

**CHECKERBOARD ASSAY TO ASSESS SYNERGISTIC ACTION OF FLUCONAZOLE AND ESSENTIAL OILS OF AJWAIN AND CORIANDER AGAINST *CANDIDA SPP.*****YOOSUF, S\*<sup>1</sup>, KAMAT, S.D.<sup>2</sup> AND KAMAT, D. V.<sup>2</sup>**<sup>1</sup>Department of Biotechnology, SIES College of Arts, Science and Commerce, Sion (W), Mumbai-400022, India<sup>2</sup> Department of Microbiology, SVKM's Mithibai College of Arts, Chauhan Institute of Science, & Amrutben Jivanlal College of Commerce & Economics, Vile Parle (West), Mumbai 400056, India**ABSTRACT**

*Candida* a commensal fungus, represents one of the major causes of mucosal and systemic infection. They show remarkable resistance to various first-line and second-line antifungal medications, namely, Fluconazole and Echinocandins. Checkerboard Assay was used to establish synergistic action of Fluconazole and essential oil of *Coriandrum sativum* and Fluconazole and essential oil of *Trachyspermum ammi* against *Candida* spp. The  $\Sigma$ FIC value obtained by Checkerboard Assay was 0.375 and 0.5 for *Candida* spp. tested in combination for essential oils of Ajwain and Coriander oil. Thus, indicating synergistic interaction between Fluconazole and the tested essential oils. The active components present in the essential can be used in combination with the antifungal under study. There is a need for in-vivo studies in order to assess the safety of the combination, though.

**KEYWORDS:** Fluconazole, *Trachyspermum ammi*, *Coriandrum sativum*, Ajwain oil, Coriander oil, Checkerboard Assay, Synergistic interaction.



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## INTRODUCTION

In recent years, fungal infections have increased prevalently in immune-compromised hosts as a consequence of HIV infection, aggressive therapies for cancer, autoimmune disease and organ or tissue transplantation. *Candida* spp., a commensal fungus of the oral cavity and gastrointestinal tract in humans, represents one of the major causes of mucosal infection and systemic infection, which can be life threatening if not treated<sup>1</sup>. What's also concerning is that some types of *Candida* are becoming increasingly resistant to first-line and second-line antifungal medications, namely, Fluconazole and Echinocandins. Approximately 7% of all *Candida* bloodstream isolates are resistant to Fluconazole<sup>2-3</sup> and the proportion of resistant *Candida* isolates has remained fairly constant over the past twenty years<sup>4</sup>. Many essential oils have been advocated for use in complementary medicine for bacterial and fungal infections including boils, acne, gingivitis and candidiasis. Coriander (*Coriandrum sativum* L.) is an annual herbaceous plant originally from the Mediterranean and Middle Eastern regions, cultivated for its culinary, aromatic and medicinal use. The essential oil and various extracts from coriander have been shown to possess antibacterial, antioxidant and free radical scavenging activities<sup>5-6</sup>. *Trachyspermum ammi* (L.) (syn *Carum copticum*) is an annual herbaceous plant belonging to the highly valued medicinally important family, *Apiaceae* and the seeds of Ajwain were highly administered by traditional healers for different ailments<sup>7</sup>. One strategy employed to overcome the resistance mechanism is the use of combination of drugs. However, it has been poorly explored<sup>8</sup>. The aim of this study was to evaluate synergy between Fluconazole and essential oils of *Coriandrum sativum* (Coriander oil) and *Trachyspermum ammi* (Ajwain oil) using checkerboard assay.

## MATERIALS AND METHODS

### Organisms and culture conditions

The Fluconazole resistant isolates of *C. albicans*, *C. tropicalis* and *C. parapsilosis* used in this study were obtained by sub culturing clinical specimens from the Microbiology department at the Breach Candy Hospital, Mumbai. The strains were maintained on Sabouraud's Dextrose medium prior to the experiments in presence of 20 µg/mL Fluconazole (Forcan, Cipla). Cultures were propagated for two days in Sabouraud's medium at 37°C on an orbital shaker at 200 rpm. Cells were harvested by centrifugation, suspended in sterile saline and inoculum equal to 0.5 of McFarland tube was prepared.

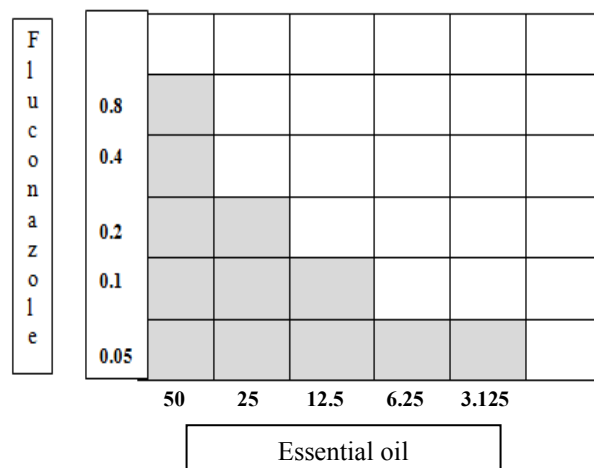
### Essential oils

Coriander oil was obtained by steam distillation from *Coriandrum sativum* seeds (examined for its authenticity by the Botany Department, Mithibai College, Mumbai). Seeds were coarsely grounded using mixer (Nirlep, Accent 9001, 280V, 18000 rpm). Hundred grams of these ground seeds were distilled in 500 mL distilled water for 90 mins using hydro-distiller. The oil was then extracted overnight using equal amount of diethylether (LobaChemie, Mumbai). Ajwain oil used in this study was kindly provided by Konark Herbals and Healthcare, Mumbai.

### Antimicrobial Activity of Fluconazole and Coriander oil and Fluconazole and Ajwain oil using Checkerboard assay

The antifungal Fluconazole stock (2 mg/mL) was used for the Checkerboard assay. It was stored at 4°C until use. The stock solution of Fluconazole ranged from 0.05 mg/mL to 0.8 mg/mL. The Coriander oil used ranged from 100 mg/mL to 3.125 mg/mL and Ajwain oil used ranged from 50 mg/mL to 3.125 mg/mL. The stock solutions and serial twofold dilutions of each drug to at least double the MIC were prepared according to the recommendations of CLSI immediately prior to testing<sup>9</sup>. A total of 500 µL of RPMI-1640 broth supplemented with 0.5% Tween 80<sup>10</sup> was distributed into each vial. Tubes containing only Tween 80 (without plant essential oil and antifungal) were kept as control. The first antifungal of the combination was serially diluted along the ordinate, while the essential oil was diluted along the abscissa (Fig.1). Each vial was inoculated with 100 µL of the inoculum, and were incubated at 37°C for 48 h under aerobic conditions. After incubation, the growth was observed by streaking a loopful from each vial on sterile Sabouraud's agar plates. The plates were then incubated at 37°C for 48 hours. The plates were observed for growth of the test organisms. The combination of the drugs in which the growth is completely inhibited was considered as effective MIC for the combination. According to the CLSI guidelines for broth micro-dilution, the MIC was defined as the lowest concentration of antibiotic that completely inhibited the growth of the organism. Synergy is more likely to be expressed when the ratio of the concentration of each drug to the MIC of that drug was same for all components of the mixture. The ΣFICs were calculated as follows: ΣFIC = FIC A + FIC B, where FIC A is the MIC of drug A (Fluconazole) in the combination/MIC of drug A (Fluconazole) alone, and FIC B is the MIC of drug B (Essential oil) in the combination/MIC of drug B (Essential oil) alone. The combination is considered synergistic when the ΣFIC is ≤0.5, indifferent when the ΣFIC is >0.5 to <2, and antagonistic when the ΣFIC is ≥2<sup>11</sup>.

Figure 1  
The checkerboard method showing the synergy of a two-drug combination



## RESULTS AND DISCUSSION

Worldwide prevalence and incidence rates of Candidiasis is increasing in the last several decades, particularly among the populations of HIV-immuno compromised and hospitalized individuals<sup>12</sup>. It is estimated that *Candida albicans* accounts for over 42% of fungal infections worldwide, followed by non-albicans species such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. guilliermondii*, *C. rugosa* and *C. dubliniensis*<sup>13-14</sup>. Fluconazole is the first line of antifungal medication used to treat candidiasis, and the isolates obtained from Breach Candy Hospital were resistant to Fluconazole. Also, previous study by us has indicated the effects of Coriander oil on *Candida* isolates<sup>15</sup>. Also, researchers have reported the antifungal properties of Ajwain oil against *Candida*<sup>16</sup>. Hence, present study considered the use of Fluconazole in combination with Ajwain and Coriander essential oils. Checkerboard assay was performed to assess the synergy between essential oil and Fluconazole against *Candida* isolates. Fluconazole in combination with Ajwain oil showed synergistic antifungal action against all the three isolates of *Candida spp.* FIC A was 0.125 whereas FIC B was 0.25. Thus,  $\Sigma$ FIC value was found to be 0.375 for all the *Candida* isolates, indicating that the activity of the antifungals in combination is greater than the sum of their independent activities. Inhibition of fungal growth was recorded at lower concentrations compared to the individually tested values. However,  $\Sigma$ FIC value was found to be 0.5 with FIC A value 0.25 and FIC B value 0.25, for Fluconazole in combination with Coriander oil,

indicating that the combination also behaves synergistically for all the three isolates. Ergosterol is a known target of Azole family. Hence, Ajwain oil along with Fluconazole may have a pronounced effect on ergosterol biosynthesis, inhibiting the process. The ability of *in vitro* combination testing to predict clinical synergy is unknown. Further clinical studies determining the relevance of these data are warranted. The clinical benefits of these combinations *in vivo* can only be determined by assessing synergies through carefully designed pharmacokinetic studies and through multicenter randomized clinical trials<sup>17</sup>.

## CONCLUSION

Checkerboard Assay showed synergistic interaction between Fluconazole and the essential oils of Ajwain and Coriander against *C. tropicalis*, *C. albicans* and *C. parapsilosis*. Thus, this study indicates that the combination of some medicinal plant extracts and known antifungals offers significant potential for the development of novel antimicrobial therapies for Candidiasis. However, pharmacokinetic studies and clinical trials are needed to evaluate the safety aspects.

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