

ORIGINAL ARTICLE

Anti-candidal activity of homoeopathic drugs: An *in-vitro* evaluation

Girish Gupta, A. K. Srivastava, Naveen Gupta, Gaurang Gupta, Sunil Mishra

ABSTRACT

Background: *Candida albicans* is an opportunistic pathogenic fungus accounting for up to 75% of all candidal infections in human beings. Generally *Candida* grow and survive as commensals but slight modification of the host defense system can transform *Candida albicans* into a pathogen.

Materials and Methods: Samples collected from the oral cavity and tongue of the patients suspected of suffering from oral candidiasis were incubated for growth of *Candida*. Fermentation and assimilation test confirmed the species as *Candida albicans*. 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".

Results: 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.

Conclusion: The results of these experiments support the concept of "evidence based medicine" depicting that homoeopathic medicines not only work in *in-vivo* but are equally effective in *in-vitro* conditions having definite inhibitory activity against *Candida albicans*.

Keywords: Antifungal, *Candida albicans*, homoeopathic drugs, *In-vitro* inhibitory activity

INTRODUCTION

Fungal infections are of great significance in tropical countries like India where heat and humidity provide conditions favorable for onset, growth and persistence of fungus. *Candida albicans* is an opportunistic pathogenic fungus found as a part of normal microflora in human digestive tract causing episodic, acute, sub-acute and chronic localized or opportunistic systemic infection in human beings.^[1-3]

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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*.^[4-6]

Candida albicans is the most important species in the genus *Candida* and accounts for up to 75% of all candidal infections. In general, innate and acquired host defense mechanisms act in concert with the resident bacterial flora such that *Candida* organisms grow and survive as commensals. Even a slight modification of the host defense system, or host ecological environment, can assist the transformation of *C. albicans* into a pathogen capable of causing infections that may be lethal. The most common body sites showing asymptomatic colonization by *Candida* are the alimentary tract and muco-cutaneous regions, viz. oral cavity, rectum vagina etc.^[7-10] Oral swabs or rinses are positive for *C. albicans* in up to 40% of healthy adult subjects, while 20–25% of healthy women carry *C. albicans* in the vagina. Colonization by *Candida* is thought to occur at an early age with the organisms being acquired during passage through the birth canal, during nursing or from food. Long-term colonization is probably responsible for eliciting the circulating Immunoglobulin G and mucosal secretory Immunoglobulin A antibodies to *C. albicans* that are detectable in most healthy individuals. It is these acquired host responses in conjunction with the anti-*Candida* activities of polymorphonuclear leukocytes and macrophages that probably play a significant part in normally restricting *C. albicans* to superficial growth at mucosal sites.

Although recent evidence suggests that some hospital-acquired (nosocomial) *Candida* infections may behave like minor epidemics with selection of more virulent strains^[11] it is often the commensal (endogenous) organisms that are believed to be the initial sources of infection. However, it is important to recognize that *C. albicans* have the ability to live in harmony with the host, for a lifetime, within the resident complex microflora present on mucosal surfaces. In the oral cavity, *C. albicans* grows and survives by competing and cooperating with an estimated 300 or more species of bacteria. There is compelling evidence that *C. albicans* and *C. dubliniensis* form tight associations with specific oral bacterial species and that these promote adhesion and

colonization by mixed-species communities.^[12] Thus, when *Candida* infections arise, they often occur in association with bacteria. On the other hand, there is also strong evidence to suggest that components of the resident microflora, present in the oral cavity and at other mucosal sites, perform to check *C. albicans* growth. That is why factors that perturb the normal microflora, such as antibiotic therapy, or changes in hormonal or mucosal secretions, may encourage *C. albicans* overgrowth.

A considerable number of experimental studies have been conducted to test the efficacy of homoeopathic drugs against fungal and viral diseases of plants, animals^[13-25] and humans.^[26-32] However, there seems to be few reports on *in-vitro* antimycotic effect of homoeopathic drugs against human pathogenic fungi in general and *C. albicans* in particular. The present study was, therefore, undertaken to determine the inhibitory effect of various homoeopathic drugs against human pathogenic fungi confirming the biological activity of homoeopathic drugs in higher dilutions.

Objective

To determine the inhibitory effect of various homoeopathic drugs against human pathogenic fungus *C. albicans* confirming the biological activity of potentized drugs in higher dilutions.

MATERIALS AND METHODS

Isolation of Human Pathogenic *Candida albicans*

The samples were collected from the oral cavity and tongue of the patients suffering from oral candidiasis who presented themselves at Gaurang Clinic and Centre for Homoeopathic Research for treatment. Part of the oral swab was examined directly in Potassium hydroxide (KOH) (10%) slide mount for the presence of yeast cells. KOH preparation of swab showed fair number of yeast-like cells and fungal mycelium. For isolation, rest part of swab was inoculated in petri dishes poured with Sabouraud's Dextrose Agar (SDA)-Emmons modified (HI Media B. No. 9039) incubated at 37°C ± 1°C for 72 hours. Microscopic examination of 4 days old culture showed globose, short, ovoid sometimes elongated blastoconidia (3–6 μm) on corn meal agar. Reynold's braude phenomenon was observed by incubating blastoconidia in human serum at 37°C and germination were found to be more than 70%.

Fermentation and assimilation test further confirmed the identity of the species as *C. albicans*. Oral swab from healthy persons was kept as control. However, for checking contamination, if any, petri dishes poured with SDA in four replicates were exposed to the environment, gave several mycelial fungi dominated by species of *Aspergillus* but there were no *C. albicans* in the working environment.

From the colonies of *C. albicans* obtained, solitary colony of *C. albicans* was taken with the help of loop, inoculated in a test tube containing 5 ml Sabouraud's dextrose broth (Test tube 1) and incubated at 37°C ± 1°C for 24 hours. Now 1 ml of this broth containing *C. albicans* was taken and added in another test tube containing 4 ml of plain Sabouraud's dextrose broth (Test tube 2). Similarly, 1 ml of this broth containing *C. albicans* was taken and added in another test tube containing 4 ml of plain Sabouraud's dextrose broth (Test tube 3). The same procedure was repeated and Test tube 4 and 5 were prepared. Now this Test tube 5 was kept at room temperature for 24 hours. Broth from Test tube 5 was taken with the help of cotton swab stick and plated on pre-prepared petridishes poured with SDA.

Table 1: *In-vitro* inhibitory effect of homoeopathic drugs in 30C potency against *C. albicans* by "inhibition zone technique" (diameter of disc= 12 mm)

Control	Homoeopathic drug potencies	Zone of inhibition against <i>C. albicans</i> (in mm diameter)
Distilled water (control/vehicle)	-	12
Rectified spirit (control/vehicle)	-	14
Standard antifungal ketoconazole (control) ((100 µg/ml)/disc)	-	40
-	<i>Acid benzoicum</i> 30	16
-	<i>Apis mellifica</i> 30	14
-	<i>Graphites</i> 30	15
-	<i>Kali iodatum</i> 30	13
-	<i>Mezereum</i> 30	27
-	<i>Petroleum</i> 30	13
-	<i>Sepia</i> 30	14
-	<i>Silicea</i> 30	14
-	<i>Sulphur</i> 30	19
-	<i>Sulphur iodatum</i> 30	14
-	<i>Tellurium</i> 30	18
-	<i>T. occidentalis</i> 30	14

C. albicans: *Candida albicans*; *T. occidentalis*: *Thuja occidentalis*

***In-vitro* Inhibitory Effect of Homoeopathic Drugs Against Human Pathogenic Fungi**

Disc method was used to assess the *in-vitro* anti-candidal effect of homoeopathic drugs [Tables 1 and 2] against human pathogenic *C. albicans*. Homoeopathic drugs namely *Acid benzoicum*, *Apis mellifica*, *Kali iodatum*, *Mezereum*, *Petroleum*, *Sulphur*, *Tellurium*, *Sulphur iodatum*, *Graphites*, *Sepia*, *Silicea* and *Thuja occidentalis* in 30C and 200C potencies were tested against *C. albicans* under *in-vitro* conditions and compared with standard antifungal drug ketoconazole (control), rectified spirit (control/vehicle) and distilled water (vehicle). Testing was done by 'inhibition zone technique'.^[33,34]

A volume of 20 ml sterilized SDA was plated on 27 sterilized petridishes and allowed to solidify. 1 ml SDA medium was seeded with the culture broth, mixed well and poured over the surface of all the petri dishes already plated with the medium. Discs (12 mm in diameter) of sterilized Whatman No. 1 filter paper were dipped in different homoeopathic drug potencies, standard antifungal

Table 2: *In-vitro* inhibitory effect of homoeopathic drugs in 200C potency against *C. albicans* by "inhibition zone technique" (diameter of disc= 12 mm)

Control	Homoeopathic drug potencies	Zone of inhibition against <i>C. albicans</i> (in mm diameter)
Distilled water (control/vehicle)	-	12
Rectified spirit (control/vehicle)	-	14
Standard antifungal ketoconazole (control) ((100 µg/ml)/disc)	-	40
-	<i>Acid benzoicum</i> 200	14
-	<i>Apis mellifica</i> 200	14
-	<i>Graphites</i> 200	14
-	<i>Kali iodatum</i> 200	25
-	<i>Mezereum</i> 200	37
-	<i>Petroleum</i> 200	14
-	<i>Sepia</i> 200	16
-	<i>Silicea</i> 200	16
-	<i>Sulphur</i> 200	16
-	<i>Sulphur iodatum</i> 200	14
-	<i>Tellurium</i> 200	17
-	<i>T. occidentalis</i> 200	16

C. albicans: *Candida albicans*; *T. occidentalis*: *Thuja occidentalis*

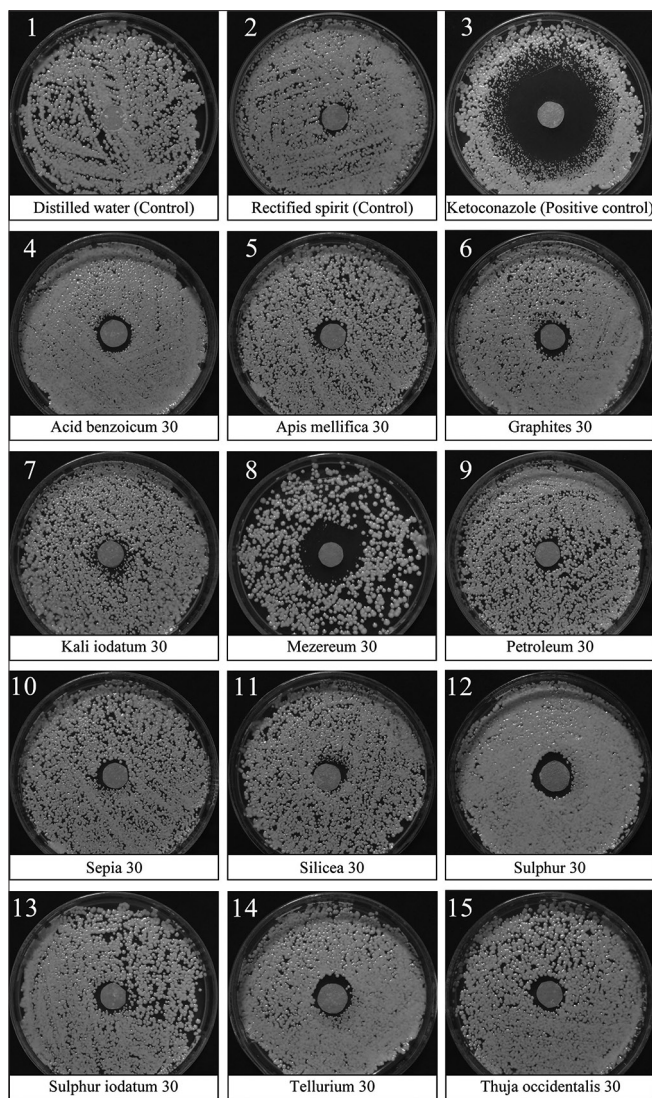


Figure 1: *In-vitro* anti-candidal activity of homoeopathic drugs in 30 potency

drug ketoconazole ($100 \mu\text{g/ml}$ /disc) and rectified spirit (control/vehicle) were placed on the center of each petridish separately.

Petridishes were then incubated at $37^\circ\text{C} \pm 1^\circ\text{C}$ for 72 hours. Inhibition of growth or no growth of *C. albicans* indicated the effectiveness of homoeopathic drugs in different potencies. Ketoconazole rectified spirit and distilled water were used for comparison as controls. The experiments were repeated 3 times and the mean effective area of the zone of inhibition was calculated.

RESULTS

Different potencies of *Mezereum*, *Kali iodatum*, *Acid benzoicum*, *Petroleum*, *Sulphur iodatum*, *Sulphur* and *Tellurium* [Tables 1 and 2 and Figures 1 and 2] have

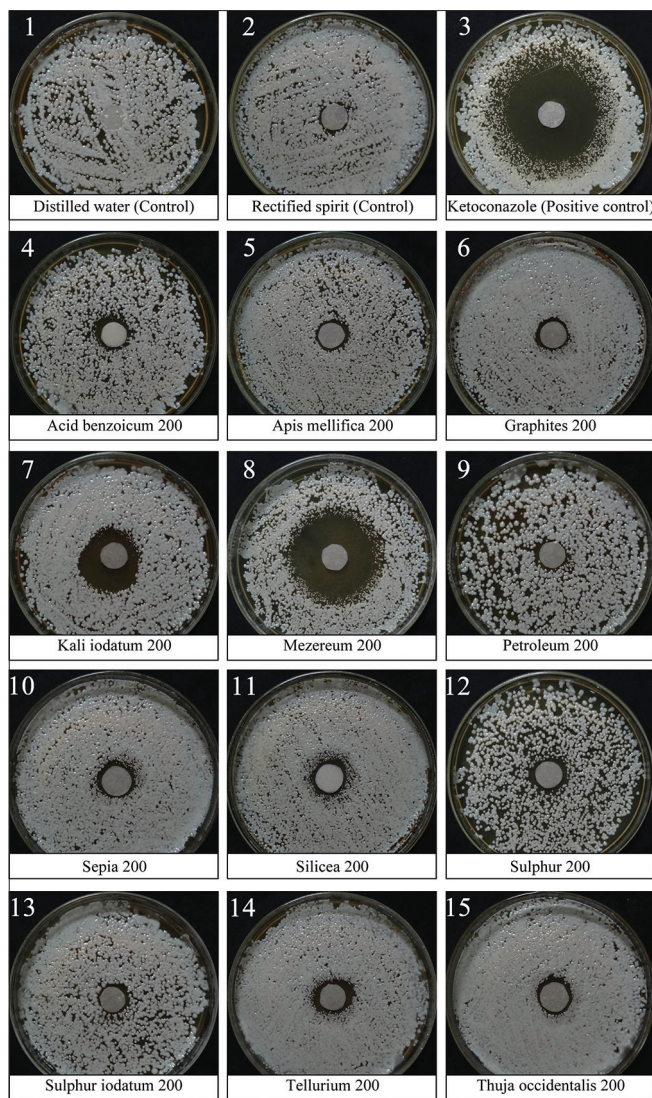


Figure 2: *In-vitro* anti-candidal activity of homoeopathic drugs in 200 potency

variable inhibitory effect against *C. albicans*. Among different drug potencies, *Mezereum* in 200C and 30C showed maximum inhibition of growth of *C. albicans* followed by *Kali iodatum* 200C while *Kali iodatum* 30C and *Petroleum* 30C had minimum. However, ketoconazole showed maximum inhibition. The difference in inhibition of different drug formulations may be ascribed to variations in drug compositions and medicament.

DISCUSSION

Before the inception of nanoparticle, it was believed that homoeopathic medicines do not have any traces of the original drug substance in dilutions beyond '24 X' or '12 C' potency because repeated dilution steps leave progressively fewer and fewer molecules of bulk-form source material in a true solution,

until eventually none should persist in solution diluted past Avogadro's number (6.023×10^{23}), that is, potencies higher than '24X' or '12C'. As a result, conventional medical scientists and chemists rejected the plausibility of Homoeopathy because of the presumptive lack of sufficient bulk-form source material to exert a 'usual' pharmacological dose-response effect. In typical clinical pharmacology, lower bulk-form "doses" should exert lesser effects, but contrary to this, the trituration and succussion procedures used in the preparation of homoeopathic remedies generates nanoparticles of the source material. Trituration with mortar and pestle is a manual method for mechanical grinding or milling, similar to ball milling used in modern nanotechnology.^[35,36] Similarly, manual succussions introduce intense turbulence, particle collisions, and shear forces into solution that break off smaller and smaller particles of remedy source material as well as Silica from the walls of the glass containers or vials^[37] as in modern nanotechnology methods of microfluidization,^[38,39] sonication^[40,41] and vortexing.^[42] The combined impact of these mechanical nanosizing procedures^[41] would be able to modify the properties of the remedy,^[43-45] generating remedy source nanoparticles,^[46,47] as well as Silica crystals and amorphous nanoparticles.^[45,47,48]

The results of these *in-vitro* studies are highly encouraging and have shown that homoeopathic drugs have definite inhibitory activity against *C. albicans* in culture plate confirming that these medicines not only work *in-vivo* but are equally effective in *in-vitro* conditions. The results of these experiments in culture plate have proved that though the homoeopathic medicines are biologically active as persistent remedy source nanoparticles have been demonstrated with high resolution types of electron microscopy in metal and plant homoeopathic remedies prepared both below and above Avogadro's number^[46,47] suggesting that nanoparticles are different from bulk-form materials as a function of their small size, including acquired adsorptive,^[49,50] electromagnetic, optical, thermal, and quantum properties.^[51-55] However, the mode of inhibitory action of homoeopathic medicines is a matter of further research and need of the hour.

CONCLUSION

The results achieved in these *in-vitro* experiments

support the concept of the 'evidence based medicine' clearly depicting that homoeopathic drugs have definite inhibitory activity against *C. albicans*. No doubt the effect is less than that of ketoconazole, but ketoconazole being known to cause many side-effects there is need to test more homoeopathic drugs to find out effective homoeopathic drugs against *C. albicans* and various other human pathogenic fungi. Further studies may open new vistas even in the treatment of resistant strains as well.

These results will definitely clear the misconceptions that Homoeopathy is a placebo therapy and it will have to be accepted that if modern medicine is the science of today Homoeopathy is the science of future.

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Conflicts of Interest

There are no conflicts of interest.

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होम्योपैथी औषधियों की कैंडिडा-रोधी गतिविधि: एक पात्रे मूल्यांकन

सार

पृष्ठभूमि

कैंडिडा एलबिकेन्स एक अवसरवादी रोगजनक कवक है जो मनुष्य के सभी कैंडिडा संक्रमणों के 75 प्रतिशत के लिए उत्तरदायी है। आमतौर पर कैंडिडा सहभोजी के रूप में बढ़ते और जीवित रहते हैं लेकिन मेजबान रक्षा प्रणाली में मामूली संशोधन इन्हें रोगजनक कैंडिडा एलबिकेन्स में बदल सकते हैं।

समाग्री एवं विधियाँ

मौखिक कैंडिडासिस से पीड़ित संदिग्ध मरीजों के मुख और जीभ से एकत्र नमूने, कैंडिडा के विकास के लिए इनक्यूबेट किये गये। किण्वन और आत्मसात परीक्षण की कैंडिडा एलबिकेन्स के रूप में पुष्टि की गयी। मनुष्यों में रोगजनक कैंडिडा एलबिकेन्स के विरुद्ध कुछ होम्योपैथी औषधियों का 30 एवं 200 की शक्ति में परीक्षण किया गया। इसके लिए चक्रिका विधि का उपयोग करते हुए, होम्योपैथी औषधियों द्वारा उपचारित संवर्ध पट्टिकाओं में और, नियंत्रण के रूप में मानक कवकरोधी औषधि कीटोकोनाज़ोल (धनात्मक नियंत्रण), परिशोधित स्पिरिट एवं आसुत जल द्वारा उपचारित संवर्ध पट्टिकाओं में कवक की वृद्धि के लिए "प्रावरोधन परिधि" की तुलना की गई।

परिणाम

30 एवं 200 की शक्ति में एसिड बेंजोइकम, एपिस मेलिफिका, ग्रेफाइट्स, काली आयोडेटम, मेजेरियम, पेट्रोलियम, सीपिया, सिलिशिया, सल्फर, सल्फर आयोडेटम, टेलुरियम एवं थूजा ऑक्सिडेंटालिस के सी. एल्बिकेन्स के विरुद्ध परिवर्तनशील प्रावरोधी प्रभाव हैं। विभिन्न औषध शक्तियों में से, 200 एवं 30 की शक्ति में मेजेरियम ने वृद्धि का सर्वाधिक प्रावरोध दर्शाया जिसके बाद काली आयोडेटम 200 का स्थान रहा, जबकि काली आयोडेटम 30 एवं पेट्रोलियम 30 इस क्रम में सबसे कम रहीं।

निष्कर्ष

इन प्रयोगों के परिणाम अत्यधिक प्रोत्साहक हैं एवं इन्होंने दर्शा दिया है कि होम्योपैथी औषधियों में कैंडिडा के विरुद्ध निश्चित प्रावरोधक गतिविधि है जिससे पुष्टि हुई है कि ये औषधियां न केवल जीव स्थितियों में कार्य करती हैं बल्कि पात्रे स्थितियों में भी समान रूप से प्रभावी हैं।