

**The *in vivo* effect of Methyl Chavicol, on the *Candida albicans* Germ Tube Induction**¹Sani. Z.R., ²Jabbi, A.M., and ²Abdullahi, R.T.¹Department of Biological Sciences, Faculty of Science, Federal University Gusau, Zamfara State.²Department of Biological Sciences, Faculty of Science, Nigeria Police Academy, Wudil Kano State.Corresponding author: rszurme@gmail.com

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Abstract

Yeast (Candida albicans) is the term for the fungus that exists as a single celled organism rather than as hyphae. In the present study Methyl chavicol a major constituent of Holy Basil (Ocimum sanctum) essential oil has been evaluated against the induction of germ tubes in Candida albicans. Ocimum Sanctum leaves and seeds were used for extraction of basil oil through steam distillation process. Candida cells were also grown in yeast extract, peptone and dextrose (1:2:2) medium. Primary culture (0.5%) of Candida albicans was mixed with 25ml of Lee's modified media which was grown for 72 h at 25°C and was centrifuged at 5000 rpm for 5 min. The cells obtained in the form of pellet were washed with distilled water and then reinoculated into fresh 50ml LMM and the test compound was added. pH of the solution was adjusted to 6.5 at 37°C by adding 0.1 N NaOH solution. Then slides were prepared after every 90 min interval for 24 h and observed under inverted microscope. After 90 min in all the three sets, germ tube induction begins but the length of the germ tube greatly differs. In the control the maximum length observed is 38.3 μm but in MC1 and MC2 it is 9.4 μm and 5.7 μm, respectively. This clearly shows the inhibitory effects of methyl chavicol on germ tube induction. Similar results of decreasing length of germ tubes in MC2 at different time periods, proved that MC2 i.e. 70 μg/mL methyl chavicol is a potent inhibitor of germ tubes induction.

Keywords: *Candida albicans; Germ Tube; Inhibition; Methyl Chavicol; Ocimum sanctum.***1.0 INTRODUCTION**

Yeast (*Candida albicans*) is the term for the fungus that exists as a single celled organism rather than as hyphae. There are about 500 species of yeast or yeast-like organisms. *Candida* is a dimorphic fungus i.e. it can take two forms. Most of the time it exists as oval, single yeast cells, which reproduce by

budding. Normal room temperature favours the yeast form (blastocnoidal) of the organism, but under physiological conditions (temperature, pH and the presence of serum) it may develop into hyphal form (Gupta and Thomas, 2003). Pseudohyphal, composed of chains of cells, are also common.

Candida frequently encountered as a harmless commensal of digestive system and vaginal tract and less frequently as opportunistic pathogen. Mycelial form of *Candida albicans* is considered more invasive, difficult to phagocytosed and therefore more pathogenic. In addition to its dimorphism, *C. albicans* possesses well organized phenotypic switching system that probably helps the pathogen to invade the host immune defense (D'Souza and Heitman, 2001). It is responsible for Candidiasis, a disease that can vary from superficial mucosal to life threatening systemic disorders. The frequency of both superficial and invasive candidiasis has increased markedly over the last few decades. Patients that become severely immune-compromised because of underlying disease such as leukemia, acquired immunodeficiency syndrome or patients who undergo cancer therapy, organ transplant are particularly susceptible to opportunistic fungal infections. A number of antifungal agents are available for the treatment of candidal infection (Gupta and Thomas, 2003; Carrillo-Munoz *et al.*, 2006). The major agents belong either to Polyenes, such as Amphotericin B and Nystatin or to the azoles, such as Itraconazole and Fluconazole. Currently, use of standard antifungal therapies can be limited because of toxicity, low efficacy rates, and drug resistance. Recent studies have indicated *Candida* resistance to azoles or hepatotoxicity and nephrotoxicity linked to Polyenes use, particularly Amphotericin B. In order to avoid these drawbacks, a search for new and effective product is needed for the treatment of this fungal infection. Various new antifungals have demonstrated therapeutic potential. This new agents may provide additional options for the treatment of superficial fungal infections and they may help to overcome the limitations of current treatments.

Herbs have now long been recognized for their medicinal values. The holy basil (*Ocimum sanctum*) is native to Indian and is commonly known as Tulsi. It has been now widely grown all over the globe. In India, Tulsi (meaning 'Incomparable One') is cultivated almost everywhere (Dixon *et al.*, 1996). The herb is bitter and pungent there are many varieties of basil, found all over the world. White and

black basil are the most commonly found varieties. The black Tulsi is the best as regards its properties. Basil acts as an appetizer & help digestion, it is beneficial to heart, suppresses excessive bile, cough and flatulence remove blood impurities. All the parts of the plants i.e seeds, leaves, shoots, flowers, stems and even the roots have medicinal value and are helpful in eradicating diseases. It belongs to the family Labiatae and is known as Holy Basil in English, which constituents are chiefly fenchol, methyl chavicol, linalool, eugenol and β -caryophyllene, responsible for its antibacterial, anti-septic and anti-fungal action. Inhibitory effects of aqueous and alcoholic extracts of Tulsi have been observed on enteric pathogens by Agar diffusion method (Geeta *et al.*, 2001). Tulsi is also known to have numerous pharmacological activities, such as antidiabetic, antistress, immunomodulatory, analgesic, antipyretic, anti inflammatory, antiulcerogenic, antihypertensive, CNS depressant, hepatoprotective, radioprotective, antitumor and antimicrobial activities (Archana and Namasivayam, 2000).

Basil oil has a clear color, thin consistency and a sweet, herbaceous, licorice like, slightly camphorous. Linalool, Fenchol, Eugenol, Methyl chavicol and Beta-caryophyllene are the active components present in basil oil. It has been used over the century as medicated oils and ointments for respiratory infections especially for children. It is used in flavouring, cosmetics, soap, pharmaceuticals and perfumery. Candidiasis is mostly an endogenous infection, arising from overgrowth of the fungus inhabiting in the normal flora. However, it may occasionally be acquired from exogenous sources (such as catheters or prosthetic devices) (Band *et al.*, 1979) or by person-to-person transmission (such as oral candidiasis in neonates of mothers with vaginal candidiasis or endophthalmitis following corneal transplantation from an infected donor) (Behrens *et al.*, 1991).

In time, a weakened immune system may relinquish control, giving *Candida* an opportunity to proliferate and transform into a harmful fungus, leading to candidiasis which can severely harm one's overall health status. In several cases for instance, when candidiasis is present, it can break down the mucosal lining between the bloodstream and the

allergens into the bloodstream. Some seventy-nine (79) different toxic substances have been identified in the blood. Once in the bloodstream these toxins are free to travel to

all parts of your body, where they can produce a host of adverse symptoms and further weaken your immune system.

1.1 Objectives of the study

1. To grow and maintain the culture of *Candida albicans* ATCC 90028.

2. To study the effect of different concentrations of Methyl Chavicol on Germ Tube Induction in *Candida albicans* ATCC 90028.

2.0 METHODOLOGY

2.1 Extraction Process of the essential oil:

Ocimum Sanctum leaves and seeds were used for extraction of basil oil through steam distillation process. The process is summarized below precisely:

1. Fresh or dried botanicals of Tulsi were placed in the retort or still.
2. Pressurized steam was introduced into a lower chamber of the retort and passed through the botanical ingredients to vaporize the volatile oils in the plant material.
3. The steam and oil vapor mixture was then passed through a condenser.
4. The essential oil was then extracted from the floral water or hydrolat in the separator.

Growth and Maintenance

Candida cells were grown in yeast extract, peptone and dextrose (1:2:2) medium.

2.2 Preparation of Yeast Stock Culture

- Yeast culture (YEPD) medium was prepared using yeast extracts, peptone and dextrose in the ratio of 1:2:2.
- Agar (Nutrient agar 28g/L) was also added to the YEPD medium to prepare solid medium.
- YEPD Agar plate was streaked with *Candida albicans* ATCC 90028 and streaking was done in the laminar flow to prevent dust and microbe contamination. The plate was then incubated at 37°C for 24 h.
- The plate was covered with aluminium foil and was kept inverted in the refrigerator.

The culture was considered as stock culture.

2.3 Preparation of Primary Culture

- A loop full of *C. albicans* ATCC 90028 from the YEPD agar plate was introduced into 25 ml of autoclaved medium. This procedure was done in laminar flow.
- The medium was kept in incubated shaker at 150rpm for 6 h, till the culture reaches the stationary phase.

2.4 Effect on Germ Tube Induction

The following procedure was employed.

- Primary culture (0.5%) of *Candida albicans* was mixed with 25ml of Lee's modified media.
- This was grown for 72 h at 25°C.
- This mixture was transferred to falcon tubes and was centrifuged at 5000 rpm for 5 min.
- The cells obtained in the form of pellet were washed with distilled water.
- The pellets were reinoculated into fresh 50ml LMM and the test compound was added.
- pH of the solution was adjusted to 6.5 at 37°C by adding 0.1 N NaOH solution. The slides observed at this stage are called as of stage zero.
- Then slides were prepared after every 90 min interval for 24 h and observed under inverted microscope.

3.0 RESULTS AND DISCUSSION

Currently, the use of standard antifungal therapies can be limited because of toxicity, low efficacy rates, and drug resistance. Recent

studies have indicated *Candida* resistance to azoles, or hepatotoxicity and nephrotoxicity linked to polyene use (Carrillo and Giusiano, 2006). In order to avoid these drawbacks,

particularly the spread of multidrug-resistant strains of fungus and the reduced number of drugs available, it is necessary to discover new classes of antifungals and compounds that inhibit these resistance mechanisms. This study aims towards discovering novel

3.1 Yeast to Hyphae Transition:

Candida albicans is a diploid asexual and dimorphic fungus and depending upon environmental conditions can exist as unicellular yeast (blastospores and chlamydo spores) as well as in different filamentous forms (hypha, pseudo-hyphae) and this filamentous form is pathogenic. The hyphae penetrate the host cell membrane and causes infection. Lee's modified media containing proline as the main inducer of germ tube has been used in the current study, the

antifungal drugs of natural origin. In case of the treated *Candida* cells it was very clearly observed from the pictures that the increase in number of cells and the induction to hyphae was lesser than the control where no treatment with the compound was given.

controlling factor of the induction is pH=6.5 for this type of media. In case of control we observed that with increase in the incubation time, the number of *Candida* cells as well as its transition to hyphae increased. Also it was clear with the pictures, Fig.2 taken after every 90 minutes incubation that the length of the hyphae increased. Figures 1-16 reported yeast to mycelium transition under the influence of sub-inhibitory concentrations of methyl chavicol.

90 MINUTES EXPOSURE

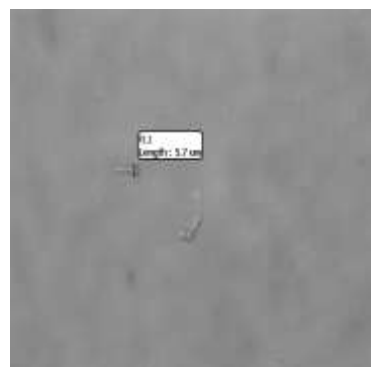
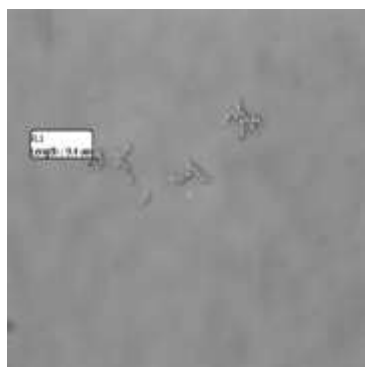
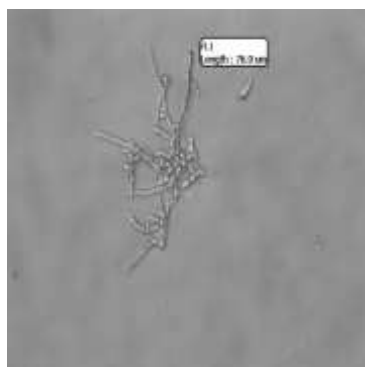


Figure 1. (plate1) CONTROL. Figure 2. (plate2) MC1. Figure 3. (plate3) MC2.

180 MINUTES EXPOSURE

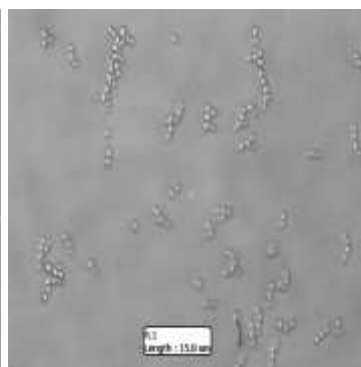
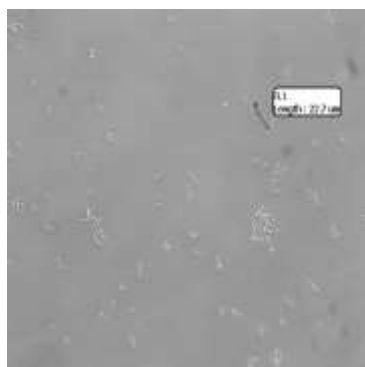
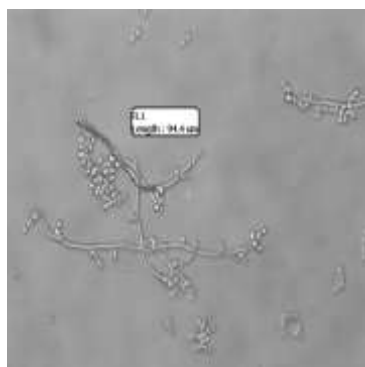


Figure 4. (plate4) CONTROL. Figure 5. (plate 5) MC1. Figure. (plate 6) MC2.

270 MINUTES EXPOSURE

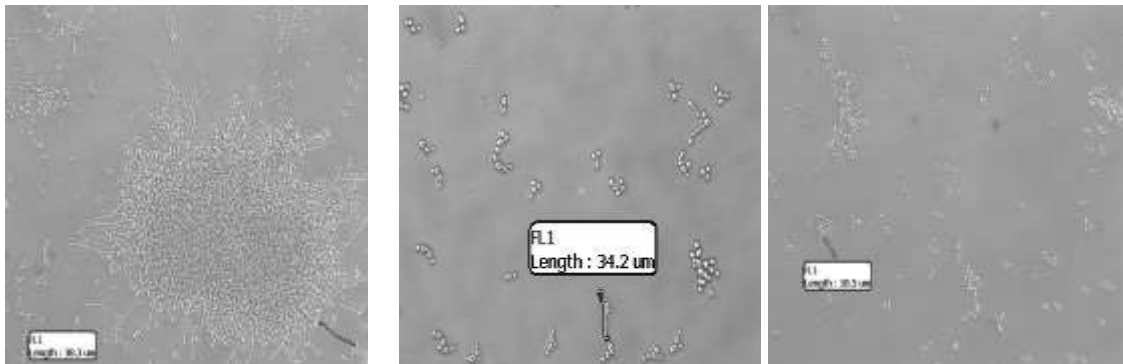


Figure 7. (plate 7) CONTROL. Figure 8. (plate 8) MC1. Figure 9. (plate 9) MC2.

360 MINUTES EXPOSURE

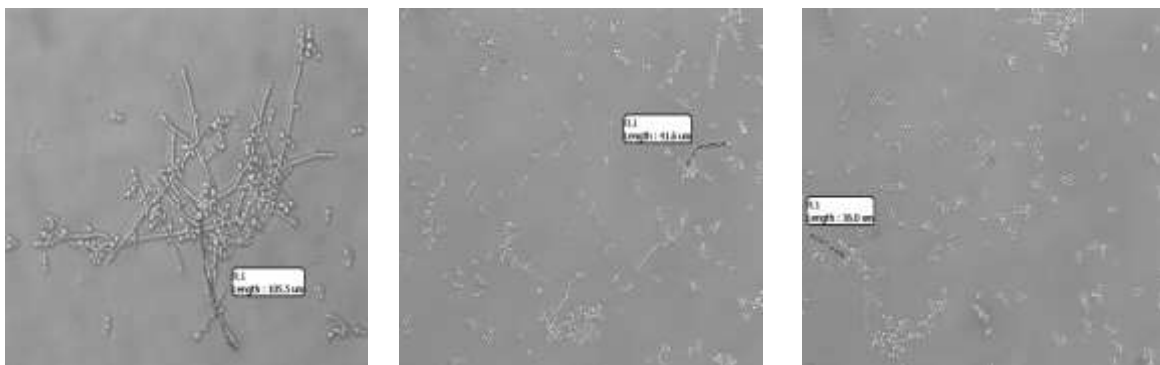


Figure 8. (plate 8) CONTROL. Figure 9. (plate 9) MC1. Fig 10. (plate 10) MC2.

450 MINUTES EXPOSURE

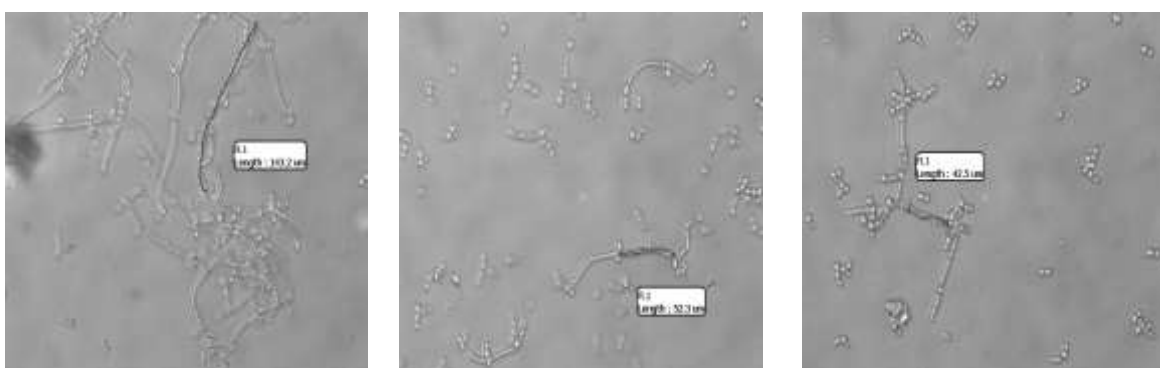


Figure 11. (plate 11) CONTROL. Figure 12. (plate 12) MC1. Figure 13. (plate 13) MC2.

24 HOURS EXPOSURE

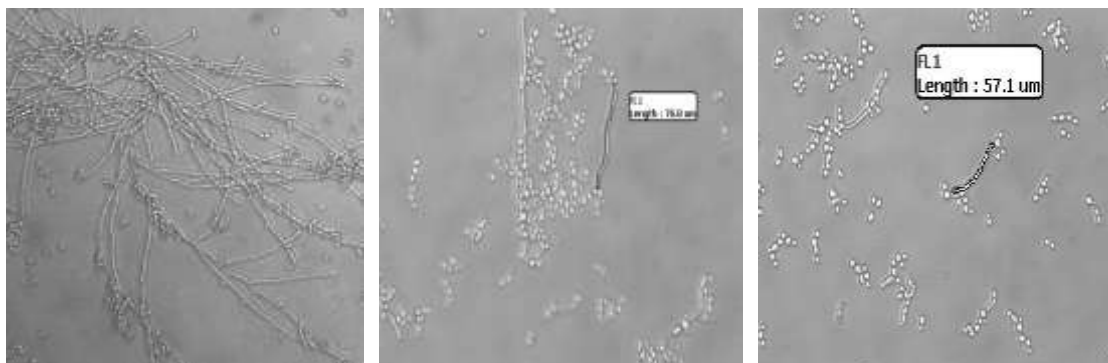


Figure 14. (plate 14) CONTROL. Figure 15(plate 15) MC1. Figure 16. (plate 16) MC2.

Table 1. Length of Hyphae Observed in Transition from yeast forms in *C. albicans* in presence of methyl chavicol at two different concentrations.

Exposure Time	CONTROL (μm)	Methyl chavicol- 35 $\mu\text{g}/\text{mL}$ (in μm) as MC1	Methyl chavicol- 70 $\mu\text{g}/\text{mL}$ (in μm)- as MC2
90 Minutes	38.3	9.4	5.7
180 Minutes	76.9	22.7	15.8
270 Minutes	94.4	34.2	18.5
360 Minutes	105.5	41.6	36
450 Minutes	143.2	52.3	42.5
24 Hours	204.3	76.8	57.1

After 90 min in all the three sets, germ tube induction begins but the length of the germ tube greatly differs. In the control the maximum length observed is 38.3 μm but in MC1 and MC2 it is 9.4 μm and 5.7 μm respectively. This clearly shows the inhibitory effects of methyl chavicol on germ tube induction. After 180 min, the germ tubes in the control grew up to 76.9 μm , but when exposed to MC1 the maximum length was found to be 22.7 μm which further decreased to 15.8 μm in

MC2. Similar results of decreasing length of germ tubes in MC2 at different time periods, proved that MC2 i.e. 70 $\mu\text{g}/\text{mL}$ methyl chavicol is a potent inhibitor of germ tubes. Though there was germ tube induction in MC2 but the percentage of germ tube induction and the length of germ tubes were much lower than of the control. After 24 hours, the germ tube length measured in MC2 was 57.1 μm which was negligible before 204.3 μm as measured in the control.

4.0 CONCLUSION

The parameters above, studied to evaluate the effect of methyl chavicol on germ tube induction show inhibitory activity of Methyl Chavicol, even at sub inhibitory concentrations in *Candida albicans*. Studies of host cell adherence and other pathogenicity markers including the production of

phospholipases and proteinase will also facilitate the possibility of methyl chavicol to be explored as an antifungal. Further animal based inhibitory activity of this compound will resolve the *in vivo* efficacy of Methyl Chavicol.

5.0 REFERENCES

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