Evaluation of antifungal activity of different homoeopathic mother tinctures against Candida albicans

Suneel Prajapati, Mahima Sharma, Pankaj Gupta*, Manoj Kumar, Binit Dwivedi, Bhopal Singh Arya
Drug Standardisation Unit, DDPR Central Research Institute for Homoeopathy, Noida, Uttar Pradesh, India

Abstract

Objective: The aim of this study is to evaluate the antifungal activity of different homoeopathic mother tinctures against the growth of Candida albicans.

Materials and Methods: Homoeopathic mother tinctures (phi) Syzygium jambolanum, Ficus religiosa, Ocimum sanctum, Allium cepa, Thuja occidentalis, Holarrhena antidysenterica and Eucalyptus globulus were evaluated for their antifungal activity against the growth of human pathogenic fungi C. albicans using paper disc agar diffusion method as per guidelines of clinical and laboratory standard (M44-A) with slight modification. The diameters of zone (mm) of inhibition were measured, and the obtained results were compared with that of the vehicle control. Ketoconazole was used as reference standard fungicide.

Results: Seven homoeopathic mother tinctures were used in this study and the results indicates that some of the homoeopathic drugs in the study showed significant inhibitory activity against the growth of C. albicans as compared to control. Out of the seven medicines tested, S. jambolanum showed a maximum zone of inhibition as compared to other mother tinctures used. 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.

Conclusion: The present study suggests the inhibitory role of homoeopathic medicines against human pathogenic fungi C. albicans.

Keywords: Anti-fungal, Candida albicans, Homoeopathic medicines, In vitro, Paper disc

INTRODUCTION

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.[1,2] 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.[3-5]

In individuals with healthy immune system, C. albicans is often harmless, kept in balance with other members of the local microbiota. However, alterations in the host microbiota (e.g., due to antibiotics), may lead to changes in the host immune response (e.g., during stress, infection by another microbe or immuno-suppressant therapy) or variations in the local environment (e.g., shifts in pH or nutritional content), can enable C. albicans to overgrow and cause infection. These infections range from superficial mucosal and dermal infections, such as thrush, vaginal yeast infections and diaper rash, to haematogenously disseminated infection with sizable mortality rates (approaching 40% in some cases).[6-8] Candida infections are most common in patients of AIDS or those undergoing anticancer or immunosuppressant therapies and healthy people with implanted medical devices.[9,10] However, the management of Candida infections faces a number of problems including limited number of effective antifungal agents, toxicity and the high cost of antifungal agents. Besides these things, the indiscriminate and prolonged use of antifungal drugs has led to therapeutic failures associated with an emergence of multi drug resistance to pathogenic organisms.

*Address for correspondence: Dr. Pankaj Gupta,
DDPR Central Research Institute for Homoeopathy,
Noida, Uttar Pradesh, India.
E-mail: pankajgupta77@gmail.com

Therefore, there is a need for the development of alternative therapies where solution for the optimal treatment of fungal infections could be found out. Medicines from plant origin could be a possible solution since herbal drugs have been used as a traditional treatment for numerous diseases.

Scientific reports revealed that some work has been done on antimicrobial activities of homoeopathic medicines from the plant origin against the different fungal and bacterial strains. However, detailed studies of antifungal activity of homoeopathic medicines against C. albicans are limited. Therefore, the homoeopathic medicines used in the present study were selected based on their clinical indications and through literature search. The present study was carried out with an objective to evaluate the antifungal activities of the homoeopathic medicines (φ) of Syzygium jambolanum, Ficus religiosa, Ocimum sanctum, Allium cepa, Thuja occidentalis, Holarrhena antidysenterica and Eucalyptus globulus against the growth of human pathogenic fungi C. albicans.

**Materials and Methods**

**Plant materials**

The raw material of plants S. jambolanum (seeds), F. religiosa (leaves), O. sanctum (whole plant), A. cepa (bulb), T. occidentalis (twig), H. antidysenterica (bark) and E. globulus (leaves) were collected from Nilgiris District of Tamil Nadu and taxonomically identified/authenticated by the Centre of Medicinal Plants Research in Homoeopathy, Ooty, Nilgiris District, Udagamandalam, Tamil Nadu.

**Preparation of homoeopathic drugs**

Homoeopathic mother tincture (φ) of S. jambolanum, F. religiosa, O. sanctum, A. cepa, T. occidentalis, H. antidysenterica and E. globulus were prepared and standardised according to the procedures mentioned in Homoeopathic Pharmacopoeia of India (HPI). Briefly, 100 g of plant material was poured in specified concentration (depending on the extractive value of plant) and volume of alcohol and kept for a certain period of time as per HPI. The filtered mother tincture was subjected to the in-house physicochemical test of batteries to check the quality and purity (data not published). Commercially available antifungal drugs Ketocanazole (10 μg/ml) was used as positive control and 90% alcohol was used as vehicle control.

**Microorganism**

The fungal culture of C. albicans (MTCC No. 3017) procured as lyophilised freeze-dried culture strain from the Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, was used to evaluate antifungal activity of homoeopathic drugs.

**Preparation of fungal culture**

The fungal test strain of C. albicans (MTCC 3017) freeze-dried culture was aseptically opened in Biosafety cabinet (Model No. AC2-4S8-NS, ESCO Micro Pvt. Ltd., Singapore) and the suspension was made as per protocol. 0.4 ml sterilised water was taken 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) (HiMedia, Mumbai, India, Catalogue No. M063) 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. Microorganisms were repeatedly subcultured using streaking method and maintained to obtain pure isolation on the SDA for further drug sensitivity assay.

**Microscopic identification of Candida albicans**

**Direct microscopy by potassium hydroxide stain**

Morphological features of C. albicans species were identified according to the method described by Okungbowa et al., 2009. One drop of potassium hydroxide (KOH) stain 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 (RTC-7, Radical scientific equipments Pvt. Ltd., Ambala, India). High-power (×45) objective was used to confirm observations [Figure 1].

**Preparation of disc for antifungal assay**

For determining antifungal activity of different homoeopathic mother tincture, agar disc diffusion method was used. Whatman 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.

**Preparation of growth media (sabouraud dextrose agar)**

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.

![Figure 1: Morphological characterisation of Candida albicans features under the microscope](image-url)
**Preparation of media (Czapek yeast extract agar) for drug-sensitivity assay**

Media containing relatively high concentration of sucrose (30%) was prepared by mixing Czapek yeast extract agar (CYEA) (HIMEDIA, Mumbai, India, Catalogue No. M1335) 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.

**Determination of antifungal activity**

The agar disc diffusion method was used to determine the antifungal activity of the selected Homoeopathic mother tinctures with measuring of diameter of growth inhibition zones.[19] To show effects of anti-Candida activity, the yeast suspension at concentration of (0.5 McFarland) (1–5) × 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, T. occidentalis, H. antidysenterica and E. globulus) 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. To get the proper statistical data and repeatability of the response, the experiments were repeated twice with five replicated in each plate. The diameter of growth inhibition zones around each paper disc was measured by scale.

**Statistical analysis**

The values were expressed as mean diameter of zone inhibition (mm), and statistical data were analysed using one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test to monitor significance among groups using the GraphPad Prism version 7.0. (GraphPad Software, Inc, USA) P < 0.05 was considered as significant as compared to vehicle control.

**Results and Discussion**

The plant-based antimicrobials serve as suitable agent to replace synthetic one in controlling the growth of human pathogenic microorganism and play major role in pharmaceuticals, especially in various alternative medicinal systems such as Ayurveda, Siddha, Unani and Homoeopathy. Homoeopathic drugs have long been used safely for curing many diseases including the ailments caused by fungi. In the present study, mother tincture of homoeopathic drugs S. jambolanum, F. religiosa, O. sanctum, A. cepa, T. occidentalis, H. antidysenterica and E. globulus were evaluated against the growth of human pathogenic fungi C. albicans. Susceptibility of each drug was tested using paper disc diffusion method and a diameter zone of growth inhibition was measured in millimetre.

Microscopy revealed the presence of spherical and oval chain of cells [Table 1 and Figure 1]. KOH preparation of the specimen revealed non-pigmented septate hyphae with characteristic dichotomous branching (at an angle of approximately 45°) confirmed the presence of C. albicans.[20] *In vitro* antifungal activity of homoeopathic mother tinctures (d) S. jambolanum, F. religiosa, O. sanctum, A. cepa, T. occidentalis, H. antidysenterica and E. globulus was evaluated by measuring the diameter of zones of growth inhibition of C. albicans and compared with the vehicle control (90% alcohol). The data obtained are depicted in Table 2 and Figure 2. Statistical data revealed that there were significant differences in diameter of zone of inhibition (mm) compared with vehicle control. However, all the seven homoeopathic medicines showed variable anti-fungal activity against C. albicans [Figure 3]. Post hoc test showed that the mother tincture of S. jambolanum was most potent against C. albicans. It exhibited maximum zone of inhibition up to 27.00 mm ± 3.81 mm followed by T. occidentalis (21.4 ± 1.52), A. cepa (16.6 ± 2.41), F. religiosa (13.8 ± 0.84), E. globulus (13.2 ± 1.30), O. sanctum (11.2 ± 1.48) and H. antidysenterica (7.0 ± 1.00). The maximum percentage of zone inhibition (285%) was detected in mother tincture of S. jambolanum as compared to other medicines used in the study. The anti-fungal activity of

<table>
<thead>
<tr>
<th>Parameters Results</th>
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<tbody>
<tr>
<td>Size (μm)</td>
</tr>
<tr>
<td>Shape</td>
</tr>
<tr>
<td>Number of buds</td>
</tr>
<tr>
<td>Attachment of buds</td>
</tr>
<tr>
<td>Thickness</td>
</tr>
<tr>
<td>Pseudohyphae and/or hyphae</td>
</tr>
<tr>
<td>Number of nuclei</td>
</tr>
</tbody>
</table>

**Table 1: Morphological characterization of Candida albicans**

<table>
<thead>
<tr>
<th>Homoeopathy tincture</th>
<th>Mean zone of inhibition (mm)</th>
<th>Percentage zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control (90% alcohol)</td>
<td>7.2±0.84</td>
<td>-</td>
</tr>
<tr>
<td>Ketoconazole (10 μg/ml)</td>
<td>20.0±1.87**</td>
<td>185</td>
</tr>
<tr>
<td>SJ (ο)</td>
<td>27.0±3.81**</td>
<td>285</td>
</tr>
<tr>
<td>FR (ο)</td>
<td>13.8±0.84*</td>
<td>97</td>
</tr>
<tr>
<td>OS (ο)</td>
<td>11.2±1.48*</td>
<td>60</td>
</tr>
<tr>
<td>AC (ο)</td>
<td>16.6±2.41*</td>
<td>137</td>
</tr>
<tr>
<td>TO (ο)</td>
<td>21.4±1.52**</td>
<td>205</td>
</tr>
<tr>
<td>HA (ο)</td>
<td>7.0±1.00</td>
<td>0</td>
</tr>
<tr>
<td>EG (ο)</td>
<td>13.2±1.30*</td>
<td>89</td>
</tr>
</tbody>
</table>

<sup>*P<0.05; **P<0.001: Significant as compared to vehicle control. S. jambolanum, F: Ficus religiosa, O: Ocimum sanctum, AC: Allium cepa, TO: Thuja occidentalis, HA: Holarrhena antidysenterica, EG: Eucalyptus globulus</sup>
reference drug Ketoconazole was promising with significant percent zone of inhibition (185%) compared to control group.

The inhibitory activity of the medicines against C. albicans may be due to the presence of several primary and/or secondary metabolites such as phenolics, polyphenols, tannins, quercetin, flavonoids, alkaloids, terpenoids, volatile oils, polypeptides and complex mixtures in respective mother tinctures. However, identification of active compounds present in the homoeopathic formulations and how homoeopathic medicines work to inhibit the growth of human pathogenic fungi C. albicans \textit{in vitro} has not been evaluated in this study which is the subject of further investigations.\textsuperscript{[21-28]}

**CONCLUSION**

The results of the study revealed that homoeopathic drugs, namely, \textit{S. jambolanum}, \textit{F. religiosa}, \textit{O. sanctum}, \textit{A. cepa}, \textit{T. occidentalis} and \textit{E. globulus} possess \textit{in vitro} antifungal effect against human pathogenic fungi C. albicans. The effectiveness of zone inhibition against the growth of human pathogenic fungi C. albicans are \textit{S. jambolanum} > \textit{T. occidentalis} > \textit{A. cepa} > \textit{F. religiosa} > \textit{E. globulus} > \textit{O. sanctum} > \textit{H. antidysenterica}. In conclusion, the findings of this experiment suggest that these homoeopathic drugs can be used to control the growth of pathogenic fungi C. albicans. However, further investigations are required to get the better understanding of the effectiveness of these medicines.

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Nil.

**Conflicts of interest**

None declared.

**REFERENCES**


Prajapati, et al.: Antifungal activity of homoeopathic mother tinctures

Beurteilung der antimykotischen Wirksamkeit verschiedener homöopathischer Urtinkturen gegen Candida albicans

**Ziel:** Beurteilung der antimykotischen Wirksamkeit verschiedener homöopathischer Urtinkturen gegen das Wachstum von Candida albicans

**Methodik:** Die Wirksamkeit der homöopathischen Urtinkturen (ф) Syzygium jambolanum, Ficus religiosa, Ocimum sanctum, Allium cepa, Thuja occidentalis, Holarrhena antidysenterica und Eucalyptus globulus wurden auf ihre antimykotische Aktivität gegen das Wachstum von humanpathogenen Pilze Candida albicans unter Verwendung der “paper disc method” nach klinischen Richtlinien und Laborstandards (M44-A) ausgewertet. Die Durchmesser der Hemmzone (mm) wurde gemessen, und die erhaltenen Ergebnisse mit der Vehikelkontrolle abgeglichen. Ketoconazol wurde als Standardreferenz-Fungizid eingesetzt.


**Fazit:** Die vorliegende Studie weist auf eine inhibitorische Funktion homöopathischer Arzneimittel gegen den humanpathogenen Pilz C. albicans hin.

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Évaluation de l’activité antifongique de différentes teintures-mères homéopathiques contre le Candida albicans

**Résumé**

**Objectif:** Évaluation de l’activité antifongique de différentes teintures-mères homéopathiques contre la prolifération du Candida albicans

**Méthodologie:** Les teintures-mères homéopathiques (ф) Syzygium jambolanum, Ficus religiosa, Ocimum sanctum, Allium cepa, Thuja occidentalis, Holarrhena antidysenterica et Eucalyptus globulus ont été évaluées pour leur activité antifongique contre la prolifération du Candida albicans, champignons pathogènes chez l’homme, à l’aide de la méthode des disques de papier conformément aux directives sur les Normes cliniques et de laboratoires (M44-A). Les diamètres des zones d’inhibition (en mm) ont été mesurés et les résultats obtenus ont été comparés à ceux du groupe témoin recevant le véhicule. Le fongicide kétoconazole a été utilisé comme fongicide de norme de référence.

**Résultats:** Sept teintures-mères homéopathiques ont été utilisées pour cette étude et le résultat indique que certaines d’entre elles ont démontré une activité inhibitrice importante contre la prolifération du C. albicans par rapport au groupe témoin. Parmi les sept médicaments testés, Syzygium jambolanum a montré une zone d’inhibition maximale par rapport aux autres teintures-mères utilisées. L’efficacité de la zone d’inhibition contre la prolifération du C. albicans, champignons pathogènes chez l’homme, est dans l’ordre suivant: Syzygium jambolanum> Thuja occidentalis> Allium cepa> Ficus religiosa> Eucalyptus globulus> Ocimum sanctum> Holarrhena antidysenterica.

**Conclusion:** La présente étude suggère un rôle inhibiteur des médicaments homéopathiques contre le C. albicans, champignons pathogènes chez l’homme.
Evalúan de la actividad antimicótica de las diferentes tinturas madre homeopáticas frente a Candida albicans

Resumen

Objetivo: Evaluación de la actividad antimicótica de diferentes tinturas madre homeopáticas frente al crecimiento de Candida albicans.

Metodología: Se evaluó la actividad antimicótica frente al crecimiento del hongo patogénico humano Candida albicans de las tinturas madre homeopáticas (ф) Syzygium jambolanum, Ficus religiosa, Ocimum sanctum, Allium cepa, Thuja occidentalis, Holarrhena antidysenterica y Eucalyptus globulus. Esta evaluación se efectuó con un método de disco de papel según las directrices del Estándar Clínico y de Laboratorio (Clinical and Laboratory Standard M44-A). Se midieron los diámetros de la zona (mm) de la inhibición y se compararon los resultados obtenidos con los del vehículo de control. Como fungicida estándar de referencia se utilizó ketoconazol.

Resultados: En este estudio, se utilizaron siete tinturas madre homeopáticas. El resultado indica que algunos medicamentos homeopáticos del estudio mostraron una actividad inhibitoria significativa frente al crecimiento de C. albicans en comparación con el control. De los siete medicamentos examinados, Syzygium jambolanum mostró la zona de inhibición máxima, en comparación con las otras tinturas madre utilizadas. La eficacia de la inhibición de zona contra el hongo patogénico humano C. albicans son Syzygium jambolanum > Thuja occidentalis > Allium cepa > Ficus religiosa > Eucalyptus globulus > Ocimum sanctum > Holarrhena antidysenterica.

Conclusiones: El presente estudio indica el papel inhibidor de los medicamentos homeopáticos frente a los hongos patogénicos humanos C. albicans.

就不同順勢療法母酊對抗白念珠菌的抗真菌活性評估

摘要

目的：就不同順勢療法母酊對抗白念珠菌的抗真菌活性評估

方法：順勢療法母酊（ф）蒲桃、菩提樹、灰羅勒、紅洋蔥、側柏、止瀉木和藍桉樹分別就其抑制人類致病真菌白念珠菌的抗真菌活性進行了評估，並以藥敏試驗紙片法為臨床和實驗室標準的指引 (M44-A)。量度被抑制範圍的直徑 (mm)，所得的結果會跟對照媒介物比對。參考的標準抗黴菌藥是酮康他索。

結果：研究中使用了7隻順勢療法母酊，結果表示研究中有一些順勢療法藥物相比安慰劑下，對白念珠菌的生長有顯著抑制作用。在7隻藥物測試中，蒲桃比其他母酊有最大抑菌區。對抗人類致病真菌的白念珠菌抑菌區的有效性是蒲桃>側柏>紅洋蔥>菩提樹>藍桉樹>灰羅勒>止瀉木。

結論：目前的研究認為順勢療法藥物對人類致病真菌的白念珠菌有抑制作用。