

Determination of Minimum Inhibitory and Fungicidal Concentrations of Potash Alum Against Clinical Isolates of *Candida albicans*



Muhammad Irshad¹

PhD

Muhamamd Younas²

FCPS

Asif Ullah Qureshi³

FDS

Amir Hameed⁴

MPH

OBJECTIVE: *Candida albicans* is an opportunistic pathogen causing oral candidiasis. Commercially available antifungal agents are effective in eliminating *C. albicans*, however, their toxicity and high cost are undesirable. Potash Alum is a naturally occurring salt with antibacterial and antifungal properties. Therefore, Potash Alum may be effective against *C. albicans*. **Objective:** The main objective of this study was to investigate the *in vitro* susceptibility of *C. albicans* to Potash Alum. **METHODOLOGY:** Swab samples from 19 patients attending the Oral medicine department of Rehman College of Dentistry were transferred to tubes containing Sabouraud Dextrose Broth. After identification of *C. albicans* by Gram-staining, a solution of $2-5 \times 10^5$ CFUs/mL *C. albicans* was prepared and subjected to MIC and MFC determination by the standard broth microdilution method. Potash alum concentrations of 5, 10 and 20 mg/mL were used. Commercially available Nystatin was used as a positive control.

RESULTS: Our results showed that 10 mg/mL of Potash Alum (PA) solution was able to inhibit growth of most of the clinical isolates of *C. albicans*. In 5 samples, even 5mg/mL was effective in inhibiting the growth of *C. albicans*. Potash alum demonstrated fungistatic rather than a fungicidal action against *C. albicans*.

CONCLUSIONS: It is concluded that potash alum has a fungistatic action against *C. albicans* *in vitro*. In addition, the optimum *in vitro* concentration of potash alum solution effective in inhibiting growth of *C. albicans* was found to be 10mg/mL.

KEYWORDS: *Candida albicans*, potash alum, nystatin, antifungal

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INTRODUCTION

Oral candidiasis (OC) is one of the most common fungal infections of oral cavity.¹ It is an opportunistic infection which most commonly affects elderly, debilitated, and immunocompromised patients.¹ *Candida albicans* (*C. albicans*) is a commensal found in oral cavity and is a part of gut microbiota and it is one of the most common opportunistic pathogen associated with OC.² Dentures provide an ideal intraoral microenvironment for

the growth of *C. albicans* and denture wearers are therefore, at a higher risk of developing OC. Suboptimal oral and denture hygiene contributes towards growth of *C. albicans*.² Factors such as biofilm formation and proteolytic enzymes help *C. albicans* to establish a successful intraoral infection.¹

Treatment options for OC are usually limited to commercially available antifungal agents.³ Most commonly, it is treated with antifungal agents such as Nystatin, Amphotericin B and Fluconazole among others.³ However, controversial results were seen with these commercial agents as toxicity is one of the main concerns about some of these antifungal agents.⁴ Others are either expensive or have an unpleasant taste. Development of antifungal resistance, particularly in the developing world, is another concern which has been on the rise due to overuse of these antifungal agents.⁵ Resistance to antifungal agents can result from their indiscriminate and widespread use in the developing world.

1. Associate Professor and HoD, Department of Oral Pathology, Rehman College of Dentistry, Peshawar.

2. Assistant Professor, Department of Oral Medicine, Peshawar Dental College, Peshawar.

3. Assistant Professor, Department of Oral Medicine, Rehman College of Dentistry, Peshawar.

4. Assistant Professor, Department of Community Dentistry, Peshawar Dental College, Peshawar.

Corresponding author: "Dr. Muhammad Irshad" <drirshad@live.nl >

Therefore, newer, safer, and cost-effective antifungal agents should be explored.

Potash Alum ($KAl(SO_4)_2 \cdot 12H_2O$) is a naturally occurring compound with antibacterial and antifungal properties.⁶ Potash Alum (PA) has been in use of Egyptian, Indian and Chinese civilizations since antiquity.⁷ It is known by the name of Phitkary in the subcontinent and is a household item. PA is odourless, cheap, and nontoxic in small quantities. Due to its lack of toxicity, the United States, Food and Drug Administration (FDA) has approved it as a food additive.⁸

The antibacterial and antifungal properties of PA have been reported earlier.⁶ We hypothesized that due to the antifungal properties of PA, it can be effective against *C. albicans* and therefore can be used as a cheap and easily available alternative to commercially available antifungal agents. Studies reporting on the antifungal properties of PA are scarce and often inconclusive.⁹ Minimum inhibitory and fungicidal concentrations (MIC & MFC) of PA against *C. albicans* have not been reported earlier. Therefore, the aim of this study was to investigate the in vitro susceptibility of *C. albicans* to Potash Alum by MIC and MFC determination.

METHODOLOGY

This quasi-experimental study was approved from the institutional ethical committee under EC (Ref. No. RCD-19-05-016). The study was carried out at the oral pathology laboratory of Rehman College of Dentistry, Peshawar during the period of May till August 2019. Patients who had used antifungal agents in the past 3 months were excluded from the study. Nineteen patients attending the oral medicine department of Rehman College of Dentistry, were sampled using convenience sampling technique, with 2 moistened sterile swabs from the hard palate and base of the prosthesis. These swabs were then added to test tubes containing Sabouraud Dextrose Broth (SDA, Difco Laboratories, France) for transfer to the laboratory. The collected samples were inoculated into 15 × 90mm disposable Petri dishes containing SDA (Difco Laboratories, USA/France), in the presence of 100µg/mL chloramphenicol (Sigma-Aldrich, St. Louis, MO, USA). After incubation at 37°C for 48 hours, *C. albicans* was Gram stained observed under light microscope. Observation of fungal hyphae confirmed presence of *C. albicans*.

C. albicans suspensions were prepared in tubes containing 5 mL of phosphate buffered saline (PBS) solution. The suspensions were vortexed for 2 mins and adjusted to 2-5 × 10⁵ CFUs/mL with the help of a haemocytometer.

Minimum Inhibitory and Fungicidal Concentrations (MIC and MFC).

Standard broth microdilution method was used to

Table 1: Results of MIC and MFC estimation of Potash Alum on *C. albicans*.

<i>C. albicans</i> clinical isolates (Sr. No.)	Potash Alum (mg/mL)				Nystatin (mg/mL)	Control of clinical isolates	Control of the culture medium
	MIC	MFC	MFC/MIC	Activity antifungal	MIC		
1.	10	20	2	Fungicidal	20	+	-
2.	10	>20	NA	Fungistatic	20	+	-
3.	10	>20	NA	Fungistatic	20	+	-
4.	5	20	4	Fungicidal	20	+	-
5.	10	>20	NA	Fungistatic	20	+	-
6.	5	>20	NA	Fungistatic	20	+	-
7.	10	>20	NA	Fungistatic	>20	+	-
8.	10	>20	NA	Fungistatic	20	+	-
9.	10	>20	NA	Fungistatic	20	+	-
10.	5	>20	NA	Fungistatic	20	+	-
11.	10	>20	NA	Fungistatic	20	+	-
12.	10	>20	NA	Fungistatic	20	+	-
13.	10	20	2	Fungicidal	20	+	-
14.	10	>20	NA	Fungistatic	20	+	-
15.	5	20	4	Fungicidal	20	+	-
16.	10	>20	NA	Fungistatic	20	+	-
17.	10	>20	NA	Fungistatic	20	+	-
18.	5	20	4	Fungicidal	20	+	-
19.	10	>20	NA	Fungistatic	20	+	-

NA: Not Available; +: growth of microorganism; -: without microorganism growth

determine the minimum inhibitory concentration (MIC) of PA and nystatin against clinical strains of *C. albicans*.¹⁰ Experiments were performed in triplicate, in sterile, 96-well microplates.

PA solution was prepared in sterile PBS and serially diluted to 5, 10 and 20 mg/mL. Commercially available Nystatin 20 mg/mL (Wyeth Pharmaceuticals, Karachi, Pakistan) was acquired from the market. We added 100µL of Sabouraud Dextrose Broth to each well of a plate. Next, 100µL of various concentrations (5, 10 and 20 mg/mL) of PA and Nystatin (positive control) were added to the respective wells. Sterile PBS was used as negative control. 10µL of the *C. albicans* inoculum containing 2-5 × 10⁵ CFUs/mL was added to each well. Viability controls were carried out on the clinical isolates of *C. albicans* under the same conditions. The plates were wrap-sealed and incubated at 37°C for 48h. The lowest concentrations of PA which visually inhibited growth of *C. albicans* in the wells was The MICs of PA and Nystatin were taken as the lowest concentrations capable of visually inhibiting the *C. albicans* growth in the wells, when compared to the growth under control conditions.

After determination of the MIC, 10µl of the supernatant from the wells were plated on SDA, and incubated at 37°C for 48h. The MFC was defined as the lowest concentration of PA that either completely inhibited growth of *C. albicans* or showed growth of fewer than three colonies. All experiments were carried out in triplicate. The MFC/MIC ratio was also calculated. Data was analysed using Microsoft Excel 2016.

RESULTS

We used 5, 10 and 20 mg/mL of PA to evaluate its antifungal activity. *C. albicans* samples of 19 denture wearing patients were used in this study. Patients included 12 males and 7 females, and the mean age of patients was 63 + 9 years. None of the patients was a current smoker.

Our results show that 10 mg/mL of Potash Alum (PA) was able to inhibit growth of most of the clinical isolates of *C. albicans*. In 5 samples, even 5mg/mL was effective in inhibiting the growth of *C. albicans*. 10mg/mL was therefore determined to be the MIC value of PA against *C. albicans*. Growth of the *C. albicans* colonies was observed on culture media even after treatment with 20 mg/mL of PA in most cases. PA showed fungicidal activity only against 5 clinical isolates. PA was therefore, fungistatic rather than fungicidal in the case of most clinical isolates of *C. albicans*.

DISCUSSION

In our study Potash Alum (PA) showed fungistatic activity at 10 mg/mL concentrations against the majority of *C. albicans* clinical strains. Only in 5 out of 19 samples, PA was able to completely eradicate *C. albicans* and thus showed fungicidal activity. We used commercially available Nystatin as a positive control in our study, which is a known fungicidal agent. Nystatin is effective in the treatment of OC¹¹; however, its nephrotoxic potential is well established and is a point of concern for its use in patients with comorbid conditions.¹² Nephrotoxic and hepatotoxic effects of other antifungal agents used in OC have also been reported.¹³

Antibacterial activity of PA on oral microbiota has been reported earlier.^{14,15} Due to its antibacterial effects PA improves periodontal health in chronic periodontitis when used as a mouth rinse.¹⁶ Human studies about antifungal activity of PA are scarce and these studies are mostly conducted on species other than *C. albicans*.¹⁷ Shalli et al¹⁸ studied antifungal properties of PA on vaginal microflora including *C. albicans* and found it an effective antifungal agent against *C. albicans*.

Studies on the MIC and MFC values of PA against *C. albicans* are scarce. We found that MIC of PA for *C. albicans* is 10mg/mL, which is consistent with another study¹⁹ where the values were reported as 20% w/v, roughly equal to our reported values. Mechanism of antimicrobial action of PA is not clear, however, it is proposed that the formation of alum ions react with free protein and thiol group molecules on the surface of microbes, resulting in protein precipitation.²⁰ The protein precipitation by PA may also partly explain its fungistatic rather than fungicidal action. Further studies are needed to elaborate on the pharmacodynamics of antimicrobial/antifungal action of PA.

Due to its easy availability and cheap cost, PA is used in a variety of applications. Its easy accessibility and global acceptability makes it an ideal antifungal agent for oral candidiasis. In addition, it has a good safety profile and milder taste than most commercially available antifungal agents.²¹ Its widespread use as a food additive and as an adjuvant in human vaccines further consolidates its lack of toxicity.²² Although, our study has shown efficacy of PA against *C. albicans* in vitro, however, conditions in the oral cavity are much different from those in the laboratory. Therefore, further clinical studies are needed to elucidate the role of PA in treatment of Oral Candidiasis.

CONCLUSION

We conclude that potash alum has a fungistatic action against *C. albicans* in vitro. In addition, the optimum in vitro concentration of potash alum effective in inhibiting growth of *C. albicans* was found to be 10mg/mL.

Since this study has been conducted on patient samples from a region, these results should be interpreted with caution when generalizations are made. For generalization of our results, further large scale in vivo and in vitro studies are needed. Based on our results and its safety profile, we recommend that general dental practitioners may recommend Potash alum solution as an alternative to commercially available antifungal agents for the treatment of oral candidiasis.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest involved in this study

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