital, Davangere, were enrolled in the study. Ethical clearance was obtained from the Institutional Review Board, and informed consent was obtained from the patients.

**Inclusion criteria**
The following criteria were included in the study:
- Clinically diagnosed with oral candidiasis
- Not under any antifungal treatment for the present lesion.

**Exclusion criteria**
The following criteria were excluded from the study:
- Patients who were unable to follow instructions regarding use of mouthwash
- Patients with HIV infections.

Tulasi powder was obtained from Isha Agro Private Limited company, Mumbai. Tulasi extract and mouthwash were prepared at Bapuji Pharmacy College.

**Preparation of tulasi extract**
1 kg of tulasi powder was mixed with 2 L of 100% ethanol. It was then subjected to filtration to attain a clear filtrate. 10 g of solid residue of tulasi extract was obtained.

**Determination of MIC of tulsi extract**
Different concentrations of tulasi extract ranging from 1% to 10% were used for determining MIC.

1 g of cold extract of tulasi was dissolved in 10 mL of ethanol to obtain a 10% concentration of extract. Then, 1 mL of the extract was transferred to a sterilized test tube labeled as 10%. The remaining 9 mL of the extract was then diluted further with ethanol to obtain 9 different concentrations of tulasi extract. 0.2% chlorhexidine (Hexide mouthwash, Dey’s) was used as positive control and ethanol as a negative control.

At 0.2% concentration of chlorhexidine, 17 mm zone of inhibition was obtained, and at 4% concentration of tulasi extract, it was 17 mm (Table 1-2).

**Preparation of tulasi mouthwash**
- 4% tulasi extract
- Distilled water - 78.469% w/w
- Sodium saccharine - 0.03%
- Glycerine - 2%
- Peppermint - 2%
- Buffer (phosphate) - 2 mL
- Preservative (benzoic acid).

Sterile cotton swabs were used to obtain smear from oral candidal lesions. Samples were streaked on Sabouraud Dextrose Agar and incubated at 37°C for 24 h and then left at room temperature for another 24 h. The growth of Candida appeared as creamy smooth pasty colonies. The culture was said to be negative if there was no growth even after 72 h of incubation. Colony counting was done using digital machine.

A total of 108 patients were randomly allocated to chlorhexidine and tulasi groups. Pre- and post-oral symptoms such as burning sensation and taste alteration were recorded on a VAS scale of 0–10.

Patients were asked to rinse either 10 mL of tulasi mouthwash diluted with equal quantity of water or 10 mL chlorhexidine mouthwash thrice daily for 2–3 min for 7 days after food. They were instructed to abstain from eating or drinking for 30 min after the use of mouthwash. On the 8th day, the smear was taken for the culture of Candida.

Analysis of the data was done using SPSS 22.0 software (paired and unpaired t-test).

**Results**
A total of 108 patients were recruited, of which 90 completed the study. Of the 18 dropouts, 8 belonged to chlorhexidine group and 10 to tulasi group. 12 did not come for the follow-up and 3 of them were referred to higher centers for their medical conditions. In three patients, the cultures were contaminated.

Mean age of patients in chlorhexidine group was 53.2 years (males - 13 and females - 22) and tulasi group was 55.2 years (males - 12 and females - 23). Most patients presented with erythematous candidiasis and pseudomembranous candidiasis followed by denture stomatitis. Following treatment clinical

<table>
<thead>
<tr>
<th>Clinical parameters and colony forming units</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
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<tbody>
<tr>
<td>Burning sensation (VAS 0–10)</td>
<td></td>
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<tr>
<td>CHX</td>
<td>4±1.56</td>
<td>1.33±1.10</td>
</tr>
<tr>
<td>Tulasi</td>
<td>5.33±1.80</td>
<td>2.44±2.10</td>
</tr>
<tr>
<td>Taste sensation (VAS 0–10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHX</td>
<td>3.13±1.48</td>
<td>1.71±1.29</td>
</tr>
<tr>
<td>Tulasi</td>
<td>5.26±1.60</td>
<td>3.28±1.29</td>
</tr>
<tr>
<td>Colony-forming units/cu mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHX</td>
<td>23.54±16.29</td>
<td>12.04±8.74</td>
</tr>
<tr>
<td>Tulasi</td>
<td>25.42±15.73</td>
<td>8.62±12.41</td>
</tr>
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**Table 1:** Inter- and intra-group comparison

<table>
<thead>
<tr>
<th>Minimum inhibitory concentration (%)</th>
<th>Diameter of zone of inhibition (mm)</th>
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<tbody>
<tr>
<td>1</td>
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<td>2</td>
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<td>3</td>
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<td>9</td>
<td>17</td>
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<td>10</td>
<td>19</td>
</tr>
</tbody>
</table>

**Table 2:** Determination of MIC of tulsi extract

MIC: Minimum inhibitory concentration
improvements of the lesions was seen in both groups. (Pictures 1 and 2).

Following treatment, the burning sensation was relieved completely in 22.22% of patients in chlorhexidine group and 26.66% in tulasi group. The mean VAS score for burning sensation was reduced from 4 ± 1.56 to 1.33 ± 1.10 in chlorhexidine group ($P = 0.00$) and from 5.33 ± 1.80 to 2.44 ± 2.10 in tulasi group ($P = 0.00$) [Graph 1].

Altered taste sensation showed an improvement from 3.13 ± 1.48 to 1.71 ± 1.29 in chlorhexidine group and from 5.26 ± 1.60 to 3.28 ± 1.29 in tulasi group [Graph 2].

Mean CFUs per cubic millimeter in chlorhexidine and tulasi groups were 23.54 ± 16.29 and 25.42 ± 15.73 which significantly reduced to 12.04 ± 8.74 and 8.62 ± 12.41, respectively ($P = 0.001$) [Graph 3].

**Intergroup comparison**

On comparing the two groups, VAS score for improvement burning sensation and taste alteration was statistically significant in patients using tulasi mouthwash as compared to chlorhexidine. The difference in pre- and post-CFUs was not statistically significant between the two groups.

**Discussion**

Tulasi, a part of the traditional Indian household, has fascinated Indian researchers for decades. The plant extract has been known to possess antimicrobial, antioxidant as well as wound healing properties. Phytochemicals such as flavonoids, linalool, alkaloids, saponins, tannins, phenols, and terpenoids abundantly present in tulasi leaves are responsible for its antifungal properties.

The results of this study indicated that both mouthwashes were effective in the management of oral candidiasis. The mean improvement in burning sensation from pre- to post-treatment in chlorhexidine group and tulasi group was 2.67 and 2.89, respectively. In both the groups, there was significant improvement in taste, but tulasi gave better results than chlorhexidine.
A widely prescribed mouth rinse, chlorhexidine has shown good clinical results as an alternative to conventional antifungal agents. Chlorhexidine inhibits adhesion of Candida to oral and mucosal surfaces. It causes coagulation of nucleoproteins and also porosities in the cell wall, resulting in extrusion of cytoplasmic components.

In an *in vitro* study, 0.2% chlorhexidine was found to be more effective than fluconazole in inhibiting growth of *Candida albicans* on heat-polymerized acrylic resins. Another *in vitro* study showed chlorhexidine to have significant antifungal activity as compared to ketoconazole on *Candida albicans* isolated from children with early childhood caries.

The antifungal action of tulasi is due to its effect on the ergosterol biosynthesis pathway. Ergosterol is a membrane sterol unique to fungi. It is necessary for the growth and normal function of the cell. It acts as a bioregulator of membrane integrity, fluidity, and asymmetry and also contributes to the proper function of membrane-bound enzymes.

Khan *et al.* in 2010 evaluated the antifungal activity of tulasi by testing the action of its two main components - methyl chavicol and linalool as well as their synergistic action with azoles. Fluconazole-resistant and non-resistant strains of Candida were exposed. Results showed that the action of tulasi extract was synergistic with fluconazole and effective even against fluconazole-resistant candidal species.

This study is the first of its kind evaluating the clinical efficacy of tulasi in the management of oral candidiasis. Therefore, early recognition of the potential risk and prophylactic treatment of oral candidiasis with tulasi mouth rinse may prevent serious morbidity and prevent fatal consequences in high-risk patients.

Tulasi because of its antimicrobial, anti-inflammatory, immunomodulatory, and antioxidant properties has shown better control over candidal microorganisms because of its combined effects as compared to chlorhexidine.

**Limitations of the study**
- The limitation of this study is short follow-up period.

**Future recommendations**
- Evaluating the efficacy of Tulasi:
  - On different species of Candida
  - Over antifungal drugs
  - On different antifungal-resistant strains
  - In HIV patients with oral candidiasis.
- Conducting different studies using tulasi extract in various forms - gels, patch, and spray.

As candidiasis is an opportunistic infection, management of underlying predisposing factor is important. The antifungal drugs do have side effects, and there is an emergence of multidrug-resistant strains of *Candida*; therefore, tulasi can be considered as a viable alternative to synthetic drugs.

Tulasi is abundantly available, easily accessible, commercially feasible, culturally acceptable, with minimal side effects and thus can be used in the management of oral candidiasis.

**Conclusion**

Antifungal activity in terms of subjective and objective evaluation of chlorhexidine and tulasi mouthwash was found to be significant in the management of oral candidiasis. However, tulasi showed better results than chlorhexidine. More clinical studies have to be conducted to validate the antifungal efficacy of tulasi.

**Acknowledgment**

This study was funded by Rajiv Gandhi University of Health Sciences, Karnataka, conducted in 2014-15. The authors declare no potential conflicts of interest.

**References**

Annigeri, et al. Tulasi in oral candidiasis


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