In vitro anti - candida activity of an indigenous mouth wash used in Ayurveda practice

Nadesakanthan S.[1], Sanjeewa A.[1], Uluwaduge D.I.[2], Peiris K.P.P.[3]* and Meedin F.[1]

Abstract

The relation between oral diseases and the activities of microbial species that form part of the micro biota of the oral cavity is well established. This study was focused to investigate anti-candida effect of an indigenous mouth wash used in Ayurveda practice which is mentioned in authentic text as *Gandusha* (mouth wash). *Candida albicans* is the most common fungal pathogen effects on oral mucosa. The study was based on anti-candida susceptibility test by using clinically isolated culture of *Candida albicans* as "standard fungal pathogen", sterile normal saline as a "negative control" and antifungal solution as "positive control" using agar well diffusion method having 8mm diameter wells. Each well was loaded with 75µl of aqueous extract of an indigenous mouth wash and Amphotericin B anti-fungal preparation as positive control against *Candida albicans*. 75µl of sterile distilled water was pipetted into the remaining well as negative control. Each sample was triplicated. The results revealed that the indigenous mouth wash inhibited the clinical isolates of *Candida albicans* (zone of inhibition=10±0.22 mm) and has considerable anti-candida activity against clinically isolated *Candida albicans.*

Keywords: Oral health, Indigenous mouth wash, *Gandusha, Candida albicans*

Introduction

Mukhapaka is the commonest disease of oral mucosa described in Indigenous medical system[1]. It is referred as stomatitis or mouth ulcers according to the modern medicine. Stomatitis is a common lesion and frequency is reported to be 10-20% in the community and is characterized by recurrent localized and painful oral ulcers which affect eating, speaking and even the quality of life[2,3]. It is curable and not considered a threat to life but have a significant negative impact on oral health.

Among the fungal pathogens *Candida albicans* (*C. albicans*) is the most prominent organism affecting oral mucosa[4]. The prevalence of oral carriers caused by *Candida albicans* in healthy dentate adult subjects was 44.4%[5] as determined by imprint culture. Moghadam et.al.[8] (2020) reported that the dental caries, periodontal diseases and oral candidiasis contribute considerably to the world's economic burden.

Recently, antifungal resistance of Candida species has been increasingly reported requiring more potent antifungal agents[8]. Further, the currently available antifungal agents cause undesirable side effects such as hepatotoxicity, nephrotoxicity and neurotoxicity. In this context, the global need for alternative prevention and treatment options and products for oral mucosal diseases which are safe, effective and economical is valued due to the rise in disease incidence specially in developing countries.

[1]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Sri Jayawardenapura, Sri Lanka.
[2]Department of Basic Sciences, Faculty of Allied Health Sciences, University of Sri Jayawardenapura, Sri Lanka.
[3]Department of Shalya – Shalakya, Faculty of Indigenous Medicine, Gampaha Wickramarachchi University of Indigenous Medicine, Sri Lanka.*

**Correspondence: Peiris K.P.P., Department of Shalya – Shalakya, Faculty of Indigenous Medicine, Gampaha Wickramarachchi University of Indigenous Medicine, Sri Lanka. Email: priyanip@gwu.ac.lk*

Ayurveda *Gandusha* and *Kavalagraha*[6] are two main oral cleansing therapies and also used to treat the oral diseases. In addition to that *Pratisarana* (local application) also described in authentic texts for oral disease management. *Gandusha* (mouth wash) involves filling the mouth completely with fluid without any movements[7].

This study was aimed to assess the anti-candida activity of an indigenous mouth wash containing *Jasminum officinale* L. (leaves), *Terminalia chebula* Retz (pericarp), *Tinospora cordifolia* (Thunb.) Miers (stem) and *Glycyrhiza glabra* (Licorice) (roots). This authentic herbal preparation is used in Ayurveda medical system as a treatment for oral mucosal and periodontal diseases from ancient time and has been mentioned in traditional medicine. However, no scientific data are available to prove its clinical efficacy and this study was undertaken to support the scientific rationale for the presumption in Ayurvedic medicine.

Materials and Methods

Plant materials

Dried pericarp of *Terminalla chebula,* dried stem of *Tinospora cordifolia*, roots of *Glycyrrhiza glabra* and tender leaves of *Jasminum officinale*, and whole plant of *Desmodium triflorum* were collected and authenticated by the Department of *Dravyaguna*, Faculty of Indigenous Medicine, Gampaha Wickramarachchi University of Indigenous Medicine, Sri Lanka.

Preparation of aqueous extract

Aqueous extract was prepared using 75 g dried plant material (*T. chebula, T. cordifolia*, *G. glabra* 75g) and fresh plant materials (*J. officinale, 7*5g and, *D. triflorum*, 150g) in 1440ml of water. This was boiled to reduce the volume 240 ml according to *Ayurveda Gandusha paribhasha* [9]. The polyherbal extract was filtered, and filtrate was freeze dried. The freeze-dried powder was used to assess the *in vitro* anti-candida activity.

Determination of antifungal activity by Agar–Well diffusion method

Inhibitory activity of the aqueous *Gandusha* extract on C. albicans was determined using an agar well diffusion method as described by Magaldi, with few modifications. Standard suspensions (0.5 McFarland) of C. albicans were prepared in sterile normal saline and inoculated on Mueller Hinton Agar (MHA) to obtain a confluent growth. Three wells of 6 mm diameter each were prepared in each agar plate and the bottoms of the wells were sealed with sterile MHA. 75µl aqueous *Gandusha* extract and sterile distilled water as the negative control were added into wells. 0.25mg/ml Amphotericin B (Sigma-Aldrich, USA) was used as the positive control. The diameter of the zone of inhibition was measured after overnight incubation of the agar plates at 37 C. In the well diffusion assay any zone of inhibition observed was considered as significant. Well diffusion assay was done in triplicates and average zone of inhibition was calculated.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was determined by using the pour plate method. 3.0g of freeze-dried powder of the plant extract was constructed to a concentration of 250 mg/ml. Briefly, doubling dilutions of the extract [Neat (concentration of 250 mg/ml), ½, ¼, 1/8, 1/16, 1/32, 1/64] were prepared in MHA using plant extract. Molten MHA (20 ml) was cooled to 50^0C, mixed well with each extract dilution, and poured into sterile petri plates. The plates were allowed to dry and 5µl of the *C. albicans* suspension (prepared to the 0.5 McFarland standards) is placed on the agar plate. The drop was allowed to stand for few minutes and the plates were incubated in the upright position for at 37^0C. Presence or absence of any growth was observed after 24 hours. The lowest concentration of the extract that inhibited the visible growth of the organism after overnight incubation was determined as MIC. Experiments were done in triplicates and the average MIC was calculated[12.]

Assessment of efficacy of the refrigerated herbal preparation

The indigenous mouth wash was made according to the *Gandusha paribhasha* described in Sharangadhaara Samhitha, was stored in the refrigerate (+4^{o}C) in an airtight sterile polypropylene container for a one month. Thereafter, this sample (one-month old indigenous mouth wash) was tested for anti-candida activity by using previously mentioned procedures[13]. The results were compared with freshly prepared sample.

Results

The indigenous mouth wash inhibited the clinical isolates of *C. albicans* (zone of inhibition=10±0.22 mm) and has considerable anti-candida activity against clinically isolated *C. albicans.* (Figure 1).

Therefore, this preparation was further tested to determine the minimum inhibitory concentration against the isolate. The MIC of the preparation against *C. albicans* was 1/32 (7.8125 mg/ml).

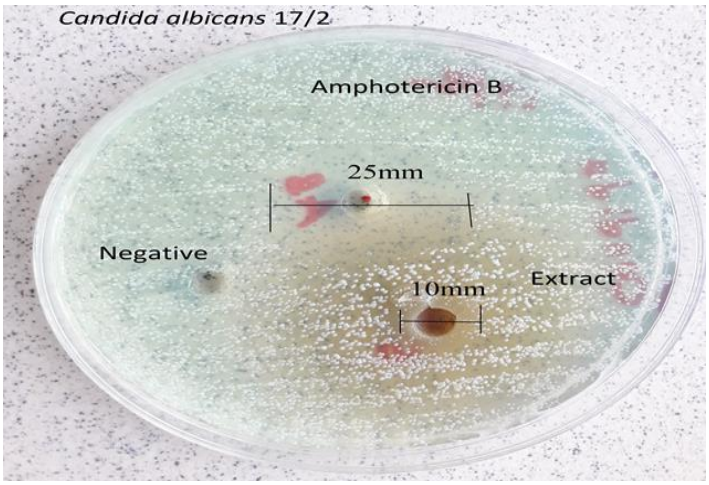

Fig.1: Inhibition of *C. albicans* by the polyherbal preparation. Inhibition zones of extract, positive control (Amphotericin B) and negative control (sterile distilled water) are shown here. Assay was done in triplicates

Determination of the stability of the polyherbal preparation

Zone of inhibition against *C. albicans* of the one month stored herbal preparation was significantly reduced when compared to the freshly prepared extract even when stored at +4^{o}C (zone of inhibition 9.67±0.58 vs 1.00±0.22 mm)

In this study, the polyherbal indigenous mouth wash exhibited anti-candida activity against infection caused by *C. albicans* in the oral cavity. *C. albicans* have been reported to be a major pathogen responsible for human candidiasis.

Recently, antifungal resistance of *Candida* species has been massively reported requiring more potent antifungal agents[14]. Further, the undesirable side effects such as hepatotoxicity of most antifungal agents are important a problem which needs to be addressed[15]. Therefore, it requires searching for alternative herbal medicines that are safe and efficacious.

This study shows that the polyherbal indigenous mouth wash has the potential to protect oral mucosa against candidiasis. Several studies have shown that an ingredient of indigenous mouth wash used in the present study possesses potent anti-candida activity. In indigenous medical system, leaves and flowers of *J. officinale* is widely used in oral ulcers and stomatitis (*Mukha Ppaka*) due to its *Sophaghna* (anti-inflammatory) *Kandughna* (antifungal), *Vrana ropana* (ulcer healing), *Krimighna* (anti-microbial) and *Sothaghna* (reduce swelling) actions. Further, the in vitro studies conducted by Hussain et al., (2013) has confirmed the anti-fungal effect of *J. officinale.*[16] This study provided evidence to prove the significant anti-fungal effect of methanol extract of whole plant against fungi, including *C. albicans*. Phytochemical studies confirmed that leaves of *J. officinale* contained, resin, salicylic acid, ascorbic acid, and alkaloids which are effective in healing oral ulcers.

Upadhyay et.al (2014) have isolated that the chebulic acid, chebulagic acid, corilagin and gallic acid as the main active components of *T. chebula* which contributes to antimicrobial activity [17,18]. In year 2012, Guptha reported that aqueous extract of the fruit possesses antifungal action against *C. albicans*[19].

In Ayurveda it has shown that, *T. cordifolia* has pharmacological actions such as *Sothahara, Sophahara,* V*rana ropana, Sodhana* and *Rakta*

sthembhana. Patil et.al., (2017) reported that aqueous extract of *T. cordifolia* contains alkaloid, flavonoids, glycosides, phenolic compounds and tannins which are known to contain antifungal activity[20]. Further, Singh et al., (2016) has reported that *T. cordifolia* has significant antifungal activity against *C. albicans.*[21]

Lai et al., (2009) confirmed that *D. triflorum* has anti-inflammatory activity[22]. Phytochemical constituents of D. *triflorum* are reported to be terpenoids, flavonoids, steroids, alkaloids, glycosides and tannins.[23]

Fatima et al., (2009) revealed that the bark of *G. glabra* was used to demonstrate antifungal activity against *C. albicans*. *G. glabra* contains glabridin which is associated with antifungal activity[24].

In the assessment of the quality, it was evident that fresh preparation is effective and with increasing the shelf life the potency also reduces. Therefore, to maximize the beneficial effects as a mouth wash a freshly prepared solution has to be used.

Conclusion

The results obtained from the current study suggest that the herbal preparation possesses significant antifungal activity against clinically isolated *C. albicans.* Further it confirmed that freshly prepared herbal preparation showed maximum potency which can be recommended to use as the mouth wash.

Conflict of Interest

Not declared.

Funding

No funding sources.

References

1. Singhal G.D., Diagnostic Considerations in Ancient Indian Surgery Vol.III, Based on Nidana sthana of Susruta Samhita Chaukhamba Sanskrit Pratishthan, 2015, 16th chapter, Verse 3, p231.
2. Kiraz S., Erdogan A., Erden A.K., Kilic L., Kalyoncu U., Bilgen S.A. and Ertenli I. Prevalence of Recurrent Aphthous Stomatitis and Related factors, Annals of the Rheumatic Diseases, 2015, 74(2) (Suppl).
3. Edgar N. R., Dahlia S.R., and Miller R.A Recurrent Aphthous Stomatitis: Review, *The Journal of Clinical and Aesthetic Dermatology*. 2017,10(3); 26-36.
4. Lijun H. U, Chun H.E, Zhao C.C, Chen X., Hue H. and Yan Z. Characterization of Oral Candidiasis and the Candida Species Profile in patients with Oral mucosal diseases. Microbial pathogenesis. 2019, 134; 103575.
5. Arendorf T.M. and Walker D.M. The Prevalence and Intra-Oral distribution of candida albicans in man. *Archives of Oral Biology*. 1980. 25(I), 1-10.
6. Charaka Samhita; (Text with English Translation and Critical Exposition based on Chakrapanidatta's Ayurveda Dipika), Ramkaran Sharma and Bhagwan Dash; Sutra Sthana, 1994, Chapter 5, Verse 75.
7. Sushruta: Sushruta Samhita of with the Nibhandhasangraha Commentary of Shri Dalhanacharya; edited by Vaidya Yadavaji Trikamji Acharya; Chikitsa Sthana, 2010, Chapter 40, Verse 62.
8. Moghadam E.T., Yazdanian M., Tahmasebi E., Tebyanian H., Ranjbar R., Yazdanian A. et.al., Current herbal medicine as an alternative treatment in dentistry: In vitro and clinical studies. *European Journal of Pharmacology*. 2020, p 889.
9. Sharangadhara, Sharangadhara Samhita of Pandita Sharanghadhara with commentaries of Adhamalla Dipika and Kashirama Gudharth Dipika, Print P.P. Shastri and U.K. Vidyasagar (Eds.), 2013, 10/2-3, Chaukhamba Publication, 352.

10. Lee S.C., Fung C.P., Lee N., See L.C., Huang J.S., Tsai C.J., Chen K.S. and Shieh W. B, Fluconazole Disk Diffusion Test with Methylene Blue-and Glaucose – Enriched Mueller Hinton Agar for Determuning Susceptibility of Candida Species. *Journal of Clinical Microbiology.* 2001. 39(4); 1615-1617.
11. Arikan S., Current Status of Antifungal Susceptibility testing methods. Medical Mycology, 2007. 45, 569-587.
12. Gunasekara T., Radhika N., Ragunathan K., Gunathilaka D., Weerasekera M., Hewageegana H., et al. Determination of antimicrobial potential of five herbs used in ayurveda practices against *Candida albicans*, Candida parapsilosis and methicillin resistant Staphylococcus aureus. *Ancient Science of Life.* 2017. 36, 187.
13. Edbeib M.F. Notarte K. I. and Yaakub H. Stability and anti-bacterial property of polyherbal mouth wash formulated using local ingredients. *Biosaintifika Journal of Biology and Biology Education*. 2020.12(3); 288-296.
14. Basu A.S. and P.K. Quality control of culture media in a microbiology Laboratory. *Indian Journal of Medical Microbiology.* 2005. 23(3); 159-63.
15. Rupasinghe R.N.N.A. and Peiris K.P.P. Comparative anti- plaque effect of herbal dental formula. *International Journal of Current Advanced Research*. 2020.9; 04 (21794-21798).
16. Hussain M., Bakhsh H., Aziz A., Majeed A. and Khan I.A., Comparative In vitro study of antimicrobial activities of flower and whole plant of Jasminum officinale against some human pathogenic microbes. *J. Pharm Altern Med.* 2013;2(4).
17. Upadhyay A., Agrahari P. and Singh D.K., A review on the pharmacological aspects of *Terminalia chebula. Int J Pharmacol*. 2014. 10(6):289–98.
18. Kannan P., Ramadevi S.R. and Hopper W., Antibacterial activity of *Terminalia chebula* fruit extract. *African J Microbiol Res*. 2009. 3(4):180–4.
19. Gupta P.C., Biological and pharmacological properties of *Terminalia chebula* Retz. (haritaki)- an overview. *Int J Pharm Pharm Sci*. 2012. 4 (SUPPL.3):62–8.
20. Patil R.C., Kulkarni C.P. and Pandey A., Antibacterial and phytochemical analysis of *Tinospora cordifolia* , *Azarchita indica* and *Ocimum santum* leaves extract against common human pathogens : An in vitro study. *J Pharmacogn Phytochem*. 2017.6(5):702–6.
21. Singh G., Saxena R.K. and Singh N.K. Screening of Potential Antimicrobial Activity of Indian Medicinal Plant of Different Solvent Extract : *Tinospora cordifolia* and *Hymenocallis littoralis. Int Res J Eng Technol.* 2016. 3(3):928–32.
22. Lai S.C., Peng W.H., Huang S.C., Ho Y.L., Huang T.H. and Lai Z.R., *et al.*, Analgesic and anti-inflammatory activities of methanol extract from *Desmodium triflorum* DC in mice., *American Journal of Chinese Medicine.* 2009. 37; 573–88.
23. Liu C., Wu Y., Zhang Q.J., Kang W.Y. Zhang L., and Zhou Q. D. Advances in studies on chemical constituents and biological activities of Desmodium species. Zhongguo Zhongyao Zazhi. 2013. 38, 4006–14.
24. Fatima A., Gupta V.K., Luqman S., Negi A.S., Kumar J.K., Shanker K., et al. Antifungal activity of *Glycyrrhiza glabra* extracts and its active constituent glabridin. Phytotherapy Research. 2009. 23;1190–3.