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HEAT STABLE COMPOUNDS IN AJWAIN (*TRACHYSPERMUM AMMI*) SALINE EXTRACT INHIBIT PROTEASE AND PREVENT GROWTH OF *STAPHYLOCOCCUS AUREUS*: AN IN VITRO STUDY

Kumar R, Bhattacharyya S*, Sarfraz A, Ansari MAA, Nitesh Jaiswal, Prasad R, Roy H, Ahmed S, Shristi

Department of Microbiology, AIIMS Patna.

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***Correspondence for Author**
Dr. Bhattacharyya S
Department of Microbiology, AIIMS Patna.

ABSTRACT

Staphylococcus aureus is a smart bacterium causing a wide range of infections in man. It is often found to be refractory to most antibiotics. This necessitates the discovery of new natural and low-cost compounds which can inhibit growth of this pathogen, and at the same time be harmless to host cells. Our experiments showed that the saline extract of *Trachyspermum ammi* (ajwain) inhibits growth of *S. aureus* at concentration of 8 gm/100 ml and inhibits protease of the same bacterium at concentration of 4 gm/100 ml. This study can show the path of synthesizing new, low cost, active pharmaceutical compounds that could kill this pathogen.

KEYWORDS: Ajwain, extract, virulence.

INTRODUCTION

Staphylococcus aureus is one of the smartest bacterial pathogens, causing a range of infections in man, like cellulitis, osteomyelitis, abscess and others.[1] It is, in fact, the commonest bacterial pathogen in inpatients and the second most common in outpatients, according to Western data.[2] This bacterium is often notoriously resistant to a wide variety of antimicrobials like Methicillin, to the tune of 12-15%, and aminoglycosides, in the order of 90-95%, and also Vancomycin.[3,4] Keeping these points in view, current research has focussed on the synthesis and discovery of new, alternative and herbal compounds that can kill *S. aureus.*[5] For example, plants like *Lycium chinense*, *Chrysanthemum indicum* etc., have been found to be very effective in inhibiting *Staphylococcus aureus* in vitro.[6] The commonly used kitchen spice, Garlic (*Allium sativum*), in a study, has been shown to inhibit

Methicillin-resistant *Staphylococcus aureus* infection in diabetic mice, in an in-vivo study.[7] *Trachyspermum ammi* (Ajwain), belonging to family Apiaceae is a highly valued medicinally important seed spice , used since many many years for treatment of gastro-intestinal ailments, lack of appetite and bronchial problems; the oil from the seeds also has antimicrobial properties.[8] Keeping all these points in mind, our study aimed at testing the inhibitory effect, if any, of saline extract of Ajwain (*Trachypermum ammi*) on growth of *S. aureus* laboratory strains, and its effect on some of its virulence factors, like protease, lipase and phospholipase (lecithinase).

Aim: To study inhibitory effect of saline extract of Ajwain (*T. ammi*) on *Staphylococcus aureus* isolates in vitro.

Objectives

i) To test effect on growth characteristics of *S. aureus*.

ii)To see effect of Ajwain on virulence traits of *S. aureus* isolates, like lipase, protease, phospholipase and biofilm formation by test tube method.

iii)To see the toxic effect of the extract on human erythrocytes and WBCs to rule out host toxicity.

MATERIALS AND METHODS

Type of study: Laboratory based observational study.

Time and place of study: The study was carried out from December 2014 to April 2015, in the Department of Microbiology of the institute.

Isolation and identification of *S. aureus* strains: The isolates were recovered from samples received in the Microbiology laboratory, like pus, urine, blood and others. They were identified by the following tests.

i)Gram positive cocci in grape-like clusters.

ii)Catalase and slide coagulase positive (using pooled human plasma).

iii) breakdown of Mannitol on Mannitol salt agar (with Andrade' indicator).

Following this, ten isolates were randomly selected for the study.

Ajwain was obtained from local grocery store.

Preparation of Ajwain saline extract: In 2 sets of experiments, two concentrations of Ajwain were prepared: One by weighing 4 gm Ajwain seeds and 0.9 grams NaCl powder in 100 ml deionised water, and in another, 8 gm Ajwain was weighed in 0.9 grams NaCl powder in 100 ml deionised water.

Extract was obtained by autoclaving the mixture in 15 lb/in2 pressure at 121 deg C for 15 min. After preparation. the sterility of the extract was checked by streaking filtrate on Chocolate agar and incubating it overnight at 37°C. For three sets of experiments, ajwain was obtained from 3 separate shops, to rule out any bias.

Tests: In each set, 1 loopful of the microbe was suspended in 2 ml of a)Peptone water, and b)Ajwain extract., and incubated overnight at 37 Deg C. Following this, 10 µl of each was streaked on: a)MacConkey agar, and b)Egg yolk agar, to see any inhibition of growth (colony), and effect on lipase, protease and lecithinase.

Same experiment was repeated with ajwain extract, heated to 100 °C for 5 minutes. Lipase activity was denoted by pearly shine of colonies. Lecithinase was interpreted by observing distinct zone of haziness around the colonies on Egg yolk agar; protease activity was indicated by zone of clearing around the colonies on the same medium.

Preparation of Egg yolk agar: Clean fresh, hen's eggs were obtained from local grocery store. Ninety ml of Nutrient agar was prepared by autoclaving. Following this, it was allowed to cool to 50 deg C. Meanwhile, taking aseptic precautions, the outer shell of the egg was cleaned with spirit swab, and using sterile metal forceps, a small nick was made in shell at one end. Egg white (Albumen) was allowed to pour off. Then this nick was widened, and remaining egg yolk (yellow part) was poured in a wide flask containing few sterile glass beads, and shaken briskly so that it mixed well. After this, the yolk was added to molten Nutrient agar, mixed and poured in plates.

Toxicity assay: Buffy coat layer was separated from routine serum samples; 10 µl of buffy coat was put on a slide. To it, 10 µl of normal saline and same volume of ajwain extract were added. The mount was prepared by cover slip, and both were observed under 40X magnification for 20 minutes, to see hemolysis or lysis of WBC, if any.

RESULTS

At 4 gm%, there was no inhibition of *S. aureus*; however, protease was inhibited. There was

in effect on golden yellow pigment formation. Biofilm formation was not inhibited.

Lecithinase and lipase were not inhibited by Ajwain extract.

At 8 gm %, however, there was complete inhibition of growth of *S. aureus* and biofilm.

The findings remained same after using heated extract.

The inhibitory, effect, was not due to cell wall damage, however, since gram stain from both growth in peptone water and in ajwain extract, showed Gram positive cocci, uniformly stained, in clusters.

At both concentrations, the extract was found to be non-toxic to human RBC and WBC.

DISCUSSION

Staphylococcus aureus is a major human pathogen, recognized ever since Sir Alexander Ogston, in 1880s, stated that it is a principal cause of wound abscess.[9] Currently, *S. aureus* is second only to coagulase-negative *Staphylococcus* spp. as a cause of hospital acquired bacteremia.[9] This pathogen can cause a wide variety of infections in man, which can be divided into 3 types: (i) superficial lesions such as wound infection, (ii) toxicoses such as food poisoning, scalded skin syndrome and toxic shock syndrome, and (iii) systemic and life-threatening conditions like endocarditis, osteomyelitis, pneumonia, brain abscesses, meningitis, and bacteremia.[10] The organism possesses a unique range of putative virulence factors like adhesins, hemolysins or exotoxins, protease, lipase and Panton-Valentine Leucocidin.[9,10] Phospholipase, specially Phospholipase C, is also a recently recognised virulence factor in *S. aureus*, especially linked with respiratory disease and coagulopathy.[11] Treatment of *S. aureus* infections, particularly bacteremia, is challenging due to emergence of resistance to beta-lactam antibiotics like Methicillin and other classes of antibiotics, more so in the western hemisphere.[12] Even increasing refractoriness to Vancomycin has been reported.[13] Also, antibiotics valuable for MRSA, Like Linezolid and Dalvabancin are too costly , which precludes their use in the poor patient.[14,15] In this regard, it is worthy to mention that turmeric (*Curcuma longa*), has been found to inhibit Sortase A, a surface protein assembler protein in *S. aureus.*[16] Our study, for the first time, highlights the protease inhibition as a killing mechanism of Ajwain against *S. aureus.* Being non-toxic to host cells, it can be used to synthesize inhibitory compounds and new antibiotics against the pathogen, which would also be expectedly cheaper. Being heat stable, the inhibitory property could be used to kill the pathogen in febrile states also. Further studies in cell lines to negate host toxicity are also required in this context.

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