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INVITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF CHEMICAL AND *ALOE VERA* MOUTHWASHES AGAINST *CANDIDA ALBICANS*

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ABSTRACT

Focusing on the scientific evidence for the effect of herbal medicines has been increased during recent decades. People are interested in herbal remedies for medication and aim to substitute herbal medicines instead of chemical drugs with limited side effects for human wellbeing. The current study was conducted to compare the *in vitro* effect of herbal and chemical mouthwashes against *Candida albicans* which is the major cause of oral candidiasis infection. A total of four mouthwashes including three chemical and one aloe vera mouthwashes were tested against *Candida albicans* by agar diffusion method. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of mouthwashes were determined by broth macro dilution method. The results showed that all the mouthwashes showed zones of inhibition against *Candida albicans.* Chlorhexidine showed higher zone of inhibition followed by Aloe vera then Colgate plax and Listerine. The results of MIC indicated that Chlorhexidine showed inhibition even at lowest dilution but Aloevera was effective at its original concentration. The findings of the study demonstrated that aloe vera mouthwash have antifungal properties as equal to the chemical mouthwashes that might represent appropriate alternative to conventional chemical mouthwashes in the management of *Candida albicans*.

KEYWORDS: Mouthwash, Aloe vera, Antifungal activity, *Candida albicans.*

INTRODUCTION

Oral diseases are recognized as major public health problem throughout the world. Oral health is an integral to general well-being and relates to the quality of life. There is

considerable evidence linking poor oral health to chronic conditions.[1] Numerous epidemiological studies showed that diseases such as oral candidiasis, tooth decay, caries, plaque and gingivitis are among the most common afflictions of mankind. Candidiasis plays a major role in the etiology of oral diseases in immunocompromised patients. Oral candidiasis is associated with multiple local and systemic factors. The subsequent dessiminated infection may be prevented by recognition and adequate management. The mainstay of preventing oral disease is the control of disease causing pathogens. Mouth rinses are generally considered as adjuncts to oral hygiene and have been recommended for the prevention and control of oral diseases. It is using as a common way to controlling the *C. albicans* population in the mouth, which are used widely in dentistry.[2] Currently, chlorhexidine is regarded the gold standard and most widely used as mouthrinse.[3, 4] But the side effects of chlorhexidine, such as tooth staining and taste alteration limit the long term use of this drug.[5] Along with increased resistance by pathogenic organisms to currently available antibiotics and chemotherapeutics, opportunistic infections in immune-compromised individuals are in rise.[6] So there is a global need for alternative treatment options and products for oral diseases that are safe, effective and economical. To overcome the side effects of such chemical drugs, the World Health Organization (WHO) has given advice for the investigation of the possible use of natural products such as herb and plant extracts.[2]

There has been increased interest in the use of natural products in the prevention and treatment of oral diseases.[7] Aloe vera is a succulent perennial plant belongs to Aloaceae family and has been used in various medical, cosmetic and neutraceutical industries. Aloe vera posses anti-inflammatory, anti-microbial, hypoglycemic and wound healing activity due to the presence of vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid.[8] Different studies have indicated that, using a mouthwash in patients with systemic infection could decrease fungal and bacterial colonization in the mouth cavity. Parker and Janu, 2011[9] investigated the Comparative evaluation of effectiveness between Aloe vera and two commercially available mouth rinses on plaque and gingival status in a randomized control trial and concluded that *A. vera* mouth rinse was as effective as two commercially popular mouth rinses in controlling plaque and gingivitis. A study by Oliveira et al., 2008 [10] indicated that the dentifrice containing *Aloe vera* was as effective *as* fluoridated dentifrice on plaque and gingivitis control and did not show any additional effect in a double blind clinical study. Chandrahas et al., 2012 [11] investigated the antiplaque and antigingivitis efficacy of Aloe vera mouth rinse

in a randomized double blind clinical study and concluded that Aloe vera mouthwash can be an effective antiplaque agent and can be an affordable herbal substitute for chlorhexidine. Thus the current study aimed to compare the antimicrobial activity of commercially available chemical mouthwashes and Aloe vera mouthwash in vitro, in order to assess their effectiveness against *Candida albicans* with respect to oral candidiasis.

MATERIALS AND METHODS

Test microorganism

The test organism *Candida albicans* (MTCC 227) was obtained from Microbial Type of Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh. The test strain was maintained on Sabouraud's Dextrose agar plates (Hi‐Media Laboratories Pvt. Limited, Mumbai, India) at 4° and sub cultured on to YPD (Yeast extract, Peptone, Dextrose) broth for 24 h prior to testing. This fungus served as test pathogen for antifungal activity assay.

Test mouthwashes

The Test mouth rinses used in this study are Hexidine ICPA Health Product (Chlorhexidine Gluconate 0.2%), Colgate plax (Cetylpyridinium chloride), Listerine mouthwash Johnson and Johnson and Aloe vera Ras. The composition of all the mouthwashes is provided in Table 1.

Anti microbial screening by agar diffusion method

A yeast suspension was made by sub culturing *Candida albicans* culture into normal saline and the OD that matched to a McFarland standard of 0.5 in which it was assumed that the number of cells were 1.5×10^8 CFU/ml. The fungal suspension was spread on Sabouraud's dextrose agar plates with a sterile swab. Subsequently, wells of 6mm in diameter were made with suitable distance using sterile cork borer on pre inoculated agar plates and filled with 100 µl of each mouthwashes then allowed to diffuse at room temperature for 2 h for proper diffusion. The presence of zone of inhibition was regarded as the presence of antimicrobial action. From the inhibition zones seen, antimicrobial activity was expressed in terms of average diameter of the zones of inhibition measured. For each mouthwash, one control plate containing mouthwash and sterilized distilled water was prepared.[12]

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations of the mouthwashes were determined using broth micro dilution method approved by the National Committee for Clinical Laboratory

Standards.[13] A serial dilution was prepared using CLSI [14] protocol. The first dilution (the highest concentration) was made by adding equal volume of stock solution to the broth in the first tube. The further dilutions were made by adding 1.5 ml of previous dilution to the next tube containing 1.5 ml broth where the v/v ratio was 1: 2, 1:4, 1:8, 1:16, 1:32, to 1:64 respectively. The final volume of broth and mouth rinse in each tube should be 1.5 ml, so the excessive amount of mouth rinse in the last test tube was discarded. Mouth rinses were thus diluted in series of exponential dilutions from undiluted to1:64 dilutions using broth dilution technique. Equal volumes (1.5 ml) of fungal suspensions and mouth rinses are mixed and incubated at 37° C for 24hrs. Controls consisted equal volume of fungal suspension and distilled water. The whole set of experiments was performed in duplicate, taking the means to get reliable results. The lowest concentration of the mouthwash for which there was no visible growth compared with the control was considered as the minimum inhibitory concentration.

Minimum fungicidal concentration (MFC)

The MFC was determined by inoculating 0.1 ml of negative growth in MIC onto sterile SDA plates. The plates were incubated at 35°C for 48 h. The lowest concentration of the mouthwash that did not demonstrate growth of the tested organism was considered as the MFC; the negative control was a plate grown with media only.[15, 16]

RESULTS

Agar diffusion method was used to screen the mouthwashes for their antimicrobial activity against *Candida albicans.* The antimicrobial activity of the tested mouthwashes against *C. albicans* had shown varying degrees of zones of inhibition and the results are depicted in Fig. 1. The mean diameter of zone of inhibition of the tested mouthwashes has been indicated in Table 2. Hexidine mouthwash showed highest anti-candidal activity and formed 19 mm of inhibition zone followed by Aloe vera ras showed 18mm zone of inhibition. Colgate plax and cool mint Listerine showed 15 mm and 12 mm zones of inhibition respectively against *C.albicans*. The comparison of inhibition zone diameter by using chemical and aloevera mouthwashes has been indicated in Fig. 2. Minimal inhibitory concentration (MIC) of the mouthwashes was determined by the broth dilution method and the results are depicted in Table 3. The lowest dilution at which growth of *C. albicans* was inhibited was recognized as the minimum inhibitory concentration. It was determined that Hexidine and Colgate plax could inhibit the growth of *C.albicans* at a lowest dilution 1:64 (0.003% and 0.001%

respectively). Listerine was able to inhibit the growth at 1:4 dilutions (12.5%) where as Aloe vera ras did not show growth at 1:1 dilution which means at 50% concentration. The lowest dilution of mouthwash that prevented the growth of *C. albicans* determined by sub-culturing of the last clear MIC tube on SDA was referred as Minimum fungicidal concentration. The MFC of the Chlorhexidine, Colgate plax Listerine and Aloevera ras were in the range of 0.003% to 0.2%, 0.001% to 0.05%, 12.5% to 100% and 50% to 100% concentration respectively.

Table 1: Ingredients of mouth washes used in the study

Mouthwash	Ingredients as stated in the package
Hexidine® ICPA health product, Ankleshwar.	Chlorhexidine gluconate 0.2%
Colgate plax freshmint Colgate-Palmolive ltd, India	Water, glycerin, propylene glycol, sorbitol, polaxomer 407, flavor, cetylpyridinium chloride, potassium sorbate, sodium fluoride, sodium saccharin, menthol, citric acid, CI 42053, CI 15985.
Cool Mint Listerine® Johnson and Johnson ltd, India	Purified water, Sorbitol, Alcohol, Poloxamer 407, Benzoic acid, Sodium Saccharin, Eucalyptol, Flavour, Methyl salicylate, Thymol, Sodium Benzoate, Menthol, CI 42053.
Amritayu Aloe vera ras, Kashmir herbal remedies, Amristar.	Aloe vera concentrate, Sodium benzoate.

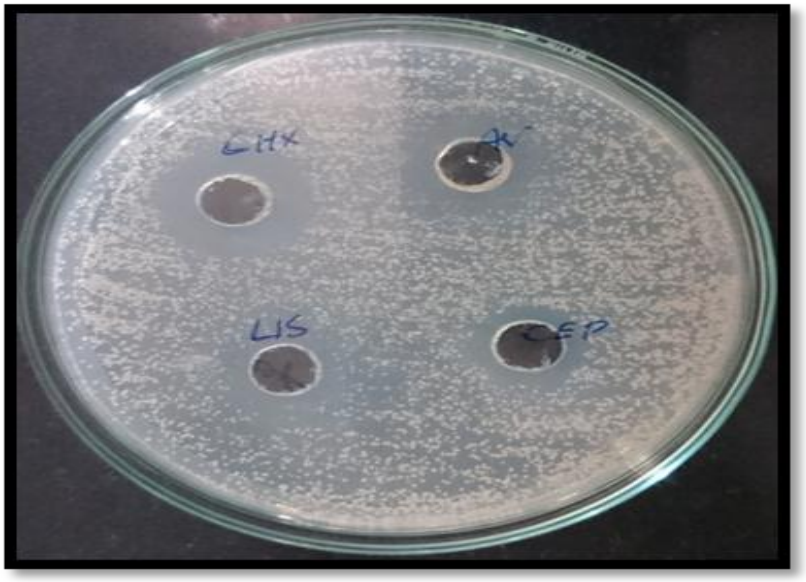

Fig 1: Anti-microbial screening of mouthwashes against *C.albicans* by Agar diffusion method

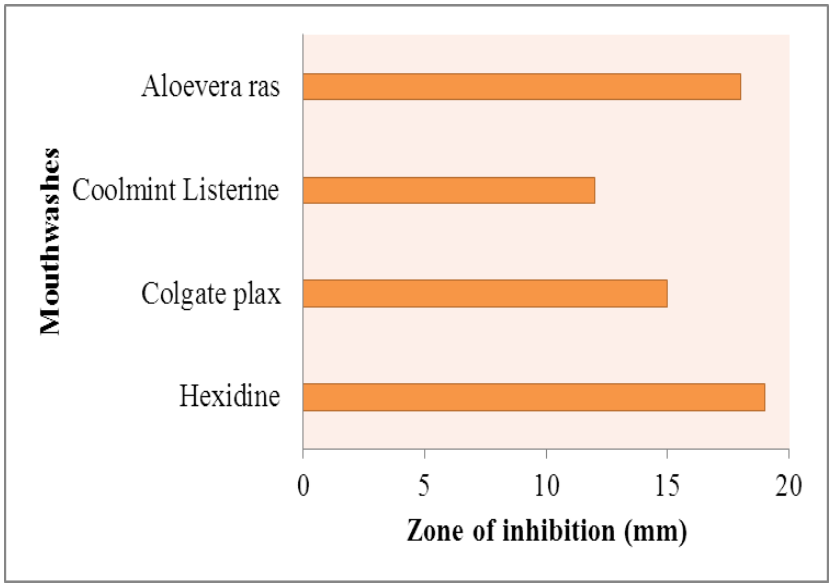

Fig 2: Comparison of mean inhibition zone diameter of mouthwashes against *C.albicans* by agar diffusion method

Table 2: Mean diameter of inhibition zone exhibited by mouthwashes against *C.albicans*

Mouthwashes	Mean in mm
Hexidine	19
Colgate plax	15
Coolmint Listerine	12
Aloevera ras	18

Table 3: Minimum inhibitory concentrations of the Mouthwashes exhibited against *Candida albicans*

Mouthwashes	Dilutions of Mouthwashes						
	1:1	1:2	1:4	1:8	1:16	1:32	1:64
Hexidine (0.2 %)	-	-	-	-	-	-	-
Colgate plax (Cetyl pyridinium chloride 0.05%)	-	-	-	-	-	-	-
Listerine	-	-	-	+	+	+	++
Aloe vera ras	-	+	+	+	++	++	+++

Key: - = No growth (turbidity), 0= MIC, + = light growth, ++ = moderate growth, +++ =high growth*

DISCUSSION

Mouthwashes are widely used as adjuncts to oral hygiene and in the delivery of active agents to the oral cavity. The results of the present study showed that all the mouth rinses used in the study were efficient on inhibition of *Candida albicans* growth. The results indicated that maximum zone of inhibition to be 19mm for Chlorhexidine, 18mm for Aloe vera ras

followed by 15mm for Colgate plax and 12mm for Listerine mouthwashes. Chlorhexidine and Aloevera ras showed least and highest MIC against *C.albicans*. The results of the present study were similar as reported by Mithun pai et al., 2013.[17] In a study conducted by Giuliana et al., 1999[18], chlorohexidine was found to be effective against *C. albicans* but cetyl pyridinium chloride received lower MIC and also showed a greater fungicidal activity than Chlorhexidine. Meiller et al., 2001[19] investigated the antifungal effect of antimicrobial mouthrinses against different species of *Candida* and reported that Listerine antiseptic showed greater efficacy against attached artificial biofilms than other antimicrobials tested. Talebi et al., 2014 [2] investigated the anti- candidal effect of various chemical and herbal mouthwashes against *C. albicans* and indicated that Chlorhexidine has a lower effect in comparison with other mouthwashes; especially Oral B. Chlorhexidine-based formulas are considered currently the golden standard for anti-microbial mouthrinses. Chlorhexidine gluconate is a cationic biguanide with broad-spectrum antimicrobial action. It's mechanism of action is that the cationic molecule binds to the negatively-charged cell walls of the microbes, destabilizing their osmotic balance causing concentration-dependent growth inhibition and cell death.[20] However, Chlorhexidine has been reported to have a number of side effects, such as unpleasant taste, staining of teeth and tongue, gingival desquamation, taste disturbance and painful mucosa for oral use as a mouthwash. Cetylpyridinium chloride (CPC) is a cationic quaternary ammonium compound. Its mechanism of action is the strong positive charge and the hydrophobic region of the CPC enable the compound to interact with the microbial cell surface and integrate into the cytoplasmic membrane which leads to disruption of membrane integrity resulting in leakage of cytoplasmic components, interference with cellular metabolism, inhibition of cell growth and cell death. Haps et al., 2008 [21] reported that staining of teeth and tongue, discomfort in taste, discomfort in sensibility, mouth burning during application and white plaque on the tongue immediately after use of CPC containing mouthwashes. Listerine is an essential oil containing mouthwash with the longest history of use that contains phenolics such as thymol, eucalyptol, menthol, and methyl salicylate. The mechanism of action of phenols is cell wall disruption and inhibition of bacterial enzymes. The phenolic compound may be capable of extracting the lipopolysacharide derived endotoxin from gram- negative bacteria.[22] In a clinical study conducted by Prashant et al., 2013[23] it was reported that, the use of CHX showed brown staining, whereas Listerine gave a burning sensation.

These drawbacks justify further research and development of antifungal agents that are safe for the host and specific for oral pathogens. There is a growing interest in the use of naturally occurring substances for the treatment of a variety of medical conditions. Use of herbs for oral care is very common in indigenous system of medicine. Aloe vera has been used for its medicinal value for several thousand years. The anti-microbial effects of *A. vera* have been attributed to the plant's natural anthraquinones: aloe emodin, aloetic acid, aloin, anthracine, anthranol, barbaloin, chrysophanic acid, ethereal oil, ester of cinnamonic acid, isobarbaloin, and resistannol.[24] Gupta et al., 2014[25] investigated the anti plaque efficacy of aloevera mouthwash on 4 day re-growth model in a randomized control trial and concluded that there was a significant reduction on plaque in Aloevera and Chlorhexidine groups with no statistically significant difference among them. He also reported that Aloe vera was as efficient as Chlorhexidine with no side effects.

CONCLUSION

The present study showed that all the tested mouth washes have antimicrobial activity against the tested organism. The results indicated that *Aloevera* mouthwash have anti fungal properties as equal as chemical mouthwashes. Therefore, with the consideration of side effects of chemical mouthwashes, socio-economic factors and preference of the population for natural products, aloe vera mouthwash might represent appropriate alternative to conventional chemical mouthwashes in the management of *Candida albicans*. In addition, more invitro and invivo studies are needed in future.

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