Original Research Article

ANTIFUNGAL EFFICACY OF XYLITOL AGAINST *CANDIDA ALBICANS*: AN IN VITRO STUDY

ABSTRACT:

AIM:

To evaluate the antifungal efficacy of xylitol against *candida albicans*

SETTINGS AND DESIGN:

The study design involves an in vitro study.

MATERIALS AND METHODS:

The fungal strain *candida albicans* was inoculated in a PDA-himedia. The sample was weighed about 10mg/ml and dissolved in Solvent (deionized water) to prepare appropriate dilution to get required concentration of about 50, 100,150, 200, 250, 300 and 350µl and standard solution as Fluconazole (10mg/ml distilled water) for fungi. Antibiogram was done by disc diffusion method using samples. Petri plates were prepared by pouring 30 ml of Potato dextrose agar (PDA) medium. The test organism was inoculated on solidified agar plate. The plates were incubated at 37 ºC for 48 hr for fungal strains.

STATISTICAL ANALYSIS USED:

Statistical analysis was performed using IBM software SPSS version 20 at one-way ANOVA.

RESULT:

The result of this study showed that the xylitol has an inhibitory effect on the growth of *candida albicans*. In the present study the xylitol had zones of inhibition against *c.albicans* at all concentrations. The highest inhibition of 14.25±0.99 was seen at the concentration of 350µl.

CONCLUSION:

According to the result Xylitol has considerable antimicrobial effects against *candida albicans*. The antimicrobial efficacy of xylitol approximates with standard fluconazole. Hence xylitol can be used as antifungal agent in clinical trials

Keywords: *Candida Albicans*, Xylitol, candidiasis, antifungal agent, fluconazole

INTRODUCTION:

The most common fungal infection among human population is candidiasis, the etiology of which is mostly *Candida albicans*. The polymorphic fungus *Candida albicans* is commonly present in the oral flora of human microbiome. In most individuals, *C.albicans* exists as a lifelong, harmless commensal. Under certain circumstance, *C.albicans* can cause infections that range from superficial infections to life-threatening systemic infections.[1]

A significant increase in the prevalence of infections caused by Candida species has been observed in the past two decades. *Candida albicans* can be found in the oral cavity, most frequently in the posterior dorsal surface of tongue, gastrointestinal tract, female genitourinary tract and sometimes the skin as the normal microflora in approximately half the population. Due to the disrupted balance of the normal flora or a compromised immune system, Candida species can become pathogenic.[2] Most Candida infections affects only the mucosal lining, but the rare systemic manifestations may have a fatal course. In the oral cavity, *C.albicans* can be seen as, various forms, such as pseudomembranous candidiasis, chronic plaque-type and nodular candidiasis, erythematous candidiasis, denture stomatitis, angular cheilitis, median rhomboid glossitis, and oral candidiasis associated with human immunodeficiency virus infection[1,20]. Before starting antifungal medication, it is necessary to eliminate any predisposing factor. Local factors are often easy to identify, but sometimes not possible to reduce or eradicate. Antifungal drugs have a primary role in such cases. The most commonly used antifungal drugs belong to the groups of polyenes, azoles. Polyenes, such as nystatin and amphotericin B are the mostly used alternatives in the treatment of oral candidiasis.[17]

Oral fluconazole is recommended for treating moderate to severe oral candidiasis, Fluconazole is characterized by its excellent bioavailability and low toxicity. The

incidence of adverse effects with fluconazole is low, among which the most frequent are nausea, vomiting, headaches, rash, abdominal pain and diarrhoea. *C.albicans* can cause two major types of infections in humans: superficial infections, such as oral or vaginal candidiasis, and life-threatening systemic infections. *C. albicans* and to a lesser extent other *Candida* species are present in the oral cavity of up to 75% of the population. In healthy individuals this colonization generally remains benign.[3] However, mildly immunocompromised individuals can frequently suffer from recalcitrant infections of the oral cavity. These oral infections with *Candida* species are termed "oral candidiasis" (OC). Such infections are predominantly caused by *C.albicans* and can affect the oropharynx and/or the esophagus of persons with dysfunctions of the adaptive immune system. Indeed, HIV is a major risk factor for developing OC.[3,4] Further risk factors for developing OC include the wearing of dentures and extremes of age.

Various in vitro surveys have shown that glucose intake is a promoter of *C. albicans* growth, whereas in vivo studies have found that xylitol can decrease the risk of candidiasis and angular cheilitis.[4]

The commonly used sugar alcohols are xylitol and sorbitol. Due to the hypo- and non-acidogenicity, they have been utilized for anticariogenic purposes. It has been known that xylitol is a better agent in reducing the incidence of dental

caries than sorbitol.[5,7] Xylitol is known to exert caries-reducing effects by inhibiting growth, metabolism, and polysaccharide production of mutan streptococci. Xylitol has no direct antibacterial properties, but it has anti-adhesive effects on microorganisms.[6] Thus, xylitol has been suggested to decrease the amount of dental plaque by decreasing the adhesivity of plaque. The combination of chlorhexidine and xylitol was more effective than each single treatment in controlling dental biofilm.[4]

Due to its similar effects on Streptococcus pneumonia, xylitol has been used for the prevention and treatment of acute otitis media.[3] Xylitol feeding also caused a shift in fecal microbial population from Gram-negative to Gram-positive bacteria in human volunteers as well as rodents.[8] Sugar alcohols also affect antifungal activity. *Candida albicans* cultured in media supplemented with xylitol showed changed susceptibility to lysozyme. The antifungal activity of Lactobacillus was enhanced in the presence of xylitol.[10]

Whereas the dietary intake of sucrose induces *C. albicans* growth in the gastrointestinal tract, xylitol intake has been reported as a possible inhibitor of *C. albicans*. In the presence of xylitol, a decrease in adhesion of *C. albicans* in the oral cavity has also been reported, suggesting inhibitory activities on fungal infections such as thrush.[9]

MATERIALS AND METHODS:

Preparation of Media:

Potato Dextrose Agar (PDA-Himedia) Media for Fungi

Composition of Media

Potatoes infusion from : 200.00g

Dextrose : 20.00g

Agar : 15.00g

Preparation of medium

Suspend 39.0 grams of PDA in 1000 ml distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15Lbs pressure (121˚c) for 15 minutes. Mix well before dispensing in specific work, when pH 3.5 is required; acidify the medium with sterile 10% tartaric acid. The amount of acid required for 100 ml of sterile cooled medium is approximately 1ml. Do not heat the medium after addition of acid.

Microorganisms

The fungal strains employed in the biological assays were *Candida albicans* (MTCC-227). Obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

Preparation of 24 hours pure culture

A loop full of the *Candida albicans* was suspended in about 10ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37ºC for 48 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8˚C until use.

Preparation of samples solutions for the experiment

Xylitol was weighed (10mg/ml) and dissolved in solvent (deionized water) to prepare appropriate dilution to get required concentration of about 50, 100,150, 200, 250, 300 and 350µl. The standard solution used was Fluconazole (10mg/ml distilled water) for fungi. They were kept under refrigerated condition unless they were used for the experiment.

Preparation of dried filter paper discs

Whattman filter paper (No:1) was used to prepare five discs approximately 6 mm in diameter, which are placed in hot air for sterilization After sterilization, the discs was loaded with 50, 100,150, 200, 250, 300 and 350µl, of xylitol sample, Standard solution as Fluconazole 30µl and control 30µl (deionized water) used to compare the test solution respectively. They were kept under refrigerated condition unless they were used for the experiment.

Antimicrobial assay

Antibiogram was done by disc diffusion method using samples. Petri plates were prepared by pouring 30 ml of Potato dextrose agar (PDA) medium. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with fungal strains from a broth culture. A sterile cotton swab is dipped into a standardized microbes test suspension and used to evenly inoculate the entire surface of the PDA plates. Briefly, inoculums containing of microbial strains were spread on PDA plates. Using sterile forceps, the sterile filter papers (6 mm diameter) containing discs were loaded on the surface of inoculated agar plate. The plates were incubated at 37 ºC for 48 hr. for fungal strains. Each sample was tested in triplicate. Results were recorded as the presence or absence of inhibition zones.

The inhibitory zone around the disc indicated the absence of tested organism. The diameters of the zones were measured using the diameter measurement scale. Triplicates were maintained and the average values were recorded for antimicrobial activity.

RESULTS

The antimicrobial potential of test compounds was determined on the basis of

mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale. The width of the zones of inhibition in each group has shown increase with increase in their concentration, as shown in table 1 and 2. The width of the zones of inhibition of xylitol approximated with that of fluconazole at concentration of about 250µl. This proves that xylitol has almost equal antifungal efficacy as fluconazole

Table.1: Antifungal activity of Xylitol against *Candida albicans*

Microbial Strains	Xylitol (µl)			Std. (30µl)	Control (30µl)
	50	100	150		
***Candida albicans* (mm)**	0.00±0.00	1.30±0.09	2.75±0.19	6.10±0.42	0.0±0.00

Values expressed as Mean ± SD for triplicates,
Standard: Fluconazole (Fungai); mm: Millimeter Control: Deionized water

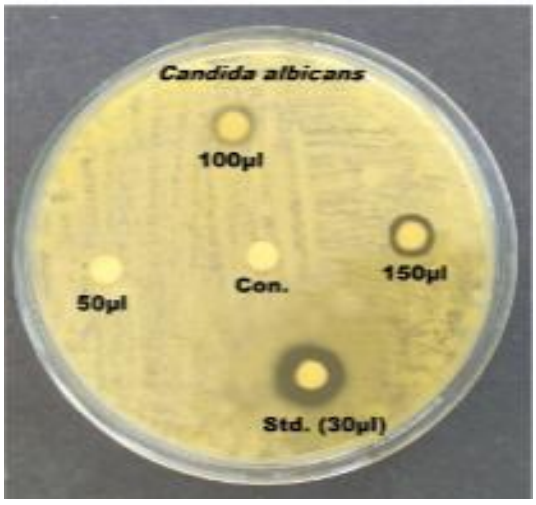


Figure 1: Shows Antifungal activity of Xylitol against *Candida albicans*

Table.2: Antifungal activity of Xylitol against *Candida albicans*

Microbial Strains	**Xylitol (µl)**			
	200	**250**	**300**	**350**
***Candida albicans* (mm)**	3.40 ± 0.23	5.15 ± 0.36	9.75 ± 0.68	14.25 ± 0.99

Values expressed as Mean ± SD for triplicates,

Standard: Fluconazole (Fungi); mm: Millimeter

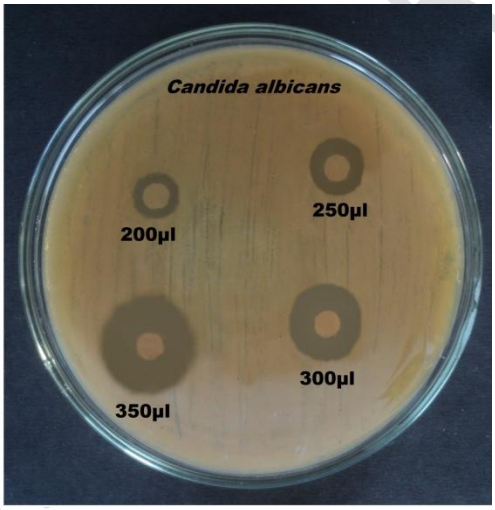
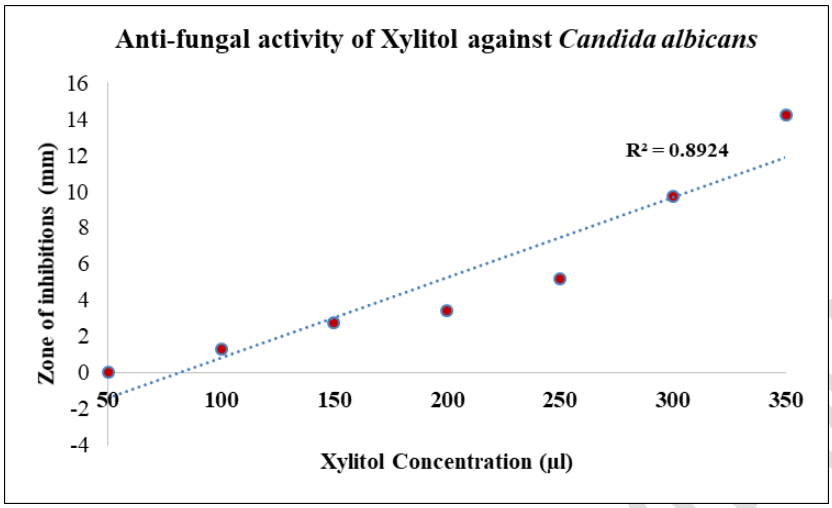


Figure 2: Shows Antifungal activity of Xylitol against *Candida albicans*



Graph 1: shows Antifungal activity of Xylitol against *Candida albicans* in concentration dependent of Xylitol (R^2 = 0.8924); In Present study zone of inhibition (mm) was concentration dependent.

DISCUSSION:

Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of Candida species, the most common being *C. albicans*. The underlying causes of oral candidiasis include extremes of age, xerostomia, antibiotic therapy, dentures, smoking, Cushing syndrome, malignancies, immune deficiencies, and diabetes mellitus.[12]

Xylitol is a natural sugar substitute derived from fruit, vegetables and other plant materials. While it is most commonly used as a sweetener, the greatest benefits of using xylitol for candida is that this substance is believed to

possess antifungal properties. Since candida feeds on sugar, replacing sugar-filled products with those containing xylitol will also help to eliminate candida's primary food source.[13] In addition to treating candida, xylitol might enhance immune system function, keep insulin levels stable, and prevent certain medical conditions[14]

Xylitol have been applied for food and pharmaceutical industries and dentistry. In food industry, sugar alcohols have been used for confectioneries and chewing gums. Most publications in dentistry regarding xylitol were about antibacterial and anticariogenic effects. Xylitol inhibit growth, metabolism, and polysaccharide production of mutans streptococci.[15] Because of their anticariogenic effects and sweet taste which can stimulate salivary secretion, xylitol and sorbitol have been included in saliva substitutes for patients with dry mouth whose susceptibility to oral candidiasis was increased. However, there have been limited numbers of studies on antifungal activities of xylitol.[11]

Xylitol can also increase enzymatic activity of salivary peroxidase which has an antifungal activity. It has been reported that salivary peroxidase activity was increased several times in individuals receiving a strict xylitol diet for 2 years.[16]

There are substantially fewer antifungal than antibacterial drugs. Clinically used antifungals are basically restricted to polyenes (e.g., amphotericin B) and azoles (e.g., fluconazole).

Although nystatin and amphotericin b were the most drugs used locally, fluconazole oral suspension is proving to be a very effective drug in the treatment of oral candidiasis.

In this study Fluconazole has been used as a standard solution, since fluconazole is used for the treatment of both systemic and superficial infection in a variety of tissues. fluconazole has much advantage over other antifungal drugs including the option of oral administration. It also has good antifungal properties, high acceptance by the patient and high efficacy compared with other antifungal drugs. But this drug is not always effective, due to the emergence of antifungal drug resistance. Fluconazole resistances have been described in some isolates of *C. albicans* and *C. dubliniensis* from HIV-infected patients with repeated episodes of oropharyngeal candidiasis treated with fluconazole.

The evolution of antimicrobial drug resistance is an almost inevitable process that is universal in the microbial world. Although fungal resistance is not as rampant as bacterial resistance, the economic facets associated with fungal infections is unacceptably high.[18] Also considering the limited number of antifungal drugs

available, one of the main strategies of improving therapy in mycoses is overcoming antifungal resistance.[15]

One of the keys to manage the emerging widespread problem of drug resistance is to develop newer drugs with newer targets, with decreased drug interactions, increased safety and tolerance and with cost-effectiveness. [15,16]

Xylitol was found to reduce the production of carcinogenic acetaldehyde from ethanol by Candida below the mutagenic level of 40 to 100 µM by Uittamo et al. Acetaldehyde is a highly toxic and mutagenic product of alcohol fermentation and metabolism, which has been classified as a class I carcinogen for humans. Many Candida species representing oral microbiota have been shown to be capable of marked acetaldehyde production. In recent years, a study found that xylitol did not augment lysozyme- and peroxidase-related candidacidal activities. However, other researchers found that xylitol combined with zinc has a more inhibitory effect on candidal growth than compared with xylitol alone. [19]

In 2008, a study revealed that benzethonium chloride had greater fungicidal effect when combined with sugars, such as xylitol than applied alone

All the studies regarding this matter point to the fact that xylitol has considerable antimicrobial effects.[20]

Samaranayake and MacFarlane et al found that the xylitol-grown *C. albicans* cells are up to two times more adherent to epithelial surfaces than control-grown cells.

Their results indicated that Candida spp. incubated in 500 mM xylitol exhibited a significant inhibition in adhesion.

From a metabolic perspective, xylitol is metabolized largely independent of insulin, so it can be safely consumed by non-insulin dependent diabetics.

Larmas, et al., conducted a study substituting xylitol for sucrose in the diet of a human test group during 8 months significantly reduced the number of persons with salivary Candida growth, while there was a slight increase in the test groups receiving sucrose and fructose.

In this study, we evaluated the effects of xylitol on the growth of *C. albicans.*

In this study we have used the disc diffusion method. The advantages of the disk method are the test simplicity that does not require any special equipment, the provision of categorical results easily interpreted by all clinicians, and flexibility in selection of disks for testing. It is the least costly of all susceptibility methods.[8]

The result of this study showed that the xylitol has an inhibitory effect on the growth of *candida albicans*. In the present study the xylitol had zones of inhibition against *c.albicans* at all concentrations. The highest inhibition of 14.25±0.99 was seen at the concentration of 350µl.

Hence xylitol can be used as an antimicrobial agent against *Candida albicans* in clinical trial.

CONCLUSION:

Our results indicate that xylitol has considerable antimicrobial effects against *candida albicans*. The antimicrobial efficacy of xylitol approximates with standard fluconazole. Xylitol will be of great beneficiary in patients who are resistant to antifungal drugs. It is also cost effective. .However this study is limited as it is an in vitro study. Further in vivo studies should be carried out to confirm the results of this study.

DISCLAIMER:

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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