

Study of Inhibitory Effect of extract of Ajwain (*Trachyspermum ammi*) on *Candida Albicans*

Sarfraz A¹, Bhattacharyya S², Sengupta A¹, Singh S³, Kumar D¹, Anjum N¹

ABSTRACT

Introduction: *Candida albicans* is a leading cause of fungemia in both developed and developing countries. Biofilm formation by *Candida albicans* impedes antifungal therapy, and the yeast is often resistant to available antifungals. These antifungals are often costly and toxic, too. So our study was planned to evaluate the anti-*Candida* effect of extract of seeds of Ajwain (*T. ammi*) on growth and biofilm formation of *Candida albicans* clinical isolates.

Material and methods: We evaluated the effect of aqueous extract of Ajwain seeds on growth and biofilm of *Candida albicans* in vitro.

Results: This extract was found to completely inhibit growth and biofilm of the pathogen at concentration of 8% (weight/volume).

Conclusion: This can be a suitable precursor to newer antifungals to treat infection by this pathogen.

Keywords: *Candida albicans*, Ajwain, biofilm.

INTRODUCTION

The use of plants and seeds in medicine is almost as old as human civilisation itself.¹ *Candida* species are currently the 4th leading cause of hospital-acquired bloodstream infections, having a mortality rate of up to 35-40% for systemic infections.² Of the disseminated fungal infections, Candidiasis is the most prevalent, with *Candida albicans* capable of causing more invasive infections than any other fungus.³ Ajwain is an Egyptian aborigine plant, which grows in arid and semiarid fields in different regions of central Europe, Asia, India (most crops are in the states of Rajasthan, Gujarat, and West Bengal), Iran (especially eastern regions of Baluchistan), Iraq, Afghanistan, and Pakistan.⁴ The therapeutic effects of seeds: this plant in treating gastrointestinal disorders, like reflux, cramps, abdominal tumors, abdominal pain, and *Helicobacter pylori* infections, as well as eye disorders, have been well demonstrated.⁴ *Candida albicans* infections, especially when associated with biofilm formation, shows marked resistance to antifungal agents.⁵ Hence researchers are now interested in studying the effect of herbal extracts and natural compounds on this dangerous yeast pathogen. Keeping in mind these points, our study was aimed to study the effect of extract of seeds of *T. ammi* (Ajwain) on growth and biofilm formation of *Candida albicans*.

MATERIAL AND METHODS

This was a lab based observational study carried out in the department of Microbiology of the institute from July 2015 to August 2016. Ten randomly selected clinical isolates of *C. albicans* identified by routine tests like Dalmau technique, sugar fermentation, growth at 44 deg C and germ tube test, were selected for the study. At first, (a) peptone water and (b) peptone water with Ajwain extract were prepared. Ajwain seeds were

procured from local market, and weighed. Every time, seeds were procured from a different shop. Peptone water with ajwain extract was prepared with 4 grams and 8 grams of Ajwain seeds in 2 sets, each in 100 ml Peptone water (peptone 1 gram, NaCl 0.5 grams, 100 ml deionised water). *C. albicans* isolates were taken and 1 loopful was inoculated in (a) 2 ml peptone water, (b) 2 ml peptone water with 4% (weight/volume) Ajwain, and (c) 2 ml peptone water with 8% (weight/volume) Ajwain. All tubes were incubated at 37 deg C overnight. Next day, 1 loopful from all liquid suspensions was inoculated on Egg yolk agar prepared in-house (Nutrient agar, autoclaved, molten, 90 ml + egg yolk 10 ml, collected by sterile precautions from unfertilised hen's egg) and plates observed next day. After that, liquid in the test tubes was discarded and tubes were washed thrice with sterile normal saline. Then, 2 ml aqueous Safranin (0.5%, weight/volume) was added to each tube and tubes were kept in vertical position for 1 minute. After that, tubes were again washed thrice with normal saline and inverted for 5 minutes for drying. Thus biofilm formation and inhibition was assessed by Test tube method. All tests were carried out thrice with each strain. For checking human toxicity, 1 drop of extracts was added to 1 drop of buffy coat from blood samples received routinely in lab for other purposes, after its intended use, made as mount, and observed microscopically after 15 minutes to check for cellular lysis. All tests were carried out thrice with each isolate.

STATISTICAL ANALYSIS

Social Science Statistics software⁶ was used for the statistical analysis. Z test was used for the statistical comparison.

RESULTS

In 4 gram% concentration, there was no inhibition of growth or biofilm formation of *C. albicans* isolates by Ajwain aqueous extract. However, in 8 gram% concentration, biofilm formation was completely inhibited in all *C. albicans* isolates by ajwain extract in test tube method (TTM) as seen visually (figure-1). Growth of *C. albicans* was also inhibited completely at this concentration by Ajwain extract, as evident by no growth of *C. albicans* colonies on subculture from colonies in Ajwain extract on Egg yolk agar. The difference in colony count was found to be significant by Z-test of statistical significance of 2 proportions, with p-value <0.05.⁶ The extract at both concentrations, was found to be non-toxic to white blood corpuscles on incubation

¹Senior Resident, ²Assistant Professor, ³Tutor, Department of Microbiology, AIIMS, Patna, India

Corresponding author: Dr Sayan Bhattacharyya, Assistant Professor, Department of Microbiology, AIIMS Patna-801507, India

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with the buffy coat preparations since there was no cell lysis. Thus, the ajwain extract was safe and potent inhibitor of growth and biofilm formation of *C. albicans* in vitro.

DISCUSSION

Candida species produce infections that range from non-life-threatening mucocutaneous illnesses to invasive processes that may involve virtually any organ.⁷ In recent years, fungal infections have increased prevalently in immunocompromised hosts as a result of HIV infection, aggressive therapies for cancer, autoimmune disease and organ or tissue transplant.⁸ Recently, fluconazole-resistant *C. albicans* strains and intrinsically resistant *Candida* species such as *C. glabrata* and *C. krusei* are emerging in immunocompromised patients treated for therapy or prophylaxis.⁸ Hence natural compounds are the topics of interest for clinicians and researchers in order to treat these infections. Known as Ajwain, *Trachyspermum ammi* (L.) is an annual herbaceous plant belonging to the highly valued medicinally important family, Apiaceae.⁹ Both acetone and aqueous extract of ajwain has been found to inhibit growth of *Staphylococcus aureus* and *Escherichia coli* in scientific studies.⁹ Ajwain oil has been found to kill *Aspergillus niger* as well.¹⁰ The main active ingredient in ajwain seeds, also known as Bishop's weeds, has been found to be Thymol, which has been documented to have antifilarial, antihyperlipidemic and antifungal effects.¹¹ *Candida albicans* readily forms biofilms on implanted medical devices, including catheters, pacemakers, dentures, and prosthetic joints, which provide a surface and sanctuary for yeast growth and often lead to refractoriness to available antifungal therapy. Given the emerging high degree of resistance of *Candida albicans* to antifungal drugs, ajwain extract definitely is a promising alternative since it could inhibit both growth and biofilm formation. It was also safe, and this toxicity assay can also be done in cell lines for better results. According to some studies, Ajwain oil has been documented to be effective against filamentous fungi like *Aspergillus niger*, *Fusarium moniliforme* and *Curvularia lunata*, with Thymol as most potent component, along with other compounds like p-cymene, γ -terpinene and β -pinene.¹² Ajwain seed oil has also been found to kill *Candida albicans* in vitro, according to some researchers, possible by membrane inhibition.² However, as far as we know, the anti-*Candida* effect of aqueous extract of seeds of Ajwain, as also its anti-biofilm activity on *C. albicans*, have not been yet assessed or published in the literature. All these findings are very interesting and can be evaluated further in future studies.

CONCLUSION

Trachyspermum ammi (Ajwain) seed aqueous extract is a potent inhibitor of growth and biofilm formation in *Candida albicans*.

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