

# Antimicrobial activity of different homoeopathic drugs and their potencies against '*Aspergillus niger*' *In vitro*

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## Abstract

**Background:** Homoeopathic remedies are widely used all over the world for different disease conditions. Approximately 70% are derived from the plant; however, their preclinical evaluation is still a major concern. **Objective:** This study was undertaken with an aim to explore the antimicrobial effect of different homoeopathic drugs and its potencies against the *Aspergillus niger*. **Materials and Methods:** Fifteen homoeopathic mother tinctures (Θ) and their potencies (3x, 6x, 12x) were tested for their biological activity against the human pathogenic fungi *A. niger* using disc diffusion method according to clinical and laboratory standard (CLSIM44-A) with slight modifications. The diameter of zone of inhibition was measured and compared with vehicle control (Alcohol 90%). The experiment was performed twice to check the reproducibility. **Results:** The marked antifungal activity was observed with Θ of *Zingiber officinale*; the growth of *A. niger* was inhibited and showed maximum zone of inhibition up to 15.4 ± 2.88 mm followed by *Holarrhena antidysenterica* (13.2 ± 1.09) and *Terminalia chebula* (10.6 ± 1.14). Different potencies (3x, 6x and 12x) were also exhibited significant zone of inhibition, especially *Allium cepa* 6x (10.4 ± 0.89), *Caesalpinia bonducella* 6x and 12x (12.8 ± 0.54 and 10.4 ± 1.14, respectively), *Eucalyptus globulus* 12x (11.3 ± 1.94), *Ruta graveolens* 12x (15.0 ± 2.23), *Thuja occidentalis* 6x (10.8 ± 0.83), and *Zingiber officinale* 3x and 6x (13.0 ± 2.73 and 11.4 ± 2.30, respectively) as compared to control. **Conclusion:** The findings of study concluded that Θ and potencies can effectively inhibit the growth of *A. niger in vitro*. This study paves the way for development of homoeopathic antifungal treatments. However, further investigations are required to get more information about the mechanistic approach, their mode of action and *in vivo* evaluation.

**Keywords:** Antifungal, *Aspergillus niger*, Disc diffusion, Homoeopathic drugs, *In vitro*

## INTRODUCTION

*Aspergillus* is airborne fungus and can cause allergy as well as invasive infection worldwide. Moulds with highest toxicity come from the *Aspergillus* genus and are considered as highly pathogenic for humans. Although since ancient time, it was thought that Invasive Aspergillosis (IA) is caused by *Aspergillus fumigatus*,<sup>[1]</sup> later on it was reported that the emergence of IA can be caused by non-fumigatus species such as: *A. flavus*, *A. ochraceus*, *A. niger*, *A. versicolor* or *A. terreus*.<sup>[2,3]</sup> *Aspergillus* species also are saprophytic, thermo tolerant fungi which are ubiquitous in the air and environment. There are 185 species of genus *Aspergillus* reported, and out of these, 20 can cause human infections. *Aspergillus fumigatus* is the most common species found in human infections all over the world.<sup>[4,5]</sup> The incidence of *A. niger* complex IA has been found to be 0.048% in organ transplantation patients and 0.16% in stem cell transplant recipients.<sup>[6,7]</sup> Although humans inhale *Aspergillus* spores at the rate of hundreds per day, they

rarely experience complications. However, under special circumstances, *Aspergillus* species has ability to produce spectrum of diseases involving lungs and later on other organs and tissues.<sup>[5]</sup>

Aspergillosis infections can also strike as sinus disease in immunocompromised hosts. If left untreated, IA can have mortality approaching 100%. In cases of suspected IA, an extensive diagnostic workup is necessary, but treatment should be initiated early to reduce morbidity and mortality.<sup>[8-10]</sup> Over the past two decades, emergence of such saprophytic fungi

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are increasing day by day, and one of the major challenges for immunocompromised patients, such as those with hematologic malignancies, bone marrow transplant and HIV infection is reported to cause cutaneous infection, paranasal aspergilloma and osteitis of the middle ear in such patients. *A. niger*, also known as black mould, are filamentous fungi having ability of fast growth with high pH tolerance ability and consist of smooth and colourless conidiophores and spores.<sup>[11]</sup> *A. niger* is also reported to cause endocarditis after heart surgery and infection of exenterated orbit even in immunocompetent patients.<sup>[12]</sup> *A. niger* was also found to cause allergic bronchopulmonary infection, IA or may be a coloniser of natural or preformed cavities of the human body. Resistance of *Aspergilli* to some clinically used antifungal agents brings a worrying clinical prognosis in people attacked by aspergillosis. For over 50 years, antibiotics have been applied for treating or inhibiting infections. The wide use and sometimes misuse of chemoantimicrobials in both human and animal medicine have been responsible for drug resistance.<sup>[13,14]</sup>

However, the management of *Aspergillus* infections faces a number of problems including limited resources and high cost of effective antifungal agents, and their adverse effects. Besides these, the indiscriminate and prolonged use of antifungal drugs has led to therapeutic failures associated with an emergence of multidrugs' resistance to pathogenic organisms. This has necessitated a search for new antifungal alternative therapeutics, which are safe and cost-effective in terms of *Aspergillus* management. Since the 20<sup>th</sup> century, the researchers have focused on the homoeopathic system due to its efficacy, minimal side effects and cost-effectiveness. Therefore, we proposed to evaluate the *in vitro* efficacy of homoeopathic medicines against the *A. niger* an opportunistic pathogen.

Although scientific reports revealed that substantial work has been done on microbial activities of many plants in other systems of medicine. Besides their traditional uses as antimicrobial agents, their antifungal activity as homoeopathic medicine has not been reported so far. The present study aims to evaluate the efficacy of antifungal activities of the selected homoeopathic medicines; (selection based on their traditional use in other systems of medicine and scientific literature survey). Thus, the particular plant materials *Allium cepa*, *Allium sativum*, *Caesalpinia bonducella*, *Calotropis gigantean*, *Cassia angustifolia*, *Eucalyptus globulus*, *Ficus religiosa*, *Holarrhena antidysenterica*, *Kalmia latifolia*, *Ocimum sanctum*, *Ruta graveolens*, *Syzygium jambolanum*, *Thuja occidentalis*, *Terminalia chebula* and *Zingiber* were processed for homoeopathic preparation, and tested for the antifungal property against the human pathogenic fungi *A. niger*.

## MATERIALS AND METHODS

### Plant material

Fifteen Plants were selected for this study based on their medicinal use, reported literature and clinical indications. Fresh plant parts were collected from the tribal villages. Plants materials of *Allium cepa* (Bulb), *Allium sativum* (Bulb),

*Caesalpinia bonducella* (fruit), *Eucalyptus globulus* (leaves), *Ficus religiosa* (leaves), *Holarrhena antidysenterica* (bark), *Ocimum sanctum* (whole plant), *Syzygium jambolanum* (Seed), *Thuja occidentalis* (leaves) and *Zingiber officinale* (Rhizome) were collected from Nilgiris District of Tamil Nadu State and taxonomically identified/authenticated by the Centre of Medicinal Plants Research in Homeopathy (CMPRH), Nilgiri District, Udagamandalam, Tamil Nadu. However, the mother tincture of *C. gigantean*, *C. angustifolia*, *K. latifolia*, *R. graveolens* and *T. chebula* were procured from a Homoeopathic pharmaceutical company (SBL, Haridwar, Uttarakhand, India).

### Processing of the plant materials

The raw plant materials were collected, sorted, washed and chopped into small pieces, wherever necessary, before drying. The materials were dried outdoors; however, leaves were dried in the shade. The dried plant materials were ground to various degrees of fineness depending on their botanical structures and further used for the preparation of alcohol-based homoeopathic mother tincture.

### Preparation of mother tinctures, potencies and standard

Mother Tinctures (MT) of Homoeopathic drugs (Θ) of *Allium cepa*, *Allium sativum*, *Caesalpinia bonducella*, *Eucalyptus globulus*, *Ficus religiosa*, *Holarrhena antidysenterica*, *Ocimum sanctum*, *Syzygium jambolanum*, *Thuja occidentalis* and *Zingiber officinale* were prepared and processed for different potencies (3x, 6x, 12x) according to the procedures mentioned in Homoeopathic Pharmacopoeia of India. For the remaining 5 homoeopathic mother tinctures potencies were prepared in house as per the standard procedure mentioned in Homoeopathic Pharmacopoeia of India. Ketoconazole (10 µg/ml) (Sigma-Aldrich, GmbH Germany) was used as standard antifungal drug and 90% alcohol (unsuccussed) used as vehicle control whereas, double-distilled water was also used as one of the negative control to highlight the effect of alcohol.

### Microorganism

For this study, the fungal culture of *A. niger* (MTCC No. 282) procured as lyophilized freeze-dried culture strain was obtained from the MTCC, Institute of Microbial Technology, Chandigarh for evaluating antifungal activity of homoeopathic drugs.

### Preparation of fungal culture

The fungal strain of *A. niger* (MTCC No. 282), freeze-dried culture, was aseptically opened in a biosafety cabinet and suspension was made in CMPRH0.4 ml sterilised water. It was then taken in a micro centrifuge tube and freeze-dried culture was transferred in water, mixed well and was left to stand for 20 minutes before transferring it on solid media. Petri plates containing Sabouraud dextrose agar (SDA; Hi Media, Mumbai, India, Catalogue No. M063) medium and Czapek Yeast Extract Agar medium (CYEA; Hi Media, Mumbai, India, Catalogue No. M1335) was incubated for 24–48 h at 30°C to give white round colonies against a yellowish/light yellowish background. Approximately, 1 mm colonies were picked up and suspended in 5 ml of sterile Sabouraud dextrose broth and kept as broth culture/stock culture. Microorganisms

were repeatedly subcultured using spreading method and maintained to obtain pure isolation on the CYEA for further drug sensitivity assay.

### Morphological Identification of *Aspergillus niger* (direct microscopy by KOH stain)

Morphological features of *A. niger* species were identified according to the method previously described.<sup>[15]</sup> In brief, one drop of KOH stain was placed on centre of clean grease free glass microscope slide and transferred a loop of culture growth from CYEA media containing *A. niger* and mixed gently with the stain and covered with a cover slip. The preparation was examined using the low power (10x, 20x) objective of Inverted phase contrast microscope (RTC-7, Radical scientific equipment's Pvt., Ltd., Ambala, India). High power (45x) objective was then used to confirm observations [Figure 1].

### Preparation of disc for antifungal assay

For determining antifungal activity of different homoeopathic mother tinctures and potencies, agar disc diffusion method was used. Filter paper (Whatman no. 1) was used to prepare discs approximately 6 mm in diameter, which were placed in a petri dish and sterilised in a hot air oven. Sterilised discs of filter paper were soaked in homoeopathic mother tincture, as well as different potencies of same drugs and allowed to stand for 30 min. Commercially available antifungal drug Ketoconazole was used as positive control and 90% alcohol was used as vehicle control. Drug-impregnated discs were used for further drug sensitivity assay.

### Preparation of growth media (Sabouraud dextrose agar/Czapek Yeast Extract Agar)

Media with pH  $5.6 \pm 0.2$  containing relatively high concentration of glucose (40%) were prepared by mixing SDA and distilled water, and CYEA medium containing relatively high concentration of sucrose (30%) was prepared by mixing with double-distilled water at 121°C for 15 min. Twenty ml 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 (Model No. AC2-4S8-NS, ESCO Micro Pvt. Ltd., Singapore).

### Preparation of Muller Hinton Agar, 2% Glucose with Methylene Blue for Drug sensitivity assay

Media containing relatively high concentration of Glucose (20%) were prepared by mixing Muller Hinton Agar, 2% Glucose with Methylene Blue (MHAGMB) (HIMEDIA,

Mumbai, India, Catalogue No: M1825) in distilled water and autoclaved at 121°C for 15 min. Twenty ml of molten (45°C) MHAGMB medium was aseptically transferred into each sterile petri plates (100 mm × 15 mm) and allowed to solidify in a biological safety cabinet.

### Inoculum suspension

Spore suspensions of *A. niger* were prepared in sterile saline from fresh colonies grown on CYEA media at 35°C. Cell concentration was adjusted to final concentrations of 0.5 McFarland (1–5) × 10<sup>6</sup> CFU/ml. These suspensions were used directly for the inoculation purpose. The fungal strains were allowed to grow in respective agar medium, and the fungal mycelia before the spore formation was used for antifungal assay to avoid the spreading of spores throughout the plate which will interrupt in the radial growth measurement.

### Disc diffusion assay

*In vitro* antifungal activities were examined as per CLSI document M 44-A (CLSI/NCCLS 2004), with minor modifications. Antifungal activities of homoeopathic drug against the pathogenic *Aspergillus niger* were investigated by the agar disk diffusion method.<sup>[16]</sup> Ketoconazole (10µg/ml) were used as standard drug. Antifungal activity of homoeopathic medicines and their potencies were determined by measuring the diameter of zone of inhibition. To screen the antifungal activity, a sterile cotton swab was dipped into the adjusted suspension and swabbed over MHAGMB media with sterile cotton bud on the entire dried surface of a MHAGMB plate. Then, filter paper discs containing the 15 homoeopathic mother tinctures, as well as their different potencies were placed on the agar surface. 90% alcohol was used as vehicle control for the antifungal activity against the *A. niger*.

The plates were inverted and placed in an incubator set at 35°C ± 2°C for 15 min after the discs were applied. Zone diameters (mm) were determined after 24 hours of incubation at 35°C and measured the point at which total growth inhibition zone was noted. Each assay was performed in duplicate on two different days, and the mean diameters were reported.

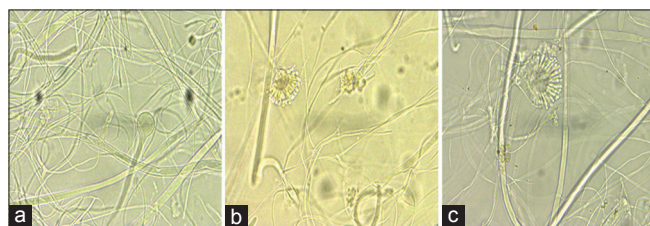
### Determination of percentage of zone of inhibition

Following the observation for fungal inhibition by homoeopathic medicines, the diameter of zone inhibition values were determined according to the equation as below:

$$(\%) \text{inhibition of diameter growth} = \frac{(\text{Diameter of sample} - \text{Diameter of control})}{\text{Diameter of control}} \times 100$$

### Statistical analysis

Experiment was performed twice to check the reproducibility of the results. Data were expressed as mean diameter of zone inhibition (mm) and analysed using one-way analysis of variance followed by Dunnett's *post hoc* test to monitor significance among groups using the Graph Pad prism version 7.0. The statistical comparison having standard



**Figure 1:** Identification and characterization of fungal strain *Aspergillus niger* at (a) 10X, (b) 20X and (c) 45X magnification

deviation  $P < 0.05$  was considered as significant. Ethical approval was not required for this study.

## RESULTS

In the present study, mother tinctures and their different potencies (3x, 6x and 12x) of homoeopathic drugs *Allium cepa*, *Allium sativum*, *Caesalpinia bonducella*, *Calotropis gigantea*, *Cassia angustifolia*, *Eucalyptus globulus*, *Ficus religiosa*, *Holarrhena antidysenterica*, *Kalmia latifolia*, *Ocimum sanctum*, *Ruta graveolens*, *Syzygium jambolanum*, *Thuja occidentalis*, *Terminalia chebula* and *Zingiber officinale* have shown varying results against the growth of *A. niger*.

Microscopy recorded under the different objectives [Figure 1] reveals fungal hyphae with conidial head biserial, radiate, conidia in chains or detached and dispersed. Single or paired conidia (at an angle of approximately 45°) confirmed the characteristic identification of *A. niger*.

Antimicrobial properties of medicinal plants are being reported from different parts of the world. World Health Organization estimates that plant extract or their active constituent's preparation are used as folk medicine in traditional therapies of 80% of the world's population. In the present work, homoeopathic drugs were prepared from different plant material, and some of them show strong activity against fungal strains. In this screening work, the antifungal activity was accessed by disc diffusion assay; this method is most suited to Homoeopathy system and most acceptable as well. Broth microdilution assay

is not feasible to perform with homoeopathic drugs because in homoeopathic drug dilution, the concentration of the active constituents is unknown; hence, determination of concentration of the compound is difficult. Therefore, the *in vitro* antifungal activity of homoeopathic mother tinctures and their potencies were tested using the disc diffusion method according to clinical and laboratory standard (CLSIM44-A) with slight modifications against the *A. niger*. The diameter (mm) of zone of inhibition was measured and compared with standard (Ketoconazole) [Table 1 and Figure 2]. The per cent zone of inhibition for each medicine was also calculated and depicted in Figure 3. Mother tincture of *Z. officinale* was found to be the most potent against *A. niger* and showed maximum zone of inhibition up to  $15.4 \pm 2.88$  mm followed by *H. antidysenterica* ( $13.2 \pm 1.09$ ) and *T. chebula* ( $10.6 \pm 1.14$ ). Different potencies (3x, 6x and 12x) exhibited significant zone of inhibition, especially *A. cepa* with 6x ( $10.4 \pm 0.89$ ), *C. bonducella* with 6x and 12x ( $12.8 \pm 0.54$  and  $10.4 \pm 1.14$ , respectively), *E. globulus* with 12x ( $11.4 \pm 1.94$ ), *R. graveolens* with 12x ( $15.0 \pm 2.23$ ), *T. occidentalis* with 6x ( $10.8 \pm 0.83$ ) and *Z. officinale* with 3x and 6x ( $13.0 \pm 2.73$ ,  $11.4 \pm 2.30$ ) as compared to vehicle control. The antifungal activity of reference drug Ketoconazole showed zone of inhibition ( $11.9 \pm 0.22$ ) compared to control group.

The maximum percentage of zone inhibition (92.5%) was found in mother tincture of *Zingiber officinalis* compared to other medicines used in the study. In case of different potencies (3x, 6x and 12x), the maximum percentage of zone of inhibition was recorded in *R. graveolens* with 12x (87.50%), *E. globulus* with 12x (42.50%), *T. occidentalis*

**Table 1: Zone of inhibition of the homoeopathic mother tincture (Ø) and their potencies (3x, 6x and 12x) against fungal strain *Aspergillus niger***

Name of plant	Family	Concentration of alcohol in mother tincture (%)	Average zone of inhibition (mm)			
			Mother tincture	Potency (90% alcohol) (3x)	Potency (90% alcohol) (6x)	Potency (90% alcohol) (12x)
<i>Allium cepa</i>	<i>Amaryllidaceae</i>	56.7	6.4±0.58	7.4±0.89	10.4±0.89*	6.6±0.74
<i>Allium sativum</i>	<i>Amaryllidaceae</i>	68.0	8.2±0.84	8.0±1.00	6.7±0.79	9.4±1.14
<i>Caesalpinia bonducella</i>	<i>Caesalpinaceae</i>	58.0	6.0±0.00	9.6±1.14	10.±0.54*	10.4±1.14*
<i>Calotropis gigantea</i>	<i>Apocynaceae</i>	65.0	6.0±0.00	7.2±0.83	9.2±1.64	8.2±0.84
<i>Cassia angustifolia</i>	<i>Caesalpinaceae</i>	51.0	6.0±0.00	6.5±0.40	10.0±1.73	6.8±0.82
<i>Eucalyptus globulus</i>	<i>Myrtaceae</i>	86.0	6.6±0.56	6.94±0.68	6.5±0.30	11.3±1.94*
<i>Ficus religiosa</i>	<i>Moraceae</i>	71.0	7.8±1.64	9.8±2.16	9.2±0.83	7.0±0.70
<i>Holarrhena antidysenterica</i>	<i>Apocynaceae</i>	60.0	13.2±1.09*	8.2±1.30	6.6±0.54	7.0±0.83
<i>Kalmia latifolia</i>	<i>Ericaceae</i>	57.0	6.0±0.00	8.4±1.67	8.8±1.64	7.8±0.44
<i>Ocimum sanctum</i>	<i>Lamiaceae</i>	75.0	6.46±0.31	8.0±1.00	7.8±1.60	9.0±2.34
<i>Ruta graveolens</i>	<i>Rutaceae</i>	68.0	6.0±0.00	7.4±0.89	9.6±1.81	15.0±2.23**
<i>Syzygium jambolanum</i>	<i>Myrtaceae</i>	86.0	6.2±0.15	6.54±0.45	8.2±2.16	6.84±0.70
<i>Terminalia chebula</i>	<i>Combretaceae</i>	84.0	10.6±1.14*	7.0±0.00	10.4±2.07	10.2±1.10
<i>Thuja occidentalis</i>	<i>Cupressaceae</i>	59.0	6.0±0.00	10.4±3.78	10.8±0.83*	8.2±1.10
<i>Zingiber officinale</i>	<i>Zingiberaceae</i>	92.0	15.4±2.88**	13.0±2.73**	11.4±2.30*	8.2±1.09
Ketoconazole 10 µg/ml					11.9±0.22	
Vehicle control (90% alcohol) (unsuccused)					8.0±0.70	
Water control					6.0±0.00	

The values were expressed as mean±SD of diameter of zone inhibition (mm) and statistical data were analysed using one-way ANOVA followed by Dunnett's *post hoc* test. \* $P < 0.05$ ; \*\* $P < 0.01$  compared to vehicle control. SD: Standard deviation; ANOVA: Analysis of variance

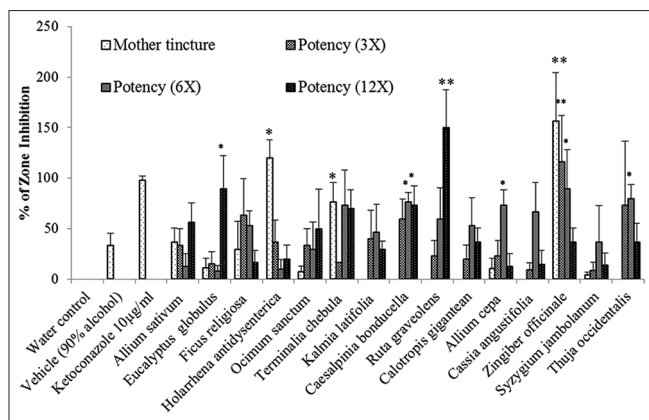


**Figure 2:** Effect of homoeopathic medicines against fungal strain *Aspergillus niger*

with 6x (35%) and *Z. officinale* with 3x and 6x (62.50% and 42.50%, respectively).

Published research data have suggested that antimicrobial components of the plant extracts (terpenoid, alkaloid and phenolic compounds) interact with enzymes and proteins of the microbial cell membrane and cause cell wall disruption to disperse a flux of protons toward cell exterior which induces

cell death or may inhibit enzymes necessary for amino acids biosynthesis.<sup>[17,18]</sup> Other research studies attributed the inhibitory effect of these plant extracts to hydrophobicity characters may enable them to react with protein of microbial cell membrane and mitochondria disturbing their structures and changing their permeability.<sup>[19,20]</sup> The result of present study showed variable antifungal activity of tested homoeopathic



**Figure 3:** Per cent zone of inhibition of homeopathic medicine against the *Aspergillus niger*

mother tincture and their potencies against the growth of *A. niger*. However, justification about the observed activity could not be explained as no uniform pattern (decreasing or increasing order) of zone of inhibition was seen in any of the ultrahigh dilutions potencies of homeopathic drugs. Hence, correlation between the effect of mother tincture and different potencies could not be established. Some previous studies about homeopathic medicine also revealed that these homeopathic preparations have capability to inhibit the growth of pathogenic microbes;<sup>[21-23]</sup> however, their mechanism of action is unknown till date. This study suggests that homeopathic drugs would be helpful for treating diseases of human beings caused by *A. niger*.

## CONCLUSION

The results of the present study demonstrated that homeopathic drugs, namely *Z. officinale*, *H. antidysenterica*, *T. chebula*, *A. cepa*, *C. bonducella*, *E. globulus*, *R. graveolens* and *T. occidentalis* have significant antifungal activity against human pathogenic fungi *A. niger*. In conclusion, the findings of this experiment confirmed that homeopathic drugs can be used as natural fungi toxicant to control the growth of pathogenic fungi (*A. niger*) and reduce the dependence on the synthetic fungicides. Further, *in vivo* experiments are required to confirm their fungicidal activity, mechanism of action to recognise them as better alternative for antifungal treatment in the current scenario.

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## Conflicts of interest

None declared.

## REFERENCES

- Hope WW, Walsh TJ, Denning DW. Laboratory diagnosis of invasive aspergillosis. *Lancet Infect Dis* 2005;5:609-22.
- Ugurlu S, Maden A, Sefi N, Sener G, Yulug N. *Aspergillus niger* infection of exenterated orbit. *Ophthalmic Plast Reconstr Surg* 2001;17:452-3.
- Aviño-Martínez JA, España-Gregori E, Peris-Martínez CP, Blanes M. Successful boric acid treatment of *Aspergillus niger* infection in an exenterated orbit. *Ophthalmic Plast Reconstr Surg* 2008;24:79-81.
- Shrimali GP, Bhatt JK, Rajat R, Parmar RV, Nayak S, Chandralekha D. Isolation of aspergillus species from sputum samples: A study conducted in a tertiary care hospital. *Natl J Med Res* 2013;3:289-91. Available from: <https://www.bibliomed.org/?mno=45259>. [Last accessed on 2018 Aug 23].
- Xess I, Mohanty S, Jain N, Banerjee U. Prevalence of *Aspergillus* species in clinical samples isolated in an Indian tertiary care hospital. *Indian J Med Sci* 2004;58:513-9.
- Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasive fungal infections among organ transplant recipients: Results of the transplant-associated infection surveillance network (TRANSNET). *Clin Infect Dis* 2010;50:1101-11.
- Kontoyiannis DP, Marr KA, Park BJ, Alexander BD, Anaissie EJ, Walsh TJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: Overview of the transplant-associated infection surveillance network (TRANSNET) database. *Clin Infect Dis* 2010;50:1091-100.
- Jenks JD, Hoenigl M. Treatment of aspergillosis. *J Fungi (Basel)* 2018;4: pii: E98.
- Lamoth F, Calandra T. Let's add invasive aspergillosis to the list of influenza complications. *Lancet Respir Med* 2018;6:733-5.
- Hoenigl M, Gangneux JP, Segal E, Alanio A, Chakrabarti A, Chen SC, et al. Global guidelines and initiatives from the European confederation of medical mycology to improve patient care and research worldwide: New leadership is about working together. *Mycoses* 2018;61:885-94.
- Gautam AK, Sharma S, Avasthi S, Bhaduria R. Diversity, pathogenicity and toxicology of *A. niger*: An important spoilage fungi. *Res J Microbiol* 2011;6:270-80.
- Ahmed M, Djebli N, Aissat S, Aggad H, Boucif A. Antifungal activity of a combination of Algeria honey and starch of ginger against *Aspergillus niger*. *Int J Microbiol Res* 2011;2:263-6.
- Abdul RQ, Naeem A, Al-Jabre S, AL-Akloby O, Mohammad AR. Anti-fungal activity of thymoquinone and amphotericin b against *Aspergillus niger*. *Sci J King Faisal Univ Basic Appl Sci* 2007;8:143-8.
- Shubhi A, Ajay KG, Rekha B. Antifungal activity of plant products against *Aspergillus niger*; a potential application in the control of a spoilage fungus. *Biol Forum Int J* 2010;2:53-5.
- Sun S, Lui Q, Han L, Ma Q, He S, Li X, et al. Identification and characterization of *Fusarium proliferatum*, a new species of fungi that cause fungal keratitis. *Sci Rep* 2018;8:4859.
- Prajapati S, Sharma M, Gupta P, Kumar M, Dwivedi B, Arya BS. Evaluation of antifungal activity of different homeopathic mother tinctures against *Candida albicans*. *Ind J Res Homeopathy* 2017;11:237-43.
- Burt S. Essential oils: Their antibacterial properties and potential applications in foods – A review. *Int J Food Microbiol* 2004;94:223-53.
- Gill AO, Holley RA. Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. *Int J Food Microbiol* 2006;108:1-9.
- Friedman M, Henika PR, Levin CE, Mandrell RE. Antibacterial activities of plant essential oils and their components against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juice. *J Agric Food Chem* 2004;52:6042-8.
- Tiwari BK, Valdramidis VP, O'Donnell CP, Muthukumarappan K, Bourke P, Cullen PJ, et al. Application of natural antimicrobials for food preservation. *J Agric Food Chem* 2009;57:5987-6000.
- Khanna KK, Chandra S. Effect of some homeopathic drugs on spore germination of isolates of *Alternaria alternata*. *J Indian Phytopathol* 1976;29:195-7.
- Dua VK, Atri DC. Control of tomato fruit rot caused by *Alternaria solani* with homeopathy drugs. *J Bot Soc* 2004;39:88-93.
- Shrivastava JN, Kushwaha RK. Effect of four homeopathic medicines and a biochemical compounds against dermatophytes. *Hahnemanian Gleanings* 1983;51:371-4.

### होम्योपैथिक दवाओं व उनकी विभिन्न पोटैन्सीयों का एस्पेरजिलस नाइगर पर 'इन विट्रो' में जैविक प्रभाव का आंकलन

**उद्देश्य :** मानव रोगजनक कवक एस्पेरजिलस नाइगर इन-विट्रो के प्रतिकूल होम्योपैथिक दवाओं के जैविक प्रभाव और उनकी विभिन्न पोटैन्सीयों का आंकलन

**सामग्री और विधियां :** एलियम सीपा, एलियम सैटाईवम, सिजिलपेनिया बॉन्डुसेला, कैलोट्रोपिस गिंगेशिया, कैसिया अंगुस्टिफोलिया, थूजा ऑक्सीडेन्टेलिस टर्मिलिया चेंबुला और जिंजीबर ओफिसिनेल होम्योपैथिक मदर टिक्चर और पोटेंसी (3x, 6x और 12x) के मामूली संशोधन के साथ नैदानिक और प्रयोगशाला मानक (एम 44-A) के दिशानिर्देशों के अनुसार आगार डिस्क प्रसार विधि का उपयोग करके एस्पेरजिलस नाइगर के विकास के प्रतिकूल उनकी जैविक गतिविधि के लिए मूल्यांकन किया गया। अवरोध के व्यास (मिमी) क्षेत्र को मापा गया और वाहन नियंत्रण से तुलना की गई तथा केटोकोनाजोल को संदर्भ मानक फगिसाइड के रूप में इस्तेमाल किया गया।

**परिणाम :** जिंजीबर ओफिसिनेल के मदर टिक्चर को  $15.4 \pm 2.88$  निषेध के अधिकतम क्षेत्र के साथ ए, नाइगर के प्रतिकूल सबसे अधिक प्रभावी पाया गया। इसके बाद होर्लहेना एंटीडाईसेंट्रिका ( $13.2 \pm 1.09$ ) और टर्मिनोलिया चेंबुला ( $10.6 \pm 1.14$ ) अवरोध का महत्वपूर्ण क्षेत्र एलियम सीपा 6x कैसलपिनिया बॉन्डुसेला 6x और 12x क्रमशः ( $12.8 \pm 0.54$  और  $10.4 \pm 1.14$ ) यकेलिप्टस ग्लोबुलस 12x ( $11.3 \pm 1.94$ ), रुटा विओलेस 12x ( $15.0 \pm 2.23$ ), थूजा ऑक्सीडेन्टेलिस 6x ( $10.8 \pm 0.83$ ) और जिंजीबर ओफिसिनेल 3x और 6x ( $13.02 \pm 2.73$ ,  $11.4 \pm 2.23$ ) नियंत्रण की तुलना में प्रदर्शित किया गया।

**निष्कर्ष :** इस अध्ययन के निष्कर्षों से यह पता चलता है कि होम्योपैथिक मदर टिक्चर और पोटेंसी प्रभावी रूप से एस्पेरजिलस नाइगर इन विट्रो के विकास को नियंत्रित कर सकती है। यह अध्ययन होम्योपैथिक फंगसविरोधी उपचारों के विकास का मार्ग प्रशस्त करता है। हालांकि, यंत्रवत दृष्टिकोण और उनकी क्रिया के तरीके के बारे में अधिक जानकारी प्राप्त करने के लिए आगे की जांच आवश्यक है।

### Évaluation de l'effet biologique des médicaments homéopathiques et de leurs différentes dilutions contre *Aspergillus niger* in vitro

**Objectif:** Évaluation de l'effet biologique des médicaments homéopathiques et de leurs différentes dilutions sur les champignons pathogènes humains *Aspergillus niger* in vitro.

**Matériaux et méthodes:** Les teintures mères homéopathiques (ø.) et dilutions (3x, 6x et 12x) d'*Allium cepa*, d'*Allium sativum*, de *Caesalpinia bonducella*, de *Calotropis gigantea*, de *Cassia angustifolia*, d'*Eucalyptus globulus*, de *Ficus religiosa*, d'*Holarrhena antidysenterica*, de *Kalmia latifolia*, d'*Ocimum sanctum*, de *Ruta graveolens*, de *Syzygium jambolanum*, de *Thuja occidentalis*, de *Terminalia chebula* et de *Zingiber officinale* ont été évaluées pour leur activité biologique contre la croissance d'*Aspergillus niger* en utilisant la méthode de diffusion sur disque d'agar conformément aux directives des normes cliniques et de laboratoire (M44-A) avec une légère modification. Les diamètres (mm) de la zone d'inhibition ont été mesurés et comparés au véhicule témoin et du Kétoconazole a été utilisé comme fongicide de référence.

**Résultats:** La teinture mère de *Zingiber officinale* s'est révélée être la plus puissante contre *A. niger* avec une zone d'inhibition maximale de  $15,4 \pm 2,88$  mm, suivie de *Holarrhena antidysenterica* ( $13,2 \pm 1,09$ ) et de *Terminalia chebula* ( $10,6 \pm 1,14$ ). L'*Allium cepa* 6x ( $10,4 \pm 0,89$ ), la *Caesalpinia bonducella* 6x et 12x ( $12,8 \pm 0,54$  et  $10,4 \pm 1,14$  respectivement), l'*Eucalyptus globulus* 12x ( $11,3 \pm 1,94$ ), le *Ruta graveolens* 12x ( $15,0 \pm 2,23$ ), le *Thuja occidentalis* 6x ( $10,8 \pm 0,83$ ) et le *Zingiber officinale* 3x et 6x ( $13,0 \pm 2,73$ ,  $11,4 \pm 2,30$ ) ont présenté une zone d'inhibition importante par rapport au témoin.

**Conclusion:** Les résultats de cette étude ont conclu que les teintures mères et les dilutions homéopathiques peuvent contrôler efficacement la croissance d'*Aspergillus niger* in vitro. Cette étude ouvre la voie au développement de traitements antifongiques homéopathiques. Cependant, des investigations complémentaires sont nécessaires pour obtenir plus d'informations sur l'approche mécanique et leur mode d'action.

### Evaluación del efecto biológico de los medicamentos homeopáticos y sus diferentes potencias frente al *Aspergillus niger* in vitro

**Objetivos:** Evaluación del efecto biológico de los fármacos homeopáticos y sus diferentes potencias frente al hongo patógeno humano *Aspergillus niger* in vitro.

**Materiales y métodos:** Las tinturas madre (Ø.) y las potencias homeopáticas (3x, 6x y 12x) de *Allium cepa*, *Allium sativum*, *Caesalpinia bonducella*, *Calotropis gigantea*, *Cassia angustifolia*, *Eucalyptus globulus*, *Ficus religiosa*, *Holarrhena antidysenterica*, *Kalmia latifolia*, *Ocimum sanctum*, *Ruta graveolens*, *Syzygium jambolanum*, *Thuja occidentalis*, *Terminalia chebula* y *Zingiber officinale* fueron evaluadas en cuanto a su actividad biológica contra el crecimiento del *Aspergillus niger* utilizando un método de difusión en disco de agar conforme a las directrices de los estándares clínicos y de laboratorio (M44-A) con leves modificaciones. Se midieron los diámetros (mm) de la zona de inhibición y se compararon con los controles de vehículo y con ketoconazol, que se utilizaron como fungicidas estándar de referencia.

**Resultados:** Se observó que la tintura madre de *Zingiber officinale* es la más potente contra *A. niger* con una zona de inhibición máxima de hasta 15,4±2,88 mm seguida de *Holarrhena antidysenterica* (13,2±1,09) y *Terminalia chebula* (10,6±1,14). Se observó una zona de inhibición significativa con *Allium cepa* 6x (10,4±0,89), *Caesalpinia bonducella* 6x y 12x (12,8±0,54 y 10,4±1,14 respectivamente), *Eucalyptus globulus* 12x (11,3±1,94), *Ruta graveolens* 12x (15,0±2,23), *Thuja occidentalis* 6x (10,8±0,83) y *Zingiber officinale* 3x y 6x (13,0±2,73, 11,4±2,30), en comparación con el control.

**Conclusiones:** Los hallazgos de este estudio muestran que las tinturas madre y las potencias homeopáticas puede controlar eficazmente el crecimiento de *Aspergillus niger* in vitro. Este estudio abre el camino al desarrollo de tratamientos homeopáticos antimicóticos. Sin embargo, se requieren más investigaciones para obtener más información sobre los métodos mecanicistas y su modo de acción.

### Bewertung der biologischen Wirkung homöopathischer Arzneimittel und ihrer verschiedenen Potenzen gegen *Aspergillus niger* in vitro

**Ziel:** Bewertung der biologischen Wirkung homöopathischer Arzneimittel und ihrer unterschiedlichen Potenzen gegen die humanpathogenen Pilze *Aspergillus niger* in vitro.

**Materialien und Methoden:** Homöopathische Urtinkturen (Ø.) Und Potenzen (3x, 6x und 12x) von *Allium cepa*, *Allium sativum*, *Caesalpinia bonducella*, *Calotropis gigantea*, *Cassia angustifolia*, *Eucalyptus globulus*, *Ficus religiosa*, *Holarrhena antidysenterica*, *Kalmia latifolia*, *Holarrhena*, *Syzygium jambolanum*, *Thuja occidentalis*, *Terminalia chebula* und *Zingiber officinale* wurden auf ihre biologische Aktivität gegen das Wachstum von *Aspergillus niger* unter Verwendung der Agarscheibendiffusionsmethode gemäß den Richtlinien des klinischen und Laborstandards (M44-A) mit geringfügiger Modifikation untersucht. Die Durchmesser (mm) der Hemmzone wurden gemessen und mit der Vehikelkontrolle verglichen, und Ketoconazol wurde als Referenzstandard-Fungizid verwendet.

**Ergebnisse:** Die Urtinktur von *Zingiber officinale* erwies sich als am wirksamsten gegen *A. niger* mit einer maximalen Hemmzone von bis zu 15,4 ± 2,88 mm, gefolgt von *Holarrhena antidysenterica* (13,2 ± 1,09) und *Terminalia chebula* (10,6 ± 1,14). Eine signifikante Hemmzone zeigten *Allium cepa* 6x (10,4 ± 0,89), *Caesalpinia bonducella* 6x und 12x (12,8 ± 0,54 bzw. 10,4 ± 1,14), *Eucalyptus globulus* 12x (11,3 ± 1,94), *Ruta graveolens* 12x (15,0 ± 2,23), *Thuja occidentalis* 6x (10,8 ± 0,83) und *Zingiber officinale* 3x und 6x (13,0 ± 2,73, 11,4 ± 2,30) im Vergleich zur Kontrolle.

**Schlussfolgerung:** Die Ergebnisse dieser Studie kommen zu dem Schluss, dass homöopathische Urtinkturen und Potenzen das Wachstum von *Aspergillus niger* in vitro wirksam kontrollieren können. Diese Studie ebnet den Weg für die Entwicklung homöopathischer Antimykotika. Weitere Untersuchungen sind jedoch erforderlich, um mehr Informationen über den mechanistischen Ansatz und ihre Wirkungsweise zu erhalten.



### 順勢療法藥物的生物學效應及其不同層級對體外黑麴黴的評價

**目的：**順勢療法藥物的生物學效應及其不同層級對人體致病真菌黑麴黴的評價。

**用料和方法：**根據臨床和實驗室標準（M44-A）的指導方針，使用瓊脂圓盤擴散法評估紅洋蔥、大蒜、刺果蘇木、牛角瓜、亞歷山大決明、藍桉樹、無花果、止瀉木、美國山桂、聖羅勒、芸香、海南蒲桃、側柏、訶子以及生薑的順勢療法母酊（ $\emptyset$ ）和層級（3x、6x、12x）對黑麴黴生長的生物活性，並對其進行了輕微修改。測量抑制區的直徑（mm），並與載體對照比較，並將酮康唑用作參考標準殺真菌劑。

**結果：**發現生薑的母酊對黑麴黴最有效，最大抑制區可達 $15.4 \pm 2.88$ mm，其次是止瀉木（ $13.2 \pm 1.09$ ）和訶子（ $10.6 \pm 1.14$ ）。紅洋蔥 6x（ $10.4 \pm 0.89$ ），刺果蘇木 6x和12x（分別為 $12.8 \pm 0.54$ 和 $10.4 \pm 1.14$ ），藍桉樹 12x（ $11.3 \pm 1.94$ ），芸香 12x（ $15.0 \pm 2.23$ ）表現出顯著的抑制區，與對照相比，側柏6x（ $10.8 \pm 0.83$ ）和生薑3x和6x（ $13.0 \pm 2.73, 11.4 \pm 2.30$ ）。

**結論：**本研究結果表明，順勢療法母體酊劑和經加能後的藥物可有效控制體外黑麴黴的生長。該研究為順勢療法抗真菌治療的發展鋪平了道路。但是，需要進一步調查以獲得有關機理及其作用方式的更多資料。