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Antifungal Effect of Plant Extract Against *Candida albicans*

Atul Kumar Gupta[1], Rajshree Pandey[2], Shrikant Kol[1], *Arvind K. Tripathi[1], Atul Kumar Tiwari[1]

[1]Centre for Biotechnology Studies, A.P.S.U, Rewa (M.P.)
[2]Professor , Department of Botany, Govt. Girls P.G. College, Rewa (M.P.)

Email : arvindt2584@gmail.com

ABSTRACT:

Candida albicans have a unique microbial feature that plays an essential role in early childhood caries (ECC). This study aimed to isolate *C. albicans* in children with ECC and observe the antimicrobial effect of 0.2% chlorhexidine (CHX). Children in the age group of 3–6 years were selected. The sample was collected using a sterile cotton swab from the tooth surface, streaked on sabouraud dextrose agar (HIMedia) plates, and incubated at 37°C for 24 hours. Disk diffusion and agar well diffusion methods were used to detect the susceptibility of *C. albicans* to 0.2% CHX. The mean zone of inhibition of CHX for *C. albicans* was 12.4 ± 0.59 mm showed a zone of inhibition of 20.85 ± 1.18 mm. CHX was effective against both *C. albicans*, showing more antibacterial activity than antifungal activity.

KEYWORDS: Antibacterial activity, sabouraud dextrose agar Children , Disk diffusion agar.

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INTRODUCTION:

Early childhood caries (ECC) is defined as “the presence of one or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled surfaces, in any primary teeth of a child under age six” and as “tooth decay in preschool children which is common, mostly untreated and can have a profound impact on children’s lives” (Tinanoff *et al*., 2019). The main etiological factors causing dental decay by a fungus, *Candida albicans*, plays a role in ECC. Caries usually follows the emergence of *S. mutans* in dental cavities after 6–24 months. The *Candida* species colonizes with other microflora in the oral cavity, depending upon systemic and oral conditions, and may shift to pathogenic microorganisms.*Candida albicans* make the environment acidic and decrease the pH by secreting organic acids, which are more effective than the lactic acid secreted (Forssten *et al*.,2010). Several studies have reported *Candida* species in ECC and severe early childhood caries. Therefore, *C. albicans* can cause dental decay.

Few antimicrobial agents have been found to be effective clinically and suitable against microbial growth, and recently formulated agents against microbial growth include essential oils, plant extracts, metals, and chlorhexidine (CHX). CHX is considered the gold

standard, and other antimicrobial agents' efficacy is compared against it. CHX is a digluconate agent developed in 1940 in search of an antiviral agent, but it was found to possess antibacterial properties (Sajjan *et al*., 2016). CHX can be used as bacteriostatic or a bactericidal, depending on its dose. In higher concentrations, it causes increased cell permeability leading to cell lysis; in lower concentrations, spore formation causes damage to the cellular transference ofbacterial cells (Sajjan *et al*., 2016).

MATERIAL AND METHODS:

A total of 20 samples that tested positive for *C. albicans* collected from carious portions of the teeth using sterile cotton swabs and then transferred to the microbiology laboratory in conical tubes containing sabouraud dextrose medium. The sabouraud's dextrose agar (SDA) plates were prepared and supplemented with chloramphenicol to inhibit bacterial overgrowth. The materials were inoculated for culture. Colony morphology on SDA plates was used to identify isolates. Growth emerged as smooth, convex pasty colonies, creamy, with a moldy odor after 1–2 days, and if no growth was seen after incubating it for 72 hours, the culture was considered to be negative. The present study was conducted in the Centre for Biotechnology and Microbiology Studies, A.P.S. University, Rewa (M.P.). Samples were collected from OPD patients during visiting Department of ENT at Sanjay Gandhi Memorial, Rewa, Community Health Centre, Raipur Karchulian, Rewa, with the help of govt. health officials.

Culture media used: Sabouraud Dextrose Agar (SDA), Nutrient Agar, Mac-Conkey's Agar, Blood Agar, Chocolate Agar, Yeast Nitrogen Based Medium (YNB)

- **Composition of Sabouraud Dextrose Agar (SDA)**

Ingredients	**Gms / Litre**
Dextrose (Glucose)	40.000
Mycological, peptone	10.000
Agar	15.000
Final pH (at 25°C)	5.6±0.2

- **Composition Nutrient Agar[20]**

Ingredients	**Gms / Litre**
Peptone	5.000
Sodium chloride	5.000
peptone	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

- **Composition Mac-Conkey's Agar[21]**

Ingredients	**Gms / Litre**
Peptones	3.000
Pancreatic digest of gelatin	17.000
Lactose monohydrate	10.000
Bile salts	1.500
Sodium chloride	5.000
Crystal violet	0.001

Neutral red	0.030
Agar	13.500
pH after sterilization	(at 25°C) 7.1±0.2

- **Composition of blood agar[22]**

Ingredients	**Gms / Litre**
peptone	10.000
Tryptose	10.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

- **Composition Chocolate Agar[23]**

Ingredients	**Gms / Litre**
Proteose peptone	20.000
Dextrose	0.500
Sodium chloride	5.000
Disodium phosphate	5.000
Agar	15.000
Haemoglobin	2.000
Final pH (at 25°C)	7.3±0.2

Antifungal susceptibility test:

The suspensions were made using the direct colony suspension method using an 18–24-hour-old agar plate. They were adjusted to McFarland turbidity of 0.5 and confirmed using a photometric device. The antimicrobial property of 0.2% CHX (Hexidine 0.2%, Icpa Health products Ltd, India) against *C. albicans* was investigated using Kirby Bauer's disk diffusion technique and the agar well diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) M44, 3rd ed, and M02, 13th ed, guidelines. The Mueller Hinton agar plate was swabbed across the whole surface by the inoculum. The uniform spread of inoculum was achieved, and this technique was repeated two more times, streaking each time by rotating the plate by around 60 degrees. Separately, 6-mm filter paper disks (13 µl/disk) were coated with 0.2% CHX and allowed to dry. With forceps, the disk of CHX was placed on the agar surface at equivalent distances and placed in an incubator for 24 hours at 35°C ± 2°C. Then, a sterile swab was inserted into the inoculum tube, spun several times, and pressed against the tube's wall (above the fluid level) while applying firm pressure to remove excess fluid. In the agar well diffusion method, the microbial inoculum was diffused throughout the agar plate surface to inoculate it. The zones were measured to the closest millimeter with a scale, taking the diameter of the disk and well into account. The diameter was measured and rounded to the nearest millimeter.The materials to be tested were placed within 6-mm-diameter hole that had been punched into the agar plate using a tip. The plates were incubated in an appropriate environment.

RESULTS AND DISCUSSION:

Candida albicans incidence was determined to be 60.6% among the children, having

children testing positive. *Candida albicans* was recovered from the whole surface of the carious tooth. *Candida albicans* was susceptible to CHX (Fig. 1) with the mean zone of inhibition of 12.4 ± 0.598 mm (Table 1) using the agar well diffusion method; no zone of inhibition was observed when Kirby Bauer's disk diffusion method was used. *Streptococcus mutans* cultured in the blood agar plate for 48 hours at 37°C were identified by hard raised, convex, undulate, opaque, frosty glass appearance, and final confirmation was done using VITEK II. *Streptococcus mutans* was susceptible to CHX (Fig. 2) and the mean zone of inhibition was 20.85 ± 1.18 mm.

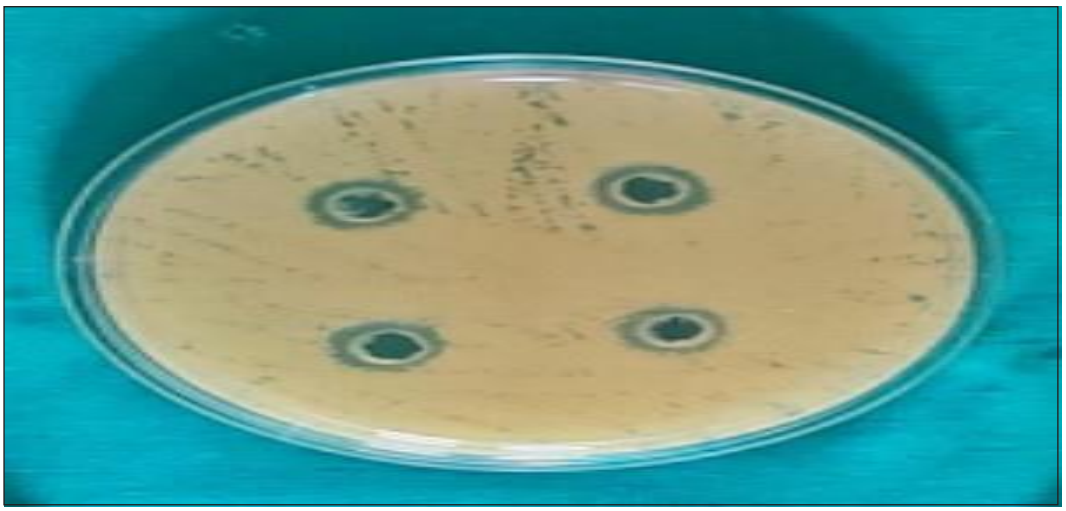

Figure 1. CHX showing zones of inhibition against *C. albicans.*

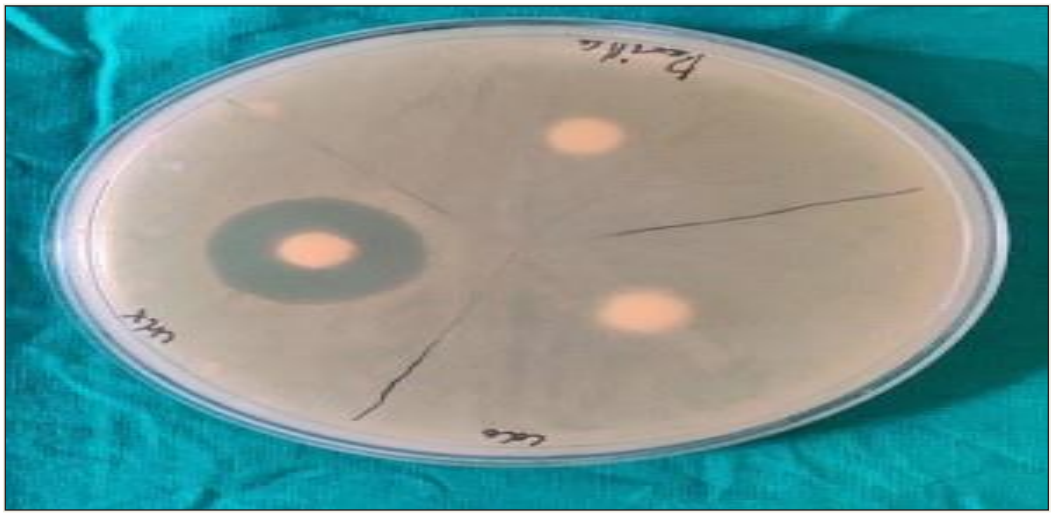

Figure 2. Zone of inhibition observed with CHX on *S. mutans.*

The comparison of CHX showed the mean of CHX against *C. albicans* (Group 1) was 14.4 mm ± 0.236 and 20.85 mm ± 1.182. The mean and median were high which was statistically significant (p-value < 0.001).

In ECC, the presence of *C. albicans* is a unique microbial feature observed (Ellepola *et al.*, 2019). However, recent research has revealed a high prevalence in biofilms harboring *C. albicans*, showing the interaction between the species and promoting caries development

(Barbieri *et al*., 2007; Jarosz *et al*., 2009). According to research by Xiao *et al*. (2016), mothers of children with ECC had significantly more numbers of *C. albicans* and the same *C. albicans* strains were observed in most child– mother dyads. *Candida albicans* is the most common *Candida* species detected in the oral cavity. Identification of *Candida* strains is crucial since they range substantially in their potential to infect and their sensitivity to antimicrobial medications. As a result, the current research was carried out to detect *C. albicans* and investigate the antibacterial activity of 0.2%CHX. Among the ECC children, the growth of *Candida* was observed to be 60.6%. This is consistent with past research by previous studies (de Carvalho *et al*., 2006; Ghasempour *et al*., 2011; Hossain *et al*., 2003; Lozano Moraga *et al*., 2017; Marchant *et al*., 2001; Rozkiewicz *et al*., 2006; Thomas *et al*., 2016; Xiao *et al*., 2016;). However, in the study by Neves *et al*. (2015) there was no correlation between the prevalence of *Candida* and carious lesions. This might be due to changes in content, buffering capacity, salivary flow rate, and the duration and amount of plaque that is present on the tooth surface as long-standing plaque will contain more *Candida* species, and in ECC, the amount of tooth surface remaining also plays a role in biofilm formation all of which affect the caries progression in the tooth. It was also observed that instead of making cultures, which is considered the gold standard for yeast identification, investigators employed other methods to detect *Candida* in the carious tooth. In this study, the candidal prevalence is lower than that in the previous investigations (de Carvalho *et al*., 2006; Ghasempour *et al*., 2011; Lozano Moraga *et al*., 2017; Marchant *et al*., 2001; Thomas *et al*., 2016) where it was observed that the frequency of *C. albicans* in infants with ECC was 85.7%, 70.8%, 100%, 63%, and 100%, respectively. Individual variability in the living environment, oral cavity ecological environment, geographical variance, and dietary preferences may have impacted the variation in candidal carriage rates between studies. In addition, the prevalence of yeast differs due to variations in age, physiological fluids, mucosal surface, hard tissue, and natural barriers to colonization.

CHX digluconate has a broad range of antibacterial action and is often used as an antiseptic mouthrinse inhibiting adherence resulting in biofilm. It acts as a fungicide by coagulating nucleoproteins in cell walls, allowing cytoplasmic components to escape via the plasmalemma. Antimicrobial action was seen against *S. mutans*, with a mean zone of inhibition of 20.85 ± 1.18 mm, and the mean zone of inhibition observed against *C. albicans* was 12.4 ± 0.59 mm, which was more than the findings of Tirali *et al*. (2013) but was lesser than observed by Shino *et al*. (2016). A study conducted by Moeintaghavi *et al*. (2012) where 0.2% CHX was used did not show any zone of inhibition against *C. albicans*.

The research design might be to blame for the disparity in results. Antibacterial investigations using agar plates are frequent. However, the microbial inhibition zone depends on the test substance's solubility and ability to permeate through agar; thus, it may not be fully effective. It was observed that CHX has more antibacterial activity (20.85 ± 1.18 mm) than antifungal activity (12.4 ± 0.59 mm), as observed by the zone of inhibition exhibited. Therefore, CHX can slow down the carious process as it will affect the attachment of pioneer species and disturb the biofilm formation, adversely affecting *Candida*'s attachment to the tooth surface and other microorganisms. *in-vivo* research is recommended for more exact results because the current study was conducted under restricted laboratory circumstances.

CONCLUSION:

Within the limitations and the experimental conditions of this study, the following conclusions can be made: CHX's antifungal effectiveness is proportional to its concentration, and 0.2% CHX is effective against the microorganisms tested andshowed more antibacterial than antifungal activity.

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