

## INHIBITORY EFFECT OF AJWAIN SEED AQUEOUS EXTRACT ON *PSEUDOMONAS AERUGINOSA* BIOFILM: AN IN-VITRO STUDY

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### ABSTRACT

Background: *Pseudomonas aeruginosa* is a non-fermentative Gram negative bacterium that is implicated in many nosocomial and community acquired infections and is often multi-drug resistant. Natural compounds and herbal extracts are hence the current topics of interest for finding new avenues to treat infection by this virulent bacterium, more so because they are non-toxic to the host. Materials and methods: We in our study assessed the inhibitory effect of aqueous extract of *Trachyspermum ammi* (Ajwain) seeds on biofilm formation by *P. aeruginosa* clinical isolates by test-tube method. Results: At concentration of 8 gram % (weight/volume), there was significant inhibition of biofilm formation by Ajwain extract. However, at concentration of 4 gram%, this inhibitory effect was less marked. Conclusion: So Ajwain extract can be the forerunner of new therapeutic compounds to treat *P. aeruginosa* infections.

**Keywords:** - Ajwain, *Pseudomonas aeruginosa*, biofilms.

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## INTRODUCTION

*Pseudomonas aeruginosa* is a Gram negative non-fermenting rod that is one of the commonest bacteria implicated in nosocomial infections, often with high mortality (Farheen et al, 2014). At present, *P. aeruginosa* isolates are increasingly becoming multi-drug resistant, which often leads to worse clinical outcomes (Hirsch et al, 2010). This bacterium is also notorious for forming complex, structured microbial communities over indwelling and bioprosthetic devices, called biofilms (Wei et al, 2013). Biofilm formation implies increased drug resistance, possibly by interference of the biofilm matrix in drug penetration to the target bacteria, and also by increased drug efflux (Estela et al). Ajwain is a medicinal herb belonging to the Apiaceae family that has many medicinal properties and is widely used as an essential kitchen ingredient since antiquity (Zarshenas et al, 2014). Hence our study was aimed at studying the anti-biofilm effect of aqueous extract of *T. ammi* (Ajwain) on *P. Aeruginosa* in vitro. Test tube method was used to study biofilms in our study.

## MATERIALS AND METHODS

This was a laboratory based observational study was carried out in the Department of Microbiology of the institute from December 2014 to December 2015. *Pseudomonas aeruginosa* isolates were recovered from routine samples processed in the laboratory, like urine, sputum, pus etc. and identified by conventional methods like pigmentation production, positive Oxidase test, fruity odour and positive Citrate

utilisation (Phillips et al, 1968). *P. aeruginosa* ATCC 27853 and ten (10) other randomly selected clinical isolates of *P. aeruginosa* were selected for the study. *T. ammi* (Ajwain) seeds were procured from local market, each time from a different vendor or shop.

At first, (a) Peptone water (Himedia labs, Delhi, India) and (b) Peptone water with 4%, and 8% (both weight/volume) of smashed Ajwain seeds were prepared by weighing in deionised water and autoclaving at 121 deg C at 15 lbs./sq. inch pressure for 15 minutes. One loopful of *P. aeruginosa* colonies from Mueller Hinton agar plate were inoculated in 2 ml each of:- (a) Peptone water, (b) Peptone water with 4% ajwain, and (c) Peptone water with 8% Ajwain. Tubes were incubated at 37 deg C in incubator and next day, the liquid content of each tube was discarded and tubes were washed thrice with sterile physiological saline. Then 2 ml of 0.5% aqueous Safranin was poured in each test tube and kept for 1 minute. Afterwards, safranin in tube was again poured off and tubes were again washed thrice with sterile physiological saline. After washing, tubes were kept standing in inverted position. After drying, they were observed visually for biofilms.

To test for toxicity of the extracts, 1 drop of extract (at both concentrations) was incubated thrice with 1 drop of buffy coat of human blood from left-over whole blood samples in the lab that were processed for other purposes, mounted and observed microscopically for cell lysis. All tests were carried out three times with each isolate.



**Fig: 1**

## RESULTS

At 4 gram %, there was no inhibitory effect of Ajwain seeds on biofilms as seen by the test tube method. However, at the concentration of 8 gram % concentration, marked inhibition of biofilm formation of *P. aeruginosa* was observed on all occasions, as seen visually, testing all isolates.

The extract was found to be completely non-toxic to the cells of buffy coat, or WBCs.

## DISCUSSION

Ajwain seeds, also known as Bishop's weed, have been widely used in the Indian kitchen and have also been documented to possess activity against numerous bacteria like *Klebsiella pneumoniae* (Pandey et al, 2014). Ajwain has probably come to India from either Middle east or Iran, and has also excellent diuretic and aphrodisiac properties besides having antimicrobial action (Jeet et al, 2012). The principal

antimicrobial moieties present in Ajwain oil, which has been used medicinally for ages, are Carvacol and Thymol (Jeet et al, 2012). *P. aeruginosa* causes protracted infections in predisposed patients like cystic fibrosis, with very high propensity of biofilm formation, causing treatment failures (Perez et al, 2011). Since this inhibitory effect was seen with autoclaved extracts, the effect is likely to be present in febrile states also. As far as we know, the inhibitory effect of aqueous extract of Ajwain seeds on *P. aeruginosa* biofilm have not yet been published in the scientific literature. Further studies are eagerly awaited in this context since this is a very interesting and promising new area of research.

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