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Original Article

Evaluation of Antifungal Activity of Ethanolic Ocimum Sanctum extract

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ABSTRACT

Aim: To determine the minimum inhibitory concentration (MIC) of Tulsi extract for Candida. **Design and setting**: Experimental design, in vitro study, Lab setting. **Material and Method:** Ethanolic extract of Tulsi was prepared by cold extraction method. Serial dilutions were prepared.(0.5%.1%,2%,3%,4%,5%,6%,7%,8%,9%,10%). Chlorhexidine was used as positive control. These were then subjected to microbiological investigation to check the minimum concentration of the extract which gives wider zone of inhibition. The zones of inhibition were measured in mm using vernier calipers. **Result**: At 0.5% concentration of Tulsi extract, zone of inhibition of 21 mm was seen. **Conclusion:** Tulsi extract showed antifungal property and 0.5% concentration of Tulsi is the MIC.

Key words: Antifungal, candida, tulsi extract.

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INTRODUCTION

Herbal medicine is both promotive and preventive in its approach. It is a comprehensive system, which uses various remedies derived from plants and their extracts to treat disorders and to maintain good health. Patients are more concerned about both their oral healthand their overall medical wellbeing. Thus, in the midst of growing evidence of the connection between oral healthand whole body health, herbal medicines offer a gentle andenduring way for restoration of health by the most trustworthy and least harmful way.[1] Chlorhexidine is a broad spectrum antimicrobial mouthrinse and most widely used over the counter mouthrinse in the market. It binds to soft and hard tissues in the mouth, enabling it to act over a long period after application of a formulation.[2] However, chlorhexidine has several side effects, such asstaining and taste alteration, which limit its long term use.[3] Therefore, chlorhexidine is used as a positive control inmany clinical trials of new mouth rinse formulations and is considered the gold standard.

The lesion is caused by Candidal species, which has clinical manifestations like pseudomembranous and erythematous forms, causing symptoms such as pain, burning sensation, and altered taste; subsequently leading to nutritional compromise.

Even the use of allopathic medicines provides temporary relief with a threat of side effects to patients Thus the use and effectiveness of antifungal activity of medicinal plants is lightening a hope against antifungal diseases.

MATERIALS AND METHODS

Preparation of tulsi extract:

Tulsi powder was procured from an Ayurvedic Shop. 500 grams of Tulsi was then mixed with 100% ethanol. The filtrate was obtained using the filter paper. This filtrate was then reduced to syrupy consistency (Figure 1) using

evaporator and the residual solvent was reduced to obtain solid residue (Figure 2).

Minimum inhibitory concentration and Zone of Inhibition Determination (MIC)

Serial Dilution Preparation:
Serial dilution of tulsi extract was prepared, 0.5%.1%,2%,3%,4%,5%, 6%,7%,8%,9%,10%. 0.2% chlorhexidine was used as positive control.
Cup and plate method was used to determine the zone of inhibition.in this method 6 circular wells which could incorporate 6 different volumes (10µl,20µl, 40µl,50µl,75 µl and 100 µl) of the test agent (tulsi extract) were cut into the Selective Media i.e. Sabouraud's Dextrose Agar media using templates.Agar plates were allowed to dry and wells of 8mm diameter were prepared with a sterile standard device. Different Volumes (10,20,30,40,50,75,100ul) of the dilutions of the extract were propelled directly into the wells of the inoculated media agar plates. The plates were allowed to stand for 10minutes for diffusion of the extract to take place and incubated at 37^{o}C for 48 hrs.The incubation zones were measured with a caliper (Figure 3) the results showed that 0.5% was the minimum concentration showing inhibitory effect on the microorganism.so 0.5% of the extract was used in preparation of the mouthwash to treat oral candidiasis in this pilot study

Preparation of Mouthwash (Figure 5)
It was carried out in the Department of Pharmaceutics; Bapuji Pharmacy College; under the guidance of Head of Department of Pharmaceutics

Formula (0.5% Tulsi mouth rinse)

- Distilled water- 78.469% w/w
- Sodium Saccharine – 0.03%
- Tulsi Extract- 0.5%
- Glycerine – 2%
- Peppermint– 2%
- Buffer (Phosphate) – 2ml
- Preservative (Benzoic Acid)

Clinical aspect of the study.

Source of data:
A pilot study was planned. Data wascollected from the patients admitted in the wards in the JJMMC hospital and Chigateri Government Hospital; Davangere.Written informed consent obtained from all the patients.Written permission was obtained from the Principal and the respective heads of the various Departments, JJMMC, Davangere.

METHOD OF DATA COLLECTION
20 patients of either gender were selected based on:

Inclusion Criteria

- Clinically diagnosed Oral candidiasis case.
- Not on any antifungal drugs before the trial.
- Mentally sound enough to answer the Questionnaire and consent for the study.

Exclusion Criteria

- Allergic to Tulsi.
- Pregnant patient.

The patients were explained about the procedure and the Visual Analogue Scale (VAS) scores (0-100).A proforma was prepared for the study where in the chief complaint and the VAS scores were recorded . Swab was collected before the mouthwash usage. Patient was asked to use 10ml of mouthwash 3times a day for three days after food, swish for 30 second and spit. For microbiological evaluation a moist swab is used to take the sample in 2ml of saline. This is then centrifuged at 2000 rpm for 15minutes.The supernatant is discarded and of sterile saline is added. 10µl of the sample is put in the SDA media plates prepared with chloramphenicol to avoid growth of other gram positive and negative organisms. The plates were streaked and incubated at 37^{0}C for 48 hours.

RESULTS:
The study included 20 subjects **.**Out of 20 patients 5 patients dropped out. The mean age of the patients was 50.9 years. On an average the patient had spent about 12 days admitted in the hospital under medication due to various health problems.
Among the 15 patients included, 12 patients had complained of burning sensation with a mean of 65.3%; in a range of 0-100 on VAS at the beginning of the study. Maximum reduction noticed after the study was about 50%.
Four patients among fifteen had also complained of metallic taste which was reduced by 1 unit in two cases.
Regarding the compliance of the mouthwash, all the subjects answered in affirmation to the taste of the mouthwash as refreshing with the duration of the freshness lasting for more than 30 minutes and easy compliability. Four patients complained of burning sensation on initial usage which was attributed to the peppermint flavour added.
Pre and post interventional candidial growth showed obvious decrease in the colonies though they could not be counted (Figure 6). There was clear clinical healing of the candidal lesions (Figure 7)

Figure 1: Ethanolic extract of tulsi reduced to syrupy consistency

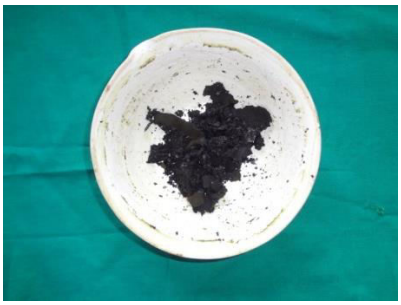

Figure 2: Solid residue.

Figure 3: Measurement of zone of inhibition by vernier callipers

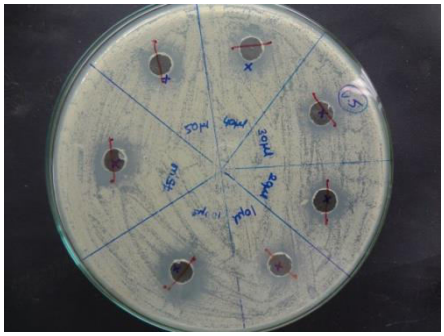

Figure 4: MIC shown by 0.5% of the tulsi extract dilution

Figure 5: Tulsi mouthwash preparation

Figure 6: Pre and Post interventional Candidal growth

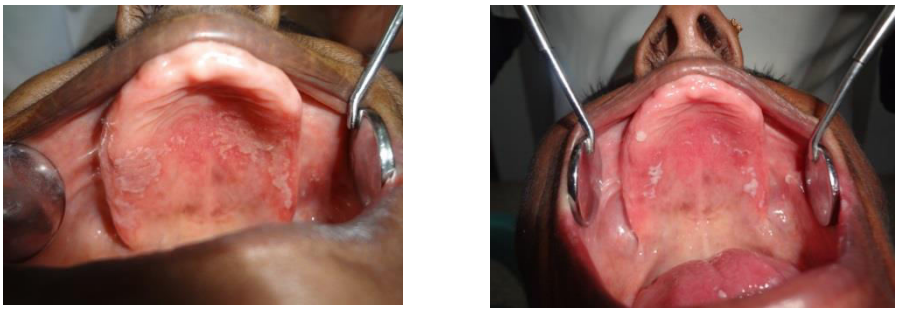

Figure 7: Pre and Post clinical presentation

DISCUSSION

Candida species occupy a predominant place in the etiologyof oral fungal infections[4].Oral candidiasis is a common problem among immunocompromised, geriatric population and chronically ill patients on antibiotic therapy. Fungal pathogens are always hard nuts to crack in medical sciences. The pathogens involved in human are more or less present all over world and are posing a severe threat due to drug resistance and through reoccurrence of diseases. It is commonly observed that these disease conditions aremore or less remains life long without cure as the tulsi extract has many beneficial effects with no side-effects, easilyaccessible, economical hence recommended for long term use.

CONCLUSION

It is found that the chlorhexidine is most potent than many antifungal drugs. It reduces the burden of consuming separate drugs to prevent fungal infections in the oral cavity. Nevertheless, there are also some adverse effects associated with its use; for example, superficial staining of the enamel, burning sensation, and altered taste sensation. As a result, alternative agents have been gaining attention .The use of such agents may be a more desirable alternative against other antifungal agents.The antifungal activity of certain bioactive compounds from medicinal plants has attracted a lot of attention within the scientific community largely as a result of the growing problem of multidrugresistance among pathogenic fungi. There are various invitro studies carried out to show the antifungal properties of various herbs. Hence a pilot study was attempted as the biological environment is different than the lab settings. Moreover this study can be further carried out at alarger scale to get more definitive results.

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