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# Assessment of anti-candidial activity of different homoeopathic medicines against *Candida albicans*

## Dr. Bhasker Sharma

**Abstract**
**Background:** Now a days, there is an increase in the number of patients affected by recurring candidiasis due to the development of antifungal resistance in pathogenic candida species. Therefore, the present study was conducted to assess the anti-candidial activity of different homeopathic medicines against *Candida albicans*.
**Material and methods:** The present study was conducted among 60 patients. Before commencement of study permission was taken from the ethical committee of the institute. Homoeopathic mother tincture (ϕ) of *S. jambolanum, F. religiosa, O. sanctum, A. cepa,* 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* procured as lyophilised freeze-dried culture strain was used to evaluate antifungal activity of homoeopathic drugs. The statistical data were analysed using one-way analysis of variance (ANOVA). $P < 0.05$ was considered as significant as compared to vehicle control.
**Results:** The results of this study shows that *S. Jambolanum* had maximum mean zone of inhibition. *S. Jambolanum* had maximum percentage zone of inhibition.
**Conclusion:** The results of our study concluded that *S. Jambolanum* was even better than Ketoconozole.

**Keywords:** *C. albicans, S. jambolanum, F. religiosa, O. sanctum, A. cepa*

## 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 and invasive candidiasis [3]. *Candida albicans* is a fungal commensal of human skin and mucosal surfaces that can transit into an invasive fungal pathogen within immune-compromised individuals. C. albicans infection results in over 400000 cases of invasive disease worldwide and systemic infection leads to high mortality rate annually [4, 5]. Like many opportunistic fungi, a key virulence factor of C. albicans has the ability to undergo a reversible morphological switch from a unicellular (yeast) to a filamentous (Hyphal or pseudohyphal) growth form. This switch, resulting in changes of both cell shape and cell physiology, is thought to allow fungal pathogens to adapt in different environmental conditions and has been correlated with pathogenicity traits [6-8]. C. albicans is one of the important nosocomial organisms that can lead to extremely life-threatening, systemic infection with 30% mortality rate [9]. There are studies citing the efficacy of homoeopathic medicines against various bacterial and fungal pathogens [10, 11]. Therefore, the present study was conducted to assess the anti-candidial activity of different homeopathic medicines against *Candida albicans*.

## Material and methods
The present study was conducted among 60 patients. Before commencement of study permission was taken from the ethical committee of the institute. Homoeopathic mother tincture (ϕ) of *S. jambolanum, F. religiosa, O. sanctum, A. cepa* 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* procured as lyophilised freeze-dried culture strain was used to evaluate antifungal activity of homoeopathic drugs. The fungal test strain of *C. albicans* freeze-dried culture was aseptically opened in Biosafety cabinet and the suspension was made as per protocol. 0.4 ml sterilised water was taken in a micro centrifuge 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. Approximately, 1-mm colonies were picked up and suspended in 5 ml of sterile SDA and kept as broth culture/stock culture.

Morphological features of C. albicans species were identified using one drop of potassium hydroxide (KOH) stain which was placed on the centre of clean grease free glass microscope slide and a loop of 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 (×10, ×20) objective of the inverted phase contrast microscope. Results were confirmed using high-power (×45) objective.

For determining antifungal activity of different homoeopathic mother tincture, agar disc diffusion method was used. Whitman filter paper No. 1 was used to prepare standard discs of approximately 6 mm in diameter and autoclaved at 121°C at 15 psi for 15 min. These 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. Media with pH 5.6 ± 0.2 containing relatively high concentration of glucose (40%) was prepared by mixing SDA and distilled water and autoclaved at 121°C for 15 min. Twenty millilitres of molten (45°C) SDA medium was aseptically transferred into each sterile Petri plates (100 mm × 15 mm) and allowed to solidify in a biological safety cabinet. Media containing relatively high concentration of sucrose (30%) was prepared by mixing C. zapek yeast extract agar (CYEA) and distilled water and autoclaved at 121°C for 15 min to dissolve completely. Twenty millilitres of molten (45°C) CYEA medium was aseptically transferred into each sterile Petri plates (100 mm × 15 mm) and allowed to solidify in a biological safety cabinet.

The agar disc diffusion method was used to determine the antifungal activity of the selected Homoeopathic mother tinctures with. To show effects of anti-Candida activity, the yeast suspension at concentration of (0.5 McFarland) (1–5) x 106 CFU/ml were adjusted. A sterile cotton swab was dipped into the adjusted suspension and swabbed over the dried surface of a CYEA media plate throughout the entire surface. Then, filter paper discs containing the different homoeopathic mother tincture (*S. jambolanum, F. religiosa, O. sanctum, A. cepa*,) were placed on the agar surface. About 90% alcohol was used as vehicle control for the antifungal activity against the C. albicans. Ketoconazole drug was used as a positive control at the concentration of 10 µg/ml. The plates were inverted and placed in an incubator set to 35 ± 2°C within 15 min after the discs were applied. After 24 h growth, inhibition zone around the paper discs was measured.

The statistical data were analysed using one-way analysis of variance (ANOVA). $P < 0.05$ was considered as significant as compared to vehicle control.

## Results

In our study 60 patients were selected for the study which were divided into six groups and each group contains 10 patients. The results of this study shows that *S. Jambolanum* had maximum mean zone of inhibition. *S. Jambolanum* had maximum percentage zone of inhibition.

**Table 1:** Morphological characteristics of C. Albicans

| Variables | Results |
|---|---|
| Size (µm) | 3-6 |
| Shape | Spherical or oval |
| Number of buds | Single; chains |
| Attachment of buds | Narrow |
| Thickness | Thin |
| Pseudo hyphae and /or hyphae | Characteristic |

**Table 2:** Antifungal activity of homeopathic tincture against C. albicans

| Homeopathic tincture | Mean zone of inhibition | Percentage zone of inhibition |
|---|---|---|
| *S. jambolanum* | 29.2±1.43 | 290 |
| *F. religiosa* | 15.6±0.58 | 98 |
| *O. sanctum* | 13.0±1.89 | 74 |
| *A. cepa* | 19.4±3.02 | 156 |
| Vehicle control | 6.9±0.94 | - |
| Ketoconazole | 22±1.44 | 180 |

## Discussion

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.[12] 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 [13-15]. The results of this study shows that *S. Jambolanum* had maximum mean zone of inhibition. *S. Jambolanum* had maximum percentage zone of inhibition.

Shinde CH found 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 [16].

Prajapati S found that the effectiveness of zone inhibition against the growth of human pathogenic fungi C. albicans are *S.jambolanum>T.occidentalis>A.cepa>F.religiosa>E. globulus>O. sanctum>H. antidysenterica* [17].

Gupta G found that 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* followed by *Kali iodatum* 200 while Kali iodatum 30 and *Petroleum* 30 had minimum inhibition.[18]

**Conclusion**

The results of our study concluded that *S. Jambolanum* was even better than Ketoconozole.

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