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Effects of antifungal to prepared herbal mouth rinses among complete denture wearers with type II diabetic mellitus

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Abstract

Objective: Effects of antifungal to prepared herbal mouth rinses among complete denture wearers with type II diabetic mellitus

Methods: Five herbal mouth rinses were prepared using parts from locally available plants, Ocimum sanctum, Aloebarbadensis miller, Elettaria cardamomum, Coffea arabica, and Salvadora persica at concentrations recommended for daily use.

Results: An 80% response rate with 96.6% compliance was achieved. No significant baseline differences were observed. All mouth rinses significantly reduced Candida albicans counts (<0.001), with Ocimum sanctum being the most effective. Ocimum sanctum exhibited highest zone of inhibition (18.34±0.11), Ocimum sanctum displayed stable colour profiles, Aloe barbadensismiller exhibited least surface roughness.

Conclusion: Findings of this study suggests promising antifungal efficacy of these herbal mouth rinses, particularly highlighting the significant reduction in Candida albicans count. Ocimum sanctum mouth rinse demonstrated the most substantial reduction in Candida albicans, followed by Coffea arabica, Aloebarbadensis Miller, Salvadora persica, and Elettaria cardamomum.

Keywords: Herbal mouth rinses, denture stomatitis, Type II diabetes mellitus, Candida albicans, antifungal effects, oral health.

Introduction

A major public health issue affecting elderly population is edentulism or teeth loss, this compromises functions of speech, mastication, and appearance leading to poor nutrition and eventually a poorer quality of life. Rehabilitation with artificial dentures have dramatically addressed these consequences but on the other hand

literature suggests an increased prevalence of oral mucosal lesions among denture wearers. Incidence of oral lesions caused by dentures is reported to vary from as low as 10.8% to as high as 62%. The most prevalent mucosal lesion associated with dentures is denture stomatitis, characterized as inflammation and erythema of oral mucosal areas covered by denture. Denture wearing elder individuals often lack physical dexterity needed to remove oral plaque from dentures this will additionally increase their risk of developing opportunistic oral mucosal infections particularly fungal ones.

Diabetes mellitus, an endocrinal disorder associated with metabolic abnormalities such as dyslipidaemia, hyperinsulinemia, and obesity promote inflammation and impairs immune functions thereby significantly delaying wound healing and increasing the risk of fungal infections among those affected. It is a global public health burden with increasing prevalence of 537 million adults in 2021 and expected to rise to 783 million by 2045. Nearly 26.5% of complete denture wearers are diabetic patients, suggesting a significant Diabetic patient with complete dentures have higher chances of developing oral candidiasis with a reported prevalence of oral of around 67%.

Antifungal medications used to treat candidiasis include Nystatin, Clotrimazole, etc. Long term antifungal therapy often leads to drug resistance and may also result in serious adverse effects, including hepatotoxicity, hormone-related effects, peripheral neuropathies, pancreatitis, and phototoxic reactions impotence, hypokalaemia, hyponatremia, adrenal insufficiency etc.

Off late, herbal compounds have gained immense popularity in the treatment of fungal infections. They form a potential source for new drugs production with less toxicity and more acceptability. This study attempts to assess antifungal effects of mouth rinses prepared from herbs among complete denture wearers with Type II diabetes mellitus.

Materials and methods

This study is a hospital based.

Identification and preparation of plant extracts: Plants of Ocimum sanctum, Aloebarbadensis miller, Elettaria cardamomum, Coffea arabicas well as sticks of Salvadora persica and were identified from the herbarium of a recognised institute and were further authenticated by an expert botanist. Leaves of Aloebarbadensis miller and Ocimum sanctum, pods of Elettaria cardamomum, Coffea arabica beans and sticks of Salvadora persica were used to prepare the extracts. Parts of the plants were further cross verified by the botanist and coded as A, B, C, D, E. Specimens were cleaned twice using distilled water and ground to fine powder using a mechanical grinder at pharmacology laboratory of the institute.

Preparation of mouth rinses: Mouth rinses were prepared by an herbal pharmacist of the institute as per a standard protocol described below. The prepared mouth rinses were coded as A, B, C, D, E by the pharmacist and were provided to the investigator.

1. Salvadora persica mouth rinse (Miswak): 25 mg of extract diluted with 100ml of distilled water to obtain 25% concentration of mouth rinse [14].
2. Aloebarbadensis miller mouth rinse (Aloe vera): 3 mg of extract diluted with 100ml of distilled water to obtain 3% concentration of mouth rinse.
3. Coffea arabica mouth rinse (Green coffee):20mg of extract diluted with 100ml of distilled water to obtain 20% concentration of mouth rinse [13]
4. Elettaria cardamomum mouth rinse (Cardamom) :25mg of extract diluted with 100ml of distilled water to obtain 25% concentration of mouth rinse [14]

5. Ocimum sanctum mouth rinse (Tulsi):50 mg of extract diluted with 100ml of distilled water to obtain 50% concentration of mouth rinse.

Selection of study participants

Sample size was calculated with 95% confidence interval, at an accepted error of 5% calculated sample was 9, a 10% of the calculated sample size was added to compensate for sampling loss if any, thus final sample size accounted to a total of 10 participants in each group. Type II diabetic patients, who have been using complete denture for a minimum of one year, harbouring 400 to 10^3 Candida albicans per millilitres of saliva, willing to participate and abide with the conditions of our study were included. Exclusions for the study were participants within the included count but having symptoms necessitating antifungal therapy. Also, individuals currently using/ with a history of use of any antifungal medications for the past two months and those currently using any other mouth rinse or therapeutic denture cleansing solutions were excluded.

Block randomisation method was employed to segregate participants to five different groups for five different mouth rinses included in the study and coded as A, B, C, D and E groups. Blinding was performed at three levels, at the first level mouth rinses were prepared and coded by the pharmacist and provided to the investigator in coded identical containers. At second level, participants were randomly allocated to different groups and each group of participants were provided with a coded mouth rinse in identical containers. Data analyst was provided with codes and respective outcome values to perform the necessary statistical analysis. Once the entire analysis was completed the codes were revealed by the pharmacist, thereby ensuring triple blindness.

Investigator provided a brief introduction about the study to the participants and informed consent from the participants were obtained. Participants were trained and instructed to use the allocated mouth rinse once daily over for a period of two weeks. Reminders were sent daily to participants and immediate family members through social media. Number of days of mouth rinse use and compliance to the study protocol was recorded by the principal investigator.

Data was collected on specially designed proforma containing three parts Part A -recorded the socio demographic details and Part B – recorded the Candida albicans count among different interventions groups, Part C- recorded acceptance of subjects to various mouth rinses. A separate section recorded invitro analysis of physical properties of denture bases that is Surface Roughness, Surface hardness, Colour Stability, Flexural Strength towards each mouth rinse.

Statistical analysis was performed using SPSS version 21 software. Descriptive statistics were calculated and expressed. Chi-square tests, one-way ANOVA, significance evaluations were conducted. Paired t-tests were employed to compare baseline and final evaluation Candida counts within each mouth rinse group. A post hoc Bonferroni test following a Repeated Measures ANOVA (RANOVA) was used to compare the mean differences between different mouth rinse groups. ANOVA followed by post hoc Bonferroni tests were employed to compare the mean differences in the zone of inhibition among various mouth rinses and the standard drug (Nystatin). The effects on the physical properties of dentures and participant's acceptance of various mouth rinses were assessed using One-way ANOVA. p-value < 0.05 was considered statistically significant.

Results

A total of 63 participants satisfying the inclusion/exclusion criteria were approached to obtain

required sample size (50 participants) thus yielding a response rate of 80%. No loss to follow up was reported. Analysis of checklist of daily mouth rinsing of participants revealed 96.6% of the participants complied with protocol of mouth rinsing.

Intra group comparison of mouth rinses for reduction in Candida albicans count(cfu/ml) at final evaluation after 15 days of use of mouth rinse revealed highly significant decrease in Candida albicans count (<0.001) amongst all mouth rinse.

Further comprehensive analysis Inter group comparison of mean differences in Candida albicans cfu reduction among various mouth rinses, Ocimum sanctum mouth rinse showed the most reduction in Candida albicans cfu followed by Coffea arabica, Aloebarbadensis miller, Salvadora persica and Elettaria cardamomum group as in Table No.3.

Zone of inhibition investigated the antifungal efficacy of various mouth rinses, alongside the standard drug Nystatin, at a dilution of 20 µl/ml. Ocimum sanctum demonstrated a significantly higher mean zone of inhibition (18.34±0.11) and Elettaria cardamomum exhibited substantial lower mean zone of inhibition (15.42±0.31) compared to all other mouth rinse, standard drug Nystatin showed the highest mean zone of inhibition (27.69±0.36) when compared to other mouth rinses.

Effects of mouth rinses on Physical Properties of Denture like Colour stability, Surface roughness, Flexural strength, Surface hardness were evaluated as shown in Table No.5. Colour stability represented by Delta E, revealed distinct characteristics among the herbal extracts. Ocimum sanctum exhibited a ▲E = 0.31, indicating a stable colour profile Elettaria cardamomum showcasing a minimal Delta E of 0.20. The Control group recorded a ▲E of 0.37, placing it within the mid-range of colour stability. Surface roughness, measured in microns, showed subtle variations. Ocimum sanctum and Salvadora persica exhibited higher surface roughness values of 0.18 microns while Aloebarbadensis miller displayed lower values of 0.15 microns when compared with Control. Flexural strength, measured in megapascals (MPa), demonstrated relatively consistent values among the herbal extracts. Aloe barbadensis miller displayed higher flexural strengths of 94.15 MPa, while Salvadora persica had lower value of 93.37 MPa when compared to Control group which exhibited a slightly higher flexural strength of 94.47 MPa. Surface hardness, measured in Vickers Hardness Number (VHN), varied subtly among the herbal extracts. Elettaria cardamomum exhibited the highest surface hardness value at 18.02 VHN, followed closely by the Control group at 18.53 VHN. Aloe Vera displayed lower surface hardness values of 17.12VHN.

Participant acceptance of various mouth rinses also showed mean taste scores ranged from 4.20 to 4.50, while the mean aroma ratings varied from 4.40 to 4.80. Overall, the taste and aroma profiles were generally consistent across the samples, indicating a high level of acceptance among participants as shown in Table no.5.

Table 1: Demographic, clinical, laboratory and candida count among participants at baseline in relation to different mouth rinse groups.

Parameters	Ocimum sanctum Group	Aloe barbadensis miller Group	Coffea arabica Group	Salvadora persica Group	Elettaria cardamomum Group	p value
Age*						
	$58^{\pm}9.5$	$60^{\pm}7.02$	$59.6^{\pm}10.2$	$51.6^{\pm 6.3}$	$58.5^{\pm 11.1}$	0.872
Gender						
Male	6(60%)	6(60%)	5(50%)	7(70%)	6(60%)	0.934
Female	4(40%)	4(40%)	5(50%)	3(30%)	4(40%)	
Systemic disorders						
Nil	7(30%)	7(30%)	7(30%)	6(30%)	7(30%)	0.831
Cardiovascular	3(30%)	3 (30%)	2 (20%)	3(30%)	2 (20%)	
Neural	1 (10%)	0	0	1(10%)	0	
Respiratory	0	0	1(10%)	0	0	
Nephrological	0	0	1 (10%)	0	1 (10%)	
Details of diabetes mellitus						
Blood sugar	$167^{\pm}15.3$	$162.8^{\pm 13.3}$	$167.3^{\pm 12.2}$	$164.6^{\pm 13.5}$	$167.30^{\pm 14.3}$	0.930
Duration *	5.2 ± 1.4	5.6 ± 2.5	4.2 ± 2.6	4.2 ± 2.6	5.4 ± 1.3	0.590
Duration of denture wear*						
	$3.5^{\pm 1.3}$	$3.0^{\pm 1.2}$	$3.9^{\pm 0.9}$	$3.8^{\pm 1.9}$	$3.3^{\pm 1.4}$	0.618
Salivary Candida count						
Candida count **	$605.8^{\pm 8.8}$	$588.4^{\pm}14.7$	$583.8^{\pm 20.2}$	$601.1^{\pm 23.5}$	$587.34^{\pm 27.8}$	0.084

Note: * Duration in years. ** Candida count reported as colony-forming units (cfu) for dilution factor of 102.

p values were determined using χ^2 test for categorical variables and One-way ANOVA for continuous variables.

p value <0.05 was statistically significant

Discussion

This study has tried to comprehensively assess the antifungal effects of various commonly available plant/herbs-based. These herbs /plants are an intricate part of daily life of people in South India. Additionally, these herbs or plants are reported to have various beneficial effects on human health and few of them have been reported to have antifungal properties.

The incidence and prevalence of diabetes have been increasing over the past 30 years, likely linked to the global obesity epidemic and demographic shifts towards older populations. Studies have shown that individuals with type 2 diabetes (T2D) have a higher prevalence of edentulism compared to those without T2D. Oropharyngeal candidiasis is common in elderly patients, with a prevalence of 13-47%. Type 2 diabetes

mellitus (T2DM) patients wearing dentures have a higher chance of developing oral candidiasis compared to non-diabetic individuals.

Conclusion

Findings of this study suggests promising antifungal efficacy of these herbal mouth rinses, particularly highlighting the significant reduction in Candida albicans count. Ocimum sanctum mouth rinse demonstrated the most substantial reduction in Candida albicans, followed by Coffea arabica, Aloebarbadensis Miller, Salvadora persica, and Elettaria cardamomum. Furthermore, Ocimum sanctum showed stable colour profiles, while Aloe barbadensis Miller displayed least surface roughness values. Importantly, all herbal mouth rinses were well-accepted by participants, as indicated by consistent taste and aroma scores.

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