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An In-Vitro Evaluation of Antifungal Effect of Tissue Conditioner Incorporated With Hydroalcoholic Leaf Extract of *Acacia Nilotica*

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Abstract

Topical antifungal therapy for denture stomatitis is challenging to apply in geriatric denture-wearers due to reduced motor activity, cognitive impairment, and memory loss. With the overuse of synthetic antimicrobials, the emergence of multi-drug resistant (MDR) strains of micro-organisms have increased. The herbal extracts of Acacia nilotica have been proven effective against MDR Candida albicans and streptococcus mutans.

Purpose: The aim of this study is to evaluate antifungal effect tissue conditioner incorporated with Acacia nilotica leaf extract.

Method: A hydroalcoholic extract of leaves of Acacia nilotica was prepared using cold maceration technique. Minimum inhibitory concentration (MIC) of the extract was evaluated using serial dilution method. The extract was incorporated into the tissue conditioner at MIC and then subjected to antifungal efficacy by disk diffusion method. A total 60 samples with dimension of 5mm diameter and 1 mm thickness were fabricated i.e. 20 control samples (CT group), 20 samples of tissue conditioner incorporated with Acacia nilotica (AT group) and 20 samples of tissue conditioner incorporated with nystatin (NT group). Each group was further subdivided according to the time interval of evaluation which is after 24 hours and after 14 days. Thus, each subgroup comprised of 10 samples.

Result: Hydroalcoholic leaf extract of Acacia nilotica have a potential antifungal activity. One-way ANOVA and Tukey's Post hoc test were used for statistical analysis.

Conclusion: Incorporation of hydroalcoholic leaf extract of Acacia nilotica into the tissue conditioner can prove to be beneficial to improve oral health status of the geriatric patients.

Keywords: Acacia nilotica, Candida albicans, denture stomatitis, streptococcus mutans, tissue conditioner.

Introduction

Denture induced stomatitis is considered the most common oral mucosal lesion which is associated with the wearing of a removable dental prosthesis with the prevalence ranging from 11% to 70% of all the denture wearers [1]. Denture stomatitis has multifactorial origin [2]. It is primarily associated with infection caused by Candida species followed by Staphylococcus species and other bacteria [3]. Candida albicans is found in 50% to 70% of all the patients [4].

Tissue conditioners can be used as drug delivery tools to the oral cavity for treatment of denture stomatitis[5], as topical antifungal therapy for denture stomatitis is challenging to apply in geriatric denture-wearers due to reduced motor activity, cognitive impairment, and memory loss[6].

Various antifungal agents like nystatin, ketoconazole, fluconazole, chlorhexidine have been effectively incorporated into tissue conditioner.[7]

However, use of synthetic antimicrobial agents have adverse effects like hepatic toxicity and nephrotoxic effects and disadvantages like drug resistance. With the overuse of synthetic antimicrobials, the emergence of multi-drug resistant (MDR) strains of micro-organisms have increased [8]. Thus, there is an increase in need to look for better drugs against MDR strains[9]. The leaf extract of Acacia nilotica have been proven effective against MDR Candida albicans and streptococcus mutans. Thus, it has a broad spectrum of action. Also, Acacia nilotica have been in use since ancient times and is well known for its antimicrobial properties[10]

Ethanolic extracts of leaves of Acacia nilotica appear to have the highest antimicrobial activities. This is because of the ability of ethanol to extract a wide range of chemical constituents (phytochemicals/ secondary metabolites) like alkaloids, sterols and tannins of the plant[11].

Aim of the study was to evaluate antifungal effect of tissue conditioner incorporated with hydroalcoholic leaf extract of Acacia nilotica. Research hypothesis states that there is significant antifungal activity exhibited by the tissue conditioner incorporated with hydroalcoholic leaf extract of Acacia nilotica.

Methodology

Preparation of the leaf extract of Acacia nilotica

The hydroalcoholic extract of leaves of Acacia nilotica was prepared by cold maceration technique. Leaves of Acacia nilotica were obtained from KLE B M Kankanwadi Ayurved Mahavidyalaya, Belagavi. The plant was authenticated from Regional Medical Research Centre, Belagavi. The leaves were kept for shed drying for 7 days. Then they were hand crushed. 70% ethanol and 30% distilled water was added to 150g of crushed leaves of Acacia nilotica. This mixture was kept for magnetic stirring for 3 days. The extract was then filtered using muslin cloth which was then subjected to evaporation using rotavapor at 35-40^{0}C. The extract was dark green in color. It was then subjected for phytochemical analysis. The resultant analysis confirmed the presence of secondary metabolites like steroids, tannins and glycosides which are responsible for antimicrobial action.

Minimum inhibitory concentration of the extract

10 ml of distilled water and 0.1 ml of DMSO was added to 10 g of extract to obtain 1g/ml of stock solution. Brain Heart Infusion broth was prepared and was autoclaved for 15 min. Serial dilution method was used to determine minimum inhibitory concentration against both the organisms. The following concentrations were prepared for the extract, using the dilution formula: 400, 200, 100, 50, 25, 12.5, 6.25µg/ml. In addition, 20µl of standard

suspension of C. albicans was added to each tube differently using Eppendorf Microtitre pipettes. The tubes were incubated at 37°C for 24 hrs. A tube containing extract and growth medium without inoculum acted as negative control whereas a tube containing growth medium and the inoculum served as a positive control. The presence of growth (turbid solution) or absence of growth (clear solution) at the end of incubation period was recorded. The lowest concentration of the extract showing no growth was regarded as the minimum inhibitory concentration[11](fig. 1). It was found that MIC for C. albicans was 75-100 µg/ml.

Fig.1 Serial dilution method to determine MIC against

Candida albicans

According to previous study nystatin was incorporated into tissue conditioner at its MIC for Candida albicans 0.032g/g.[12]

Fabrication of the specimens

The powder and liquid (with and without the extract) were mixed according to the manufacturer's instruction which was then applied to the metal die thus, 60 disk shaped specimens (5 mm in diameter and 1mm thick)[13] were fabricated i.e. 20 control samples (CT group), 20 samples of tissue conditioner incorporated with Acacia nilotica (AT group) and 20 samples of tissue conditioner incorporated with nystatin (NT group). Nystatin and the extract were incorporated into tissue conditioner at MIC. After setting, the disks were immersed in distilled water at 37^0C for 2 evaluation periods: 24 hours and 14 days.

Disk diffusion method

Mueller Hinton Agar (MHA) plates were prepared in sterile conditions in a UV chamber. Antifungal susceptibility was tested with the disk diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines.[14] then agar plates were inoculated with a Candida albicans suspension which was standardized by diluting it with sterile saline to a density of 0.5 McFarland and was spreaded over the entire agar surface. Then, a hole with a diameter of 5mm is punched aseptically with a sterile cork borer or a tip, and the samples were introduced into the well. The test groups were nystatin in sterile saline as a standard antifungal agent, Acacia nilotica as a test antifungal agent, tissue conditioner as a negative control and tissue conditioner incorporated with Acacia nilotica and tissue conditioner incorporated with nystatin. Then the plates were incubated at 35±2°c for 24 hours and 14 days. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. After the specified time interval, the diameter of inhibition zone was measured to the nearest whole millimeter (mm).[15](fig.2 and 3)

The data obtained was then subjected to statistical analysis to conclusion. Statistical analysis: one way ANOVA followed by Tukey Kramer's post hoc analysis and dependent t test.

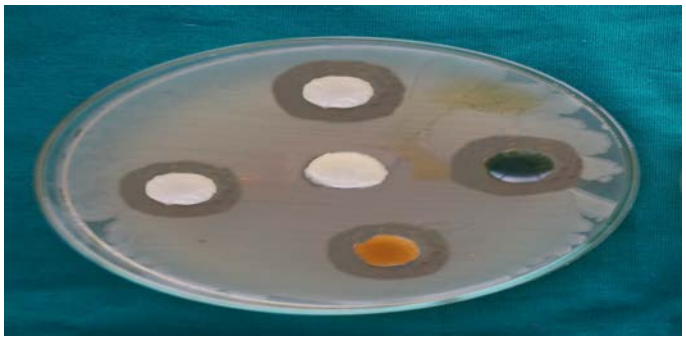


Fig 2: Diameter of inhibition zone after 24 hours

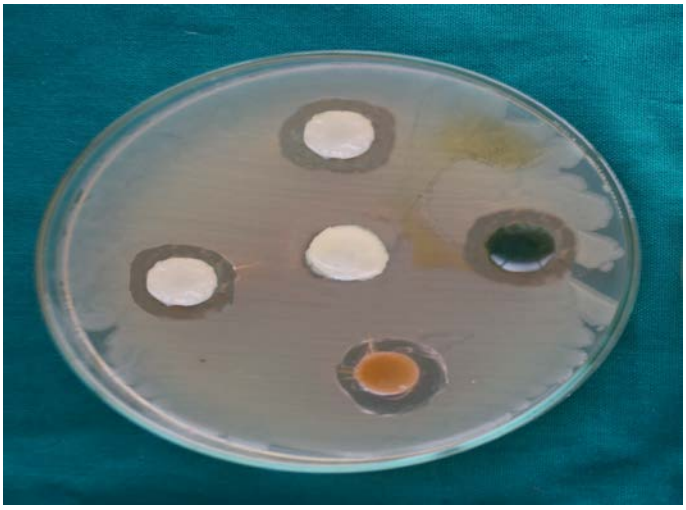


Fig 3: Diameter of inhibition zone after 14 days

Result

Tissue conditioner has no anti-fungal activity. However incorporation of nystatin, Acacia nilotica into the tissue conditioner exhibit marked antifungal activity ($p<0.05$). Highest antifungal activity was shown by Nystatin followed by NT group, Acacia nilotica and AT group. Thus, an herbal drug, hydroalcoholic extract of Acacia nilotica show antifungal activity comparable to that of nystatin. Also, the percentage change in diameter of inhibition zone is less with Acacia nilotica as compared to nystatin.

Discussion

Denture stomatitis is primarily associated with infection caused by Candida species followed by Staphylococcus species and other bacteria [3]. Topical antifungal therapy for denture stomatitis is challenging to apply in geriatric denture wearers due to reduced motor activity, cognitive impairment and memory loss[16]. In addition, maintaining an efficient dose of topical antifungals in the oral cavity is also challenging. The delivered antifungal agents fail to adhere and remain in contact with oral mucosal tissue due to factors such as regular ingestion and constant wash out by the salivary flow[17].

Various antimicrobial agents have been added to the soft liner to increase the longevity of the soft liner[18]. Antifungal agents that are added to the soft liner can be classified under natural and synthetic origins. Synthetic origins drugs include Nystatin, Amphotericin B, Miconazole, Ketoconazole, Chlorhexidine, Clotrimazole, Fluconazole, Itraconazole. Natural agents such as melaleuca alternifolia oil, origanum oil, Thai herbs C. nutans, c. sappan Linn lemongrass oil. Various inorganic antifungal medicaments have also used. They are: silver zeolite, photocatalyst, silver nanoparticles, magnesium oxide[7].

Table 1: summary of mean diameter of inhibition zones of all the groups at different time intervals

N: nystatin

A: Acacia nilotica

NT: tissue conditioner incorporated with nystatin

AT: tissue conditioner incorporated with Acacia nilotica

Time	Groups	Mean	SD	SE
24 hours	N	20.80	0.79	0.25
	A	18.00	0.82	0.26
	NT	18.50	0.71	0.22
	AT	17.00	0.94	0.30
14 days	N	16.70	0.95	0.30
	A	15.60	0.97	0.31
	NT	15.20	0.63	0.20
	AT	14.20	0.63	0.20
Difference	N	4.10	0.99	0.31
	A	2.40	1.35	0.43
	NT	3.30	0.82	0.26
	AT	2.80	0.92	0.29

Nystatin is a potent well-known antifungal agent. According to this study Acacia nilotica had a comparative antifungal activity as observed with nystatin.

Acacia nilotica is a well-known herb used since ancient times for its antimicrobial properties and minimum side effects. The herb is easily found in India and commonly

known as Babool. The extract obtained from Acacia nilotica is rich in secondary metabolites like alkali, tannins, sterols which are responsible for its antimicrobial properties[10]. However, there are various solvents used for extraction, those are ethanol, chloroform, methanol, ether. Various studies have confirmed that ethanolic extract gives better result and they fetch more phytochemicals than other solvents[11].

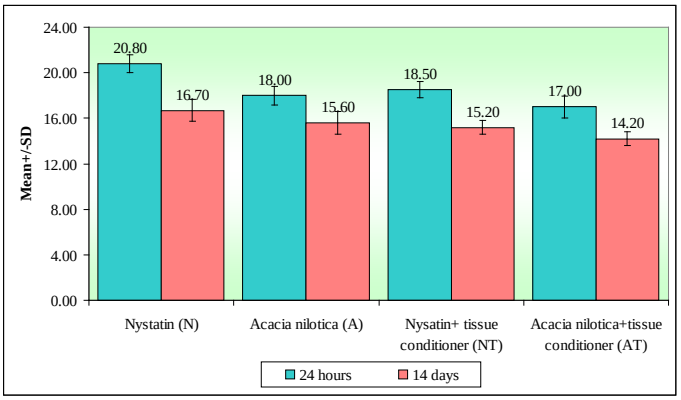
Thus, it signifies that more of secondary metabolites from the Acacia nilotica leaf extract might be leaching out of the resilient liner and produce antimicrobial effect. Also, the percentage change in diameter of inhibition zone is less with Acacia nilotica as compared to nystatin (Table 2). Thus, it reflects the sustained release of the extract from tissue conditioner. The addition of Acacia nilotica leaf extract into tissue conditioner should be considered as it can act against both on fungi as well as the bacteria because of its broad spectrum because the prosthetic Biofilm is complex formed of fungi and the bacteria[19] with minimal potential side effects. But the disadvantage being the extract causes greenish discoloration of the tissue conditioner.

Table: 2 Comparison of 24 hours (h) and 14 days' (D) time points in four study groups with mean diameter of inhibition zone by dependent t test

	Time	Mean	% change	Paired t	p-value
N	24 h	20.80			
	14 D	16.70	19.71	13.0380	0.0001*
A	24 h	18.00			
	14 D	15.60	13.33	5.6223	0.0003*
NT	24 h	18.50			
	14 D	15.20	17.84	12.6757	0.0001*
AT	24 h	17.00			
	14 D	14.20	16.47	9.6355	0.0001*

*p<0.05

Figure: 4 Comparison of 24 hours and 14 days' time points in four study groups with mean diameter of inhibition zone



Conclusion

Within the limitation of the study it can be concluded that the hydroalcoholic leaf extract of Acacia nilotica have potent antifungal activity and can be incorporated into tissue conditioner for treating denture stomatitis.

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