



Research Article

Assessment of Anti-Candidal Activity of Various Homoeopathic Mother Tinctures Against The Growth Of Candida Albicans

Singh N¹, Sidhu Gagandeep², Sharma Priyanka³, Kumar Parveen⁴

¹Resident, ²Lecturer, Department of Dentistry, MGS Dental College, Sriganaganagar

³BDS, Surendera Dental College, Sriganaganagar, ⁴Professor, Sriganaganagar Homeopathic Medical College, Sriganaganagar

ABSTRACT

Background: *Candida albicans* is an opportunistic pathogenic fungus which causes up to 75% of all candidal infections in human beings. **Aim:** The aim of this study is to evaluate the anti-candidal activity of different homoeopathic mother tinctures against the growth of *Candida albicans*. **Materials and Methods:** Homoeopathic mother tinctures (ϕ) *Syzygium jambolanum*, *Ficus religiosa*, *Allium cepa*, *Thuja occidentalis*, and *Eucalyptus globulus* were investigated for their antifungal activity against the growth of pathogenic fungi *C. albicans* using paper disc agar diffusion method. The percentage of zone of inhibition were measured, and the obtained results were compared with that of the vehicle control. Ketoconazole is used as a reference. **Results:** Homoeopathic mother tinctures used in this study shows the results that some of the homoeopathic drugs used in the study showed significant inhibitory activity against the growth of *C. albicans* as compared to control. Out of which *S. jambolanum* showed a maximum percentage of inhibition as compared to other mother tinctures used. **Conclusion:** The present study suggests that homoeopathic medicines having definite inhibitory activity against *Candida albicans*.

Keywords: Anti-candidial, *Candida albicans*, homoeopathic mother tinctures.

Correseponding Author: Sharma P, Department of Dentistry, S Dental College, Sriganaganagar

How of Cite: Singh N, Sidhu G, Sharma P, Sharma P. Assessment of Anti-Candidal Activity of Various Homoeopathic Mother Tinctures Against The Growth Of *Candida Albicans* TU J .Homo & Medi Sci. 2018; 1(1):13-16

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 Adherence, perspiration, dimorphism and/or germ tube formation, phenotype switching, interference with host defense mechanism, hormonal imbalance, synergism with bacteria and production of hydrolases from the metabolites have been identified as factors enhancing the virulence of *C. albicans*.⁶⁻⁸ 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.⁹ The present study was done to determine the inhibitory effect of various homoeopathic mother tinctures against human pathogenic fungi *C. albicans*.

MATERIALS AND METHODS

The raw material of plants *S. jambolanum* (seeds), *F. religiosa* (leaves), *A. cepa* (bulb), *T. occidentalis* (twig) and *E. globulus* (leaves) were collected. Homoeopathic mother tincture (ϕ) of *S. jambolanum*, *F. religiosa*, *A. cepa*, *T. occidentalis* and *E. globulus* were prepared. Commercially available antifungal drugs Ketoconazole (10 µg/ml) was used as positive control and 90% alcohol was used as vehicle control. The fungal culture of *C. albicans* was used to investigate antifungal activity of homoeopathic drugs. Fungal Culture is prepared by using 0.4 ml sterilised water in a microcentrifuge tube, and freeze-dried culture was transferred into it and mixed well. The mixture was allowed stand for 20 min before transferred it on solid media. Petri plates containing Sabouraud Dextrose Agar (SDA) medium and incubated for 24–48 h at 35°C to give white round colonies against a yellowish background..

Microscopic identification of *Candida albicans*

Direct microscopy by potassium hydroxide stain

One drop of potassium hydroxide (KOH) stain was placed on the centre of clean glass microscope slide and a culture growth from SDA media containing *C. albicans* was

transferred into it and mixed gently with the stain and covered with a coverslip. The preparation was examined using the low power objective microscope.

Preparation Of Disc For Antifungal Assay

For determining antifungal activity of different homoeopathic mother tincture, agar disc diffusion method was used. The discs of filter paper were soaked in selected homoeopathic mother tincture, and let stand for 30 min. After which, they were taken out and allowed to dry under aseptic condition.

Statistical analysis

The values were expressed as mean diameter of zone inhibition (mm), and statistical data were analysed using the GraphPad Prism version 7.0.(GraphPad Software, Inc, USA). $P < 0.05$ was considered as significant as compared to vehicle control.

RESULTS

Microscopy revealed the presence of spherical and oval chain of cells with size measuring 3-6 µm, having single buds with narrow attachment, showing characteristic pseudohyphae and/or hyphae and nucleus having single nuclei. KOH preparation of the specimen shows that non-pigmented septate hyphae with characteristic dichotomous branching confirmed the presence of *C. albicans*.¹⁰ *In vitro* antifungal activity of homoeopathic mother tinctures (ϕ) *S. jambolanum*, *F. religiosa*, *A. cepa*, *T. occidentalis*, and *E. globulus* was evaluated by measuring the percentage zones of growth inhibition of *C. albicans* and compared with the vehicle control (90% alcohol). The data obtained are depicted in table 1. Statistical data revealed that there were significant differences in percentage of zone of inhibition compared with vehicle control. However, all the homoeopathic medicines showed variable antifungal activity against *C. albicans*. the mother tincture of *S. jambolanum* was most potent against *C. albicans*. It exhibited maximum percentage zone of inhibition up to 287% followed by *T. occidentalis* (209%), *A. cepa* (140%), *F. religiosa* (94%), *E. globulus* (85%).

Table 1: Antifungal profile of homoeopathic medicines against pathogen *Candida albicans*

Homoeopathy tincture	Percentage zone of inhibition
Vehicle control (90% alcohol)	-
Ketoconazole (10 µg/ml)	180
FR (ø)	94
SJ (ø)	287
AC (ø)	140
EG (ø)	85
TO (ø)	209

* $P < 0.05$; ** $P < 0.001$: Significant as compared to vehicle control.

FR: *Ficus religiosa*, SJ: *Syzygium jambolanum*, AC: *Allium cepa*, EG: *Eucalyptus globules*, TO: *Thuja occidentalis*,

DISCUSSION

The aim of the current study was to evaluate the ability of homoeopathic medicines to inhibit the growth of *Candida albicans*. From the results of the study it was clear that selected medicines could inhibit *Candida albicans*, since they showed inhibition zone around the well in agar diffusion assay. The anti-fungal activity of reference drug Ketoconazole was promising with significant percent zone of inhibition (185%) compared to control group. However, all the homoeopathic medicines showed variable anti-fungal activity against *C. albicans*. the mother tincture of *S. jambolanum* was most potent against *C. albicans*. It exhibited maximum percentage zone of inhibition up to 287 followed by *T. occidentalis* (209), *A. cepa* (140), *F. religiosa* (94), *E. globulus* (85). Gupta G et al conducted a in-vitro study to evaluate anti-candidal activity of homoeopathic drugs. Samples were collected from the oral cavity and tongue of the patients suspected of

suffering from oral candidiasis were incubated for growth of *Candida*. Disc method was used to assess the *in-vitro* anti-candidal effect of few homoeopathic drugs in 30 and 200 potencies against human pathogenic *Candida albicans* under *in-vitro* conditions and compared with standard antifungal drug ketoconazole (control), rectified spirit (control/vehicle) and distilled water (vehicle) by "inhibition zone technique". Homeopathic drugs namely *Acid benzoicum*, *Apis mellifica*, *Kali iodatum*, *Mezereum*, *Petroleum*, *Sulphur*, *Tellurium*, *Sulphur iodatum*, *Graphites*, *Sepia*, *Silicea* and *Thuja occidentalis* in 30 and 200 potencies were tested against *Candida albicans*. *Mezereum* in 200 and 30 potency showed maximum inhibition of growth of *Candida albicans*.¹¹ Chetan Hanamantrao Shinde et.al. conducted in Vitro Study for the Anti - Candida Activity of Homoeopathic Medicines against *Candida Albicans* 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*.¹²

CONCLUSION

The results achieved in this study clearly depicts that homoeopathic drugs have definite inhibitory activity against *C. albicans*. The results of this study shows that these homoeopathic drugs can be used to control the growth of pathogenic fungi *C. albicans*. However, further researches are needed to know better about the effectiveness of these medicines.

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. Kabir MA, Hussain MA, Ahmad Z. *Candida albicans*: a model organism for studying fungal pathogens. ISRN microbiology. 2012 Sep 29;2012.
6. Ashman RB, Fulurija A, Apadimitriou JM. Pathogenesis of *Candida Albicans* Infection, Advances in Medical Mycology. Vol. 2. New Delhi: Aditya Books Private Limited; 1997. p. 1-28.
7. Srivastava AK, Singh KP, Ray PK. Protein A induced protection against experimental candidiasis in mice. Mycopathologia 1997;138:21-8.
8. Srivastava OP, Srivastava AK, Shukla PK, editors. Advances in Medical Mycology. Vol. 2. Lucknow: Council for Advances in Bioresearch and Evoker Research Perfecting Co.; 1997. p. 188.
9. 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.
10. Baveja C. Medical mycology. Text Book of Microbiology for Dental Students. 3rd ed. Delhi, India: Arya Publications; 2010. p. 322-3.
11. Gupta G, Srivastava AK, Gupta N, Gupta G, Mishra S. Anti-candidal activity of homoeopathic drugs: An *in-vitro* evaluation. Indian J Res Homoeopathy 2015;9:79-85.
12. 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.

Conflict of Interest: None

Source of Support: Nil

This work is licensed under a Creative Commons Attribution 4.0 International License