

Original Research Article

In Vitro Study for the Anti - Candida Activity of Homoeopathic Medicines against *Candida Albicans*

Chetan Hanamantrao Shinde¹, Pashmin Kaur Anand², Bipinraj Nirichan Kunchiraman², Arun Bhargav Jadhav¹

¹Department of Homoeopathic Pharmacy, Homoeopathic Medical College & Hospital, Bharati Vidyapeeth Deemed To Be University, Pune, India.

²Department of Microbiology, Rajiv Gandhi Institute of Information Technology and Biotechnology, Bharati Vidyapeeth Deemed To Be University, Pune, India.

Corresponding Author: Arun Bhargav Jadhav

ABSTRACT

Recently there is an increase in the number of patients affected by recurring candidiasis due to the development of antifungal resistance in pathogenic candida species. Current study has aimed to screen various homoeopathic medicines and their potencies for inhibition of *Candida albicans*. The homoeopathic medicines Azadirachta indica, Cinchona officinalis, Zincum metallicum, Iodium, Selenium, Sulphur, Acidum Benzoicum, Phosphorus, Acidum Sulphuricum and Zingiber officinale in 6C, 12C, 30C, 200C, 1M potencies were screened by agar well diffusion method using Sabouraud Dextrose Agar and found out that Iodium 6C, Zincum Metallicum 30C, Selenium 6C, Zingiber officinale 6C, Cinchona officinalis 12C, Acidum Benzoicum 12C, Phosphorus 6C, Azadirachta indica 6C, Acidum Sulphuricum 6C and Sulphur 6C could inhibit *Candida albicans* (CA-3557). These medicines could also inhibit the germ tube formation in *Candida albicans*.

Keywords – Anti-candida, homoeopathic medicines, Acidum Sulphuricum, Acidum Benzoicum, Azadirachta indica, Cinchona officinalis, Iodium, Phosphorus, Selenium, Sulphur, Zincum Metallicum, Zingiber officinale.

INTRODUCTION

Candida albicans is one of the important fungi found as a part of normal human microflora and it is an opportunistic yeast causing infection known as candidiasis under various circumstances. [1,2] Candidiasis occurs in three forms oropharyngeal candidiasis, vulvovaginal candidiasis (VVC) and invasive candidiasis. [3] It is been estimated that over 75% of women suffer from VVC once in their lifetime. [4] Moreover, *C. albicans* is one of the important nosocomial organisms that can lead to extremely life-threatening, systemic infection with 30% mortality rate. [5]

Though drugs like clotrimazole, imidazole, triazole, thiazole, allylamines,

echinocandins (antifungals), selenium sulfide, salicylic acid, fluocinolone etc. are currently being practiced worldwide for management of candidiasis, there appears hardly any formulation to have a target specific, preventive and curative role in reverting back the fungal changes. [6,7] Also the treatment modalities available are failing to prove their safety as far as their serious side-effects are concerned.

In the recent years, there is an increase in the number of treatment failure in long term anti-fungal treatment due to emergence of drug resistance in *Candida* species. *Candida* resists the antifungal compounds by changing the cell wall/membrane composition or modifying

the drug target molecule or efflux of drugs mediated by ATP binding cassette. [8]

These conditions forced scientists to screen new drugs from natural sources or modifying existing drugs. Another approach is to screen medicines used in alternative medicine such as Homoeopathy. These medicines may play a significant role in treatment of candidiasis without imparting any side effects. Homoeopathy offers constructive treatment of fungal infections particularly for Candidiasis.

Recently, there are reports citing the efficacy of homoeopathic medicines against various bacterial and fungal pathogens. [9, 10] Acidum Sulphuricum, Acidum Benzoicum, Azadirachta Indica, Cinchona Officinalis, Iodium, Phosphorus, Selenium, Sulphur, Zincum Metallicum and Zingiber Officinale are group of medicines used to treat symptoms similar to *Candida albicans* infection according to Complete, Murphy's and Synthesis Homoeopathic Repertory, but there is no clear cut study regarding significant activity or mechanism of action. [11-13] Thus, it becomes imperative to collect, identify and validate the efficacy of Homoeopathic Medicines for management of candidiasis.

In the present study was aimed to screen the ability of various homeopathic medicines to inhibit pathogenic *Candida albicans in-vitro* and to study mechanism of inhibition.

MATERIALS AND METHODS

Media and Chemicals - All media was procured from Hi Media Mumbai, India. All the reagents and chemicals used in this work were of AR grade and procured from MERK India.

Homoeopathic Medicines preparation - Homoeopathic medicines such as Acidum Sulphuricum, Acidum Benzoicum, Azadirachta indica, Cinchona officinalis, Iodium, Phosphorus, Selenium, Sulphur, Zincum Metallicum, Zingiber officinale were obtained from GMP approved Standard Homoeopathic Medicines

Manufacturer in 6C, 12C, 30 C, 200 C, 1M liquid potencies.

Organism - *Candida albicans* (NCIM-3557) was used as standard culture to examine anti-candida activity. The culture was maintained in potato dextrose agar slants and stored in refrigerator. For all studies candida cultures were grown in Sabouraud dextrose broth and incubated at 37°C for 24 h. The culture then centrifuged and the pellets were suspended in saline to get 1.0 OD at 595 nm.

Anti-candida assay

Anti-candida activity of the medicines was determined using agar diffusion assay. [9,14,15] Accordingly, candida culture suspension (100 µl) was plated on Sabouraud dextrose agar plates and incubated for 30 min. various potencies of the selected homoeopathic medicines (20 µl) were added in wells (3 mm dia.) made on the agar. It was followed by incubation at 37°C for 24 h. Clotrimazole (20µl, 100µg/ml) was added as the positive control. Anti-candida activity was scored by measuring the diameter of zone of inhibition around the wells.

MIC value

MIC value of the selected medicines was found out using 96 well plates assay. Total volume in each well was adjusted to 200 µl with Sabouraud dextrose broth, varying potencies of homoeopathic medicines and *Candida albicans* 3557 (0.5 OD). The experiment was conducted along with negative control (Sabouraud dextrose broth) and positive control (clotrimazole) and ethanol (Dispensing alcohol). [16] Optical density of the candida was measured at zero hours and after 3 hrs. of incubation at 37°C using Elisa plate reader (BIORAD) at 595 nm. The experiment was repeated three times and mean value was taken as the reading.

Germ Tube Inhibition

Germ tube inhibition was performed in coated 96 well plates. [15] Accordingly, 100µl of Candida (1.0 OD) in Sabouraud dextrose broth containing 0.2 % human

serum were added in each well followed by 100 µl of drug or clotrimazole and incubated 37°C for 4 hrs. After the incubation media were discarded by inverting the plate and wells were washed with 70% ethanol followed by distilled water. The germ tubes were stained using 200 µl of 0.1% crystal violet for 10 min. Stains were washed with distilled water and with 0.25% SDS and again by distilled water wash once. After drying the plate, mixture of isopropanol with 0.04 HCL and 50 µl of 0.25 % SDS were added in the well. Optical density of the resulting solution was measured using Elisa plate reader at 595 nm.

Lysis studies for Candida

For lysis study, the culture suspension (200µl) was mixed with 200µl of Homoeopathic medicines and incubated for 1 hr at 37°C. After the incubation 50µl of the culture mixed with 50µl Trypan blue. The mixture was then loaded on a hemocytometer and observed under microscope to observe intact as well as lysed cells.

RESULT

Anti-candida screening and MIC.

All homoeopathic medicines used were showed inhibition zone around well. The inhibition zone ranged from 0.3 to 0.9 mm for various potencies (Table.1). The MIC of the drug varied from 6C to 30C potencies as given in the Table 2.

Germ tube inhibition

Germ tube formation is a very important stage in candida pathogenicity since this initiate the biofilm formation on the epithelial tissue. An ideal anti-candida drug should be able to inhibit the formation of germ tube. All the homoeopathic medicines used at MIC value showed 100% of germ tube inhibition. Whereas the activity of control was found to be 23% (Table 3 and Figure 1)

The selected medicines when checked for lysis of candida culture did not show any lysis.

Anti fungal activity against tested Candida culture is as shown in the table,

| Table 1. Anti-candida activity of the Homoeopathic Medicines | | |
|--|-----------|-------------------------|
| Name of Medicines | Potencies | Mean Activity (in cm) ± |
| Azadirachta Indica | 6 C | 0.9 ± 0 |
| | 12C | 0.65 ± 0.07 |
| | 30 C | 0.9 ± 0 |
| | 200 C | 0.7 ± 0 |
| | 1M | 0.55 ± 0.07 |
| Acidum Benzoicum | 6 C | 0.6 ± 0 |
| | 12C | 0.6 ± 0 |
| | 30 C | 0.6 ± 0 |
| | 200 C | 0.8 ± 0.28 |
| | 1M | 0.55 ± 0.07 |
| Acidum Sulphuricum | 6 C | 0.85 ± 0.21 |
| | 12C | 0.75 ± 0.21 |
| | 30 C | 0.7 ± 0 |
| | 200 C | 0.65 ± 0.07 |
| | 1M | 0.7 ± 0.14 |
| Cinchona Officinalis | 6 C | 0.85 ± 0.21 |
| | 12C | 0.9 ± 0.42 |
| | 30 C | 0.75 ± 0.21 |
| | 200 C | 0.75 ± 0.25 |
| | 1M | 0.65 ± 0.07 |
| Iodium | 6 C | 0.65 ± 0.07 |
| | 12C | 0.75 ± 0.07 |
| | 30 C | 0.6 ± 0.14 |
| | 200 C | 0.7 ± 0 |
| | 1M | 0.6 ± 0.14 |
| Phosphorus | 6 C | 0.6 ± 0 |
| | 12C | 0.8 ± 0.14 |
| | 30 C | 0.7 ± 0.14 |
| | 200 C | 0.9 ± 0.14 |
| | 1M | 0.65 ± 0.07 |
| Selenium | 6 C | 0.65 ± 0.07 |
| | 12C | 0.6 ± 0.14 |
| | 30 C | 0.7 ± 0 |
| | 200 C | 0.8 ± 0.14 |
| | 1M | 0.55 ± 0.07 |
| Sulphur | 6 C | 0.75 ± 0.07 |
| | 12C | 0.65 ± 0.07 |
| | 30 C | 0.7 ± 0 |
| | 200 C | 0.9 ± 0.14 |
| | 1M | 0.7 ± 0.14 |
| Zincum Metallicum | 6 C | 0.6 ± 0 |
| | 12C | 0.75 ± 0.07 |
| | 30 C | 0.6 ± 0.14 |
| | 200 C | 0.85 ± 0.21 |
| | 1M | 0.7 ± 0.14 |
| Zingiber Officinale | 6 C | 0.8 ± 0.28 |
| | 12C | 0.6 ± 0 |
| | 30 C | 0.55 ± 0.07 |
| | 200 C | 0.55 ± 0.07 |
| | 1M | 0.8 ± 0.11 |
| Chemical Control (Clotrimazole) | 100µg/ml | 0 |
| Vehicle Control (Dispensing alcohol) | 90% | 0.35 ± 0.07 |

| Sr. No. | MIC of the Homoeopathic Medicines | |
|---------|-----------------------------------|------------------------------------|
| | Name of Homoeopathic Medicine | MIC value of (Homeopathic potency) |
| 1 | Azadirachta Indica | 6 C |
| 2 | Acidum Benzoicum | 12 C |
| 3 | Acidum Sulphuricum | 6 C |
| 4 | Cinchona Officinalis | 12 C |
| 5 | Iodium | 6 C |
| 6 | Phosphorus | 6 C |
| 7 | Selenium | 6 C |
| 8 | Sulphur | 6 C |
| 9 | Zincum Metallicum | 30 C |
| 10 | Zingiber Officinale | 6 C |

| Table 3: Germ tube inhibition by Homoeopathic Medicines | |
|---|--------------------------|
| Homoeopathic Medicines | Germ tube inhibition (%) |
| Azadirachta Indica 6 C | 100 |
| Acidum Benzoicum 12 C | 100 |
| Acidum Sulphuricum 6 C | 100 |
| Cinchona Officinalis 12 C | 100 |
| Iodium 6 C | 100 |
| Phosphorus 6 C | 100 |
| Selenium 6 C | 100 |
| Sulphur 6 C | 100 |
| Zincum Metallicum 30 C | 100 |
| Zingiber Officinale 6 C | 100 |
| Clotrimazole | 23 |
| Dispensing Alcohol (Ethanol) | 00 |

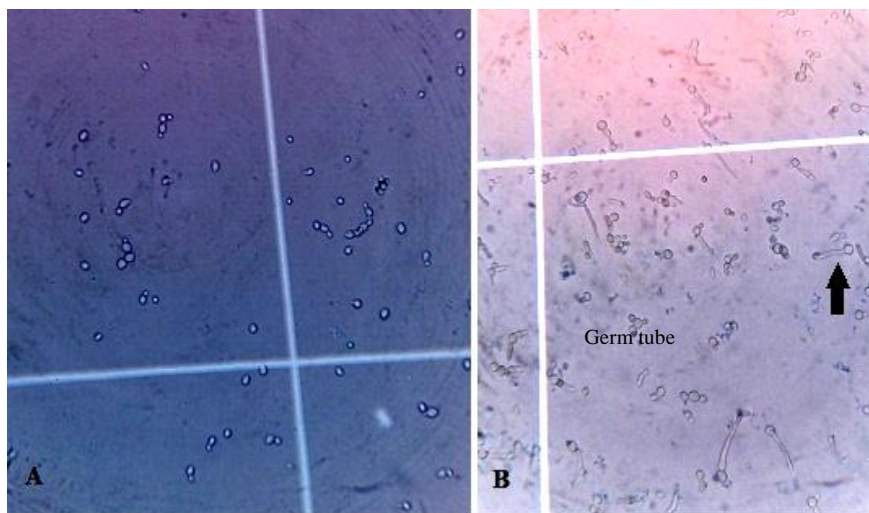


Figure 1. Inhibition of germ tube formation.
 A. In presence of Zincum Metallicum 30 C
 B. In presence of clotrimazole.

DISCUSSION

The aim of the current study was to screen the ability of homoeopathic medicines to inhibit *Candida albicans*. The medicines were selected as per the Complete and Murphy's Homoeopathic Repertory. From the results of the screening study it was clear that selected medicines could inhibit *Candida albicans in vitro*, since they showed inhibition zone around the well in agar diffusion assay. MIC value varied from 6C to 30C potencies. Presence of active principle in homoeopathic medicines with higher dilution is a topic for debate since many years. There is speculation about possible placebo effect in homoeopathic medicines, since higher dilution reduces the concentration of the active principle below the Avogadro number. Till the understanding of nanotechnology, no one could explain how homoeopathic drug works with efficiency.

The results presented in this study clearly indicating that homoeopathic medicines have activity even at lower dilutions.

Induction of biofilm formation is important part pathogenicity. In *C. albicans* germ tube formation initiates the process of biofilm formation. As a result, one of the aims of anti-candida medicines is to inhibit germ tube formation in *C. albicans*. The medicines tested in the study could inhibit formation of germ tube in *Candida albicans*. However, none of the medicines could induce lysis of yeast culture. These results clearly depicting that homoeopathic medicines control the pathogen by inhibiting the germ tube formation.

CONCLUSION

The present study reveals that homoeopathic medicines can effectively inhibit *Candida albicans* by preventing the formation of germ tube. The tested

medicines at various potencies can be an effective alternative to currently used anticandida drugs. However, further investigations needs to be performed to understand the molecular events involved in the germ tube inhibition by homoeopathic medicines.

ACKNOWLEDGEMENT

The authors are grateful to Bharati Vidyapeeth (Deemed To Be University) for providing facility to carry out the study.

REFERENCES

1. Arun S, Renuka V, Aditi M, Ashutosh A. Oral candidiasis: An overview, J Oral Maxillofac Pathol. 2014; (Sep 18(Suppl 1): S81–S85.
2. Martins N, Ferreira IC, Barros L, Silva S Henriques M. Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment, Mycopathologia. 2014; 177(5-6):223-40.
3. Parveen SD. An approach to etiology, diagnosis and management of different types of candidiasis, J. Yeast and Fungal Res. 2013; 4(6):63-74.
4. Lydia K, Pedro M, Nadja J, Stephanie W, Duncan W, Sascha B, Bernhard H. Antifungal activity of clotrimazole against Candida albicans depends on carbon sources, growth phase and morphology. J. Med. Microbiol. 2015; 64:714–723.
5. Annual MK, Asif MH, Zulfiqar A. Candida albicans: A Model Organism for Studying Fungal Pathogens. ISRN Microbiol. 2012; vol. 2012, Article ID 538694:15 pages.
6. Phyllis RS, Brogden RN, Pinder KM, Speight TM, Avery GS. Clotrimazole: A Review of its Antifungal Activity and Therapeutic Efficacy. Drugs. 1975; (9): 424-447.
7. Crowley PD, Gallagher HC. Clotrimazole as a pharmaceutical: past, present and future. J Appl Microbiol. 2014; 117(3):611-7.
8. Mishra NN, Prasad T, Sharma N, Payasi A, Prasad R, Gupta DK, Singh R. Pathogenicity and drug resistance in Candida albicans and other yeast species. A review. Acta Microbiol Immunol Hung. 2007; 54(3):201-35.
9. Suneel P, Mahima S, Pankaj G, Manoj K, Binit D, Bhopal SA, Evaluation of antifungal activity of different homoeopathic mother tinctures against Candida albicans. Ind. J. Res. Homoeopathy. 2017; 11(4):237-243.
10. Almaguer A, Gonzalez P. Antibacterial Activity of Homeopathic Medications Lycopodium clavatum and Arsenicum album Against Periodontal Bacteria. Int. J. Dental Sc., 2018; 20-2: 71-79.
11. Zandvoort RV, Complete Repertory, English (version 2003). Radar 10.0
12. Murphy R, Homoeopathic Medical Repertory, A Modern Alphabetical and Practical Repertory, Third Revised Edition. New Delhi (INDIA): B Jain Publication; 2014; 389.
13. Schroyens F, Augmented Clinical Synthesis, Repertorium Homeopathicum Syntheticum, Edition 9.1. New Delhi (INDIA): B Jain Publication; 2014; 650, 1867.
14. Letscher-Bru V, Obszynski CM, Samsoen M, Sabou M, Waller J, Candolfi E. Antifungal activity of sodium bicarbonate against fungal agents causing superficial infections. Mycopathologia. 2013; 175(1-2): 153-8.
15. Vikrant P, Rajeev M, Rahul MS, Monalisa M, Meera SM, Neelam K, Prashant KD, Prabhakar KR, Bipinraj NK. Inhibition of pathogenic strains of Candida albicans and non-albicans by Bacillus species isolated from traditional Indian fermented food preparations, Int. J. Cur. Microbiol. Appl. Sci.2015; 4(3): 691-699.
16. Homoeopathic Pharmacopoeia of India, Government of India Ministry of Health and Family welfare, Volume I; Appendix - I, New Delhi 1971; 323.

How to cite this article: Shinde CH, Anand PK, Kunchiraman BN et.al. In vitro study for the anti - candida activity of homoeopathic medicines against *candida albicans*. Int J Health Sci Res. 2018; 8(9):57-61.
