Original Article

Inhibitory effect of garlic and omam extracts on *Mucor circinelloides,* a fungus causing mucormycosis: An *in vitro* study

Nimoshini G[1], Priyadharsini Nataraj[1], Annasamy Rameshkumar[1], Rajkumar Krishnan[1], S. Savithri[2]

Departments of [1]Oral Pathology and Microbiology, [2]Microbiology, SRM Dental College, Ramapuram, Chennai, Tamil Nadu, India

Abstract

Context: *Mucor circinelloides* is reported to be the second among the most common causative agents of emerging mucormycosis. It is intrinsically resistant to most known antifungals. Further the use of antifungals cause side effects. Traditional knowledge system for treating various ailments is stronger in India and it also backs deriving various bioactive compounds from herbal sources, in the modern system of medicine. Therefore, two most commonly used culinary herbal materials viz., ginger and omam were studied *in vitro* against *M. circinelloides*, as an alternate to antifungal drugs.

Aims: To explore the traditional herbal resources as alternate to Amphotericin B to use against *M. circinelloides*, a fungus causing mucormycosis.

Methods and Material: Aqueous extracts of garlic and omam were prepared and tested against *M. circinelloides*, at different concentrations. A positive control with Amphotericin B and negative control without any supplements were also maintained. The inhibitory effect was assessed by adopting optical density (OD) measurement method in SD broth and SD Agar Well Plate using spore suspension as inoculum.

Statistical Analysis Used: Paired student *T* test was employed using SPSS Version 1.6.

Results and Conclusions: Both garlic and omam extracts were found to inhibit the *M. circinelloides* and their MICs were 600 and 700 µL/mL. It is comparable with the MIC of Amphotericin B, 200 µg/mL. Thus, the regular use of garlic and omam can reduce the risk of possible mucormycosis and these herbs can be explored for drug formulations against *M. circinelloides.*

Keywords: Amphotericin B, garlic extract, *Mucor circinelloides*, mucormycosis, omam extract

Address for correspondence: Dr. Priyadharsini Nataraj, Department of Oral Pathology, SRM Dental College, Bharathi Salai, Ramapuram, Chennai - 600 089, Tamil Nadu, India.
E-mail: drdharsiniraj@gmail.com

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INTRODUCTION

Fungi are eukaryotic heterotrophic microorganisms. There are 144,000 species of fungi named and classified so far. Among them some are known to cause diseases on organisms as pathogens. Fungal diseases to the animals are collectively termed as mycoses but it varies based on the site of the infection, route of acquisition of the pathogen, and type of virulence exhibited by the fungus.

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Mucormycosis, also known as Zygomycosis, in humans is an opportunistic mycosis mainly caused by the genus Mucor of the order Mucorales under the division Zygomycota.[1-3] There are about 50 species in the genus Mucor, but a few are virulent.[3,4] *Mucor circinelloides* is one among them with dimorphic features.[5] According to the recent report on all mucormycosis prevalence in India is 0.14 cases per 1000 population with the prevalence range between 208,177 and 137,807 cases (mean: 171504; SD: 12 365.6; 95% CI: 195,777–147,688) and a mean of 65,500 (38.2%) attributable deaths per annum.[6]

In the orofacial region, though many fungi are reported to cause mycosis with the symptom that ranges from oral thrush to fatal systemic super-infections, depending upon the health condition of the patents. The treatment of Mucormycosis is so far successful with the use of antifungals. Alongside, there are also challenges emerging due to resistance to innate host defenses, extensive angio-invasion, increased virulence; variability of susceptibility to Amphotericin B and the side effects due to the use of antifungal agents.[7]

M. circinelloides is reported to be the second among the most common causative agents of emerging mucormycosis. It is intrinsically resistant to most known antifungals as there are several mutants with alteration of gene sequences.[8] Hence, improved/alternate remedial measures are of urgent needs for overcoming these challenges.

Traditional knowledge system for treating various ailments is stronger in India and it also backs deriving various bioactive compounds from herbal sources, in the modern system of medicine. Under this background, two most commonly used culinary herbal materials viz., ginger and omam were studied *in vitro* against *M. circinelloides*, a fungus causing mycosis.

MATERIALS AND METHODS

Fungal culture, *M. circinelloides* Strain TAB 011, was obtained from the Department of Botany, Tagore Government Arts and Science College, Puducherry in slant and subcultured in SDA plates [Figure 1]. After the complete colonization, the plate was flooded sterile water with a drop of tween-20 to prepare the spore suspension, the inoculum. The spore density in the inoculum was determined by counting under microscope. Herbal materials viz. garlic cloves (*Allium sativum* L.) and omam seeds (*Trachyspermum ammi* L.) were procured from market. Peeled and washed cloves of Indian, Maharastra garlic weighing 250 gm was ground with 50 mL sterile distilled water (SDW) and filtered with Whatmann

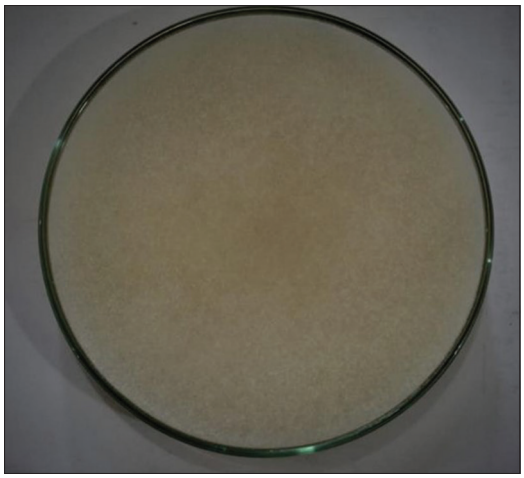

Figure 1: Pure culture of *M. circinelloides* on SDA plate

No. 1 filter paper. The filtrate was stored at 4°C. Similarly, omam seeds weighing 100 gm 25 gm was powdered, added 100 mL of SDW and kept for 24 h. Then filtered with Whatmann No. 1 filter paper and the filtrate was stored at 4°C. Amphotericin B was also procured and diluted with water to give the required concentrations.

In culture tubes containing SD broth (10 mL), the extracts of garlic and omam were incorporated separately at 100, 200, 300, 400, 500, 600, 700, and 800 µL concentrations, inoculum was added and incubated for a day. A positive control with similar concentrations of Amphotericin B and a negative control without any compound, but with different concentrations of spore suspension were also maintained in triplicates. After incubation the growth was determined by OD measurement method.[9] The significant variations in absorbance of the broth between 0 and 24 h after inoculation of the fungal spores is considered as the growth of the fungus in control and treatments (garlic and omam extracts). Agar Well Diffusion Assay[10] was performed to ascertain the activity at MICs as determined by OD measurement method, using the extracts and Amphotericin B. The study has been presented and approved by ethical committee and below is approval number SRMDC/IRB/2018/MDS/No. 605.

Statistical analysis

The variables derived in the study are expressed as mean ± standard deviation. Significance of the difference between the OD values of the culture of 0 and 24 h are found using paired student *T* test and the *P* value <0.05 is considered as significant. The analysis was done using the Statistical Package for the Social Sciences (SPSS) version 16.

RESULTS

M. circinelloides, the test fungus exhibited growth SD broth

without any supplements (negative control) and it was a significant ($P < 0.0001$) in all the concentrations of the inoculum (100–800 µL) tested [Figure 2].

In Amphotericin B (positive control), the growth could be noticed only at 100 µg and it was also statistically significant ($P < 0.001$), among the other concentrations tested (200–800 µg) [Table 1]. The growth becomes insignificant from 200 µg and thus 200 µg/mL is regarded as minimum inhibitory concentration (MIC) against *M. circinelloides*.

In aqueous extract of garlic, the fungus showed growth up to 500 µL/mL concentrations ($P < 0.001$) and failed in the further concentrations of 600–800 µL/mL. Thus the MIC of aqueous extract of garlic against *M. circinelloides* is considered as 600 µL/mL. Similarly, in the aqueous extract of Omam, the fungus showed significant ($P < 0.001$) growth up to 600 µL/mL and failed in further concentrations (700 and 800 µL/mL) tested. Accordingly, the MIC of Omam extract against *M. circinelloides* is determined as 700 µL/mL [(Table 1].

Agar Well Diffusion Assay, the activity was measured as 4.3, 4.0, and 4.4 cm diameter of inhibition zone at the MICs of 600 and 700 µg/mL of garlic and omam extracts, and 200 µg/mL of Amphotericin B, respectively [Figures 3-6].

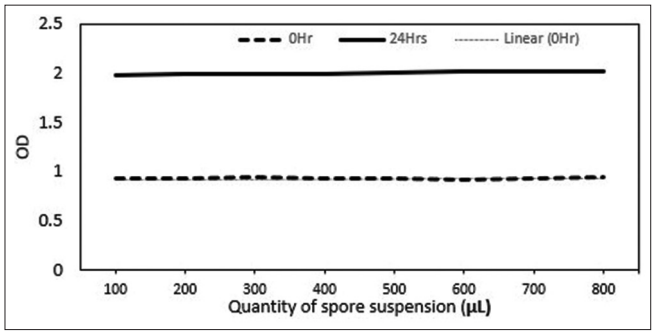

Figure 2: Mean OD values (550 nm) of the fungal culture in SD broth of negative control at 0 and 24 h

Analysis of variance formula (ANOVA) also revealed significant differences within and between the treatments (garlic and omam) and control ($P < 0.001$) at 800 µL concentration. ANOVA results showed the sum of squares value between groups as 3.71 and the mean square value as 1.24; and within the groups, the sum of the value of the square as 0.20 and the mean square value as 0.01 ($P < 0.001$).

Post hoc comparison of the analysis showed that there was a significant mean difference between all combinations of groups [Table 2]. Further, from this, the order of inhibitory effect could be identified as: garlic extract greater than omam extract.

DISCUSSION

Amphotericin B deoxycholate (AmB) was the only remedy for the treatment of mucormycosis for decades. But there is a limitation on the use of the drug because of its nephrotoxic effect. Thus, lipid formulations of AmB are in the use nowadays. These formulations are reported to have a comparatively less nephrotoxic effect and thus comparatively higher doses are administered for a longer period than AmB.[11,12] However, for a no side effect remedy, explorations of traditional plant resources are essential. The present study on the garlic and omam have given possible clues on the use as alternate remedies with no side effects.

Garlic and omam, the common culinary herbs, contain a wide array of compounds and various kinds of biological activity and hence they are being explored for the treatments of various ailments nowadays.[13,14] Antimicrobial activities of garlic[15] and omam[16-18] are also documented against various human pathogen, In this line the present study finds them as against *M. circinelloides* and comparable with the activity of Amphotericin B. Further, it is observed that though MICs of both the extracts are differing against the pathogen, both are promising and it may be due to the

Table 1: Comparison of OD values of culture broths between 0 and 24 h of incubation in the treatments with different concentrations of Amphotericin B, Garlic extract and omam extract

Quantity of Extracts (µL)/ Amphotericin B (µg)	Optical Density Value (Mean±SD)								
	In Amphotericin B			In Garlic Extract			In Omam Extract		
	0 h	24 h	*P*	0 h	24 h	*P*	0 h	24 h	*P*
100	0.95 (0.03)	1.58 (0.03)	<0.001	0.90 (0.06)	1.93 (0.09)	<0.001	1.03 (0.15)	1.90 (0.12)	<0.001
200	0.98 (0.02)	1.00 (0.03)	0.007	0.98 (0.02)	1.89 (0.32)	<0.001	0.99 (0.13)	1.89 (0.13)	<0.001
300	1.02 (0.02)	1.04 (0.04)	0.061	1.03 (0.02)	1.72 (0.03)	<0.001	0.96 (0.11)	1.88 (0.11)	<0.001
400	1.06 (0.11)	1.09 (0.11)	0.238	1.08 (0.10)	1.63 (0.09)	<0.001	0.97 (0.12)	1.86 (0.17)	<0.001
500	1.11 (0.10)	1.11 (0.09)	1.000	1.13 (0.11)	1.35 (0.04)	<0.001	0.98 (0.13)	1.82 (0.18)	<0.001
600	1.14 (0.13)	1.14 (0.19)	1.000	1.17 (0.08)	1.19 (0.12)	0.697	1.00 (0.14)	1.91 (0.07)	<0.001
700	1.18 (0.10)	1.18 (0.12)	1.000	1.26 (0.03)	1.26 (0.05)	0.866	1.14 (0.17)	1.17 (0.16)	0.053
800	1.21 (0.09)	1.21 (0.10)	1.000	1.32 (0.05)	1.32 (0.09)	1.000	1.17 (0.13)	1.26 (0.12)	0.059

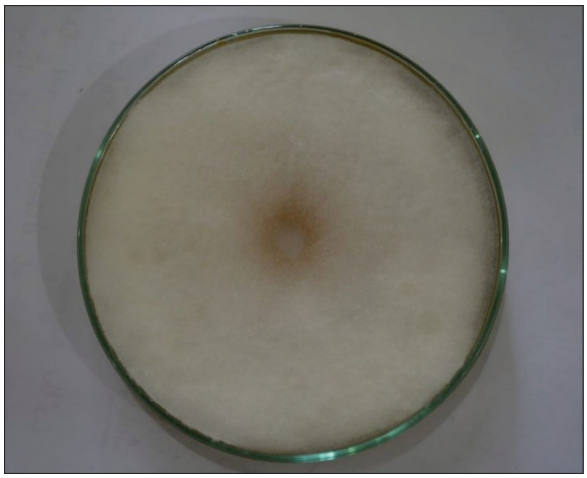

Figure 3: Growth of *M. circinelloides* on SD Agar Well – diffusion plate (negative control)

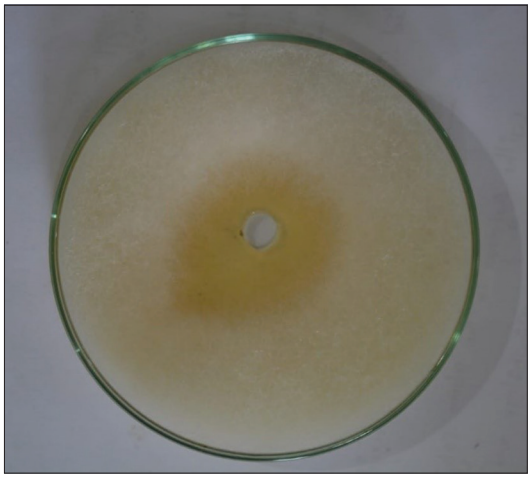

Figure 5: SD Agar Well – diffusion assay plate showing the inhibition of *M. circinelloides* by omam extract

Table 2: Comparison of optical density between negative, positive control, garlic and omam extract containing culture broth with 800 µL concentration (post hoc test)

Group (A)	Group (B)	Mean difference	*P*
Negative control	Positive control	0.79	<0.001
	Garlic extract	0.68	<0.001
	Omam extract	0.58	<0.001
Positive control	Negative control	−0.79	<0.001
	Garlic extract	−0.11	0.016
	Omam extract	−0.20	<0.001
Garlic extract	Negative control	−0.68	<0.001
	Positive control	0.11	0.016
	Omam extract	−0.10	0.041
Omam extract	Negative control	−0.58	<0.001
	Positive control	0.20	<0.001
	Garlic extract	0.10	0.041

different constituents present in them. Thus, the present study suggests that the regular use of garlic and omam can reduce the risk of mucormycosis and these herbs can be considered for drug formulations against *M. circinelloides*, a fungus stands as a major threat to human life.

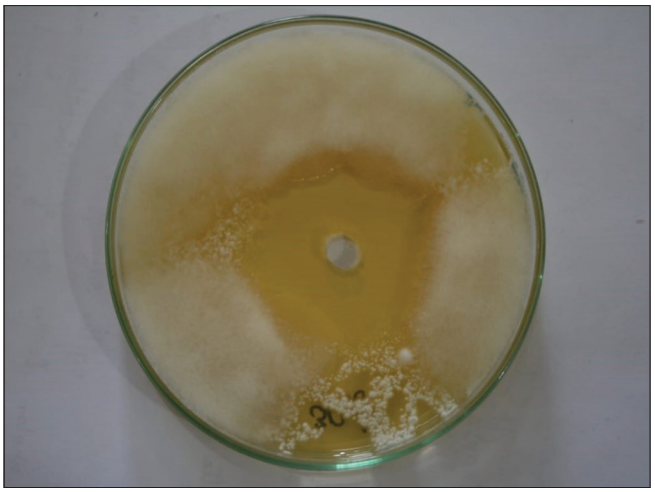

Figure 4: SD Agar Well – diffusion assay plate showing the inhibition of *M. circinelloides* by garlic extract

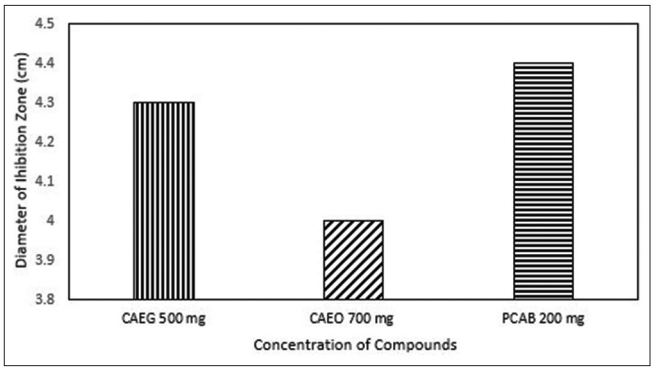

Figure 6: Diameter of inhibition zone as caused by 500, 700, and 200 mg of crude aqueous extract of garlic (CAEG), crude aqueous extract of omam (CAEO) and positive control of Amphotericin B (PCAB), respectively

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Nil.

Conflicts of interest

There are no conflicts of interest.

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