



Assessment of Antimicrobial Property Of Ethanolic Extract of *Trachyspermum ammi* (ajwain) Seeds Against *A.actinomycetemcomitans* and *P. gingivalis* – an *in vitro* study

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Abstract

Introduction: - Idea of using medicinal plants for treatment of various ailments in dentistry is gaining popularity because of its therapeutic uses with less side effects. *Trachyspermum ammi* is an ayurvedic plant with good antibacterial and antifungal property and is a topic of interest among researchers.

Aim:- To determine the minimum inhibitory concentration and minimum bactericidal concentration of ajwain seeds against *A. actinomycetemcomitans* and *P. gingivalis*.

Materials and Methods: - Followed by the extract preparation and inoculum preparation the MIC and MBC of the extract of *Trachyspermum ammi* against the organisms were checked by broth dilution method and streaking method respectively.

Results: - The MIC and MBC of the ajwain seed extract was found to be 16.66 and 33.33 respectively for both *A. actinomycetemcomitans* and *P. gingivalis*.

Conclusion: - The study concluded that ajwain seed is a good option for the treatment of chronic periodontal infections.

Keywords: *A. actinomycetemcomitans*, ajwain, minimum bactericidal concentration, minimum inhibitory concentration, *P. gingivalis*, *Trachyspermum ammi*

INTRODUCTION

Periodontitis can be defined as an inflammatory disease of supporting tissues of teeth caused by specific microorganisms or groups of microorganisms resulting in progressive destruction of periodontal ligament and alveolar bone.^[1] Because of the diverse microbial flora of the oral cavity, many bacteria can be found adhered to the biofilms on the tooth surface and tongue that can result in caries, gingival and periodontal infections. These mainly include different types of bacteria, fungi, viruses and protozoa including *A.actinomycetemcomitans*, *P.gingivalis*, *P.intermedia*, *Tannerella forsythia*, *Treponema denticola*, *Peptostreptococcus*, *Lactobacillus*, *Candida* species etc. Occurrence and progression of these diseases can be prevented by inhibiting the growth of these

microorganisms with the use of mouthwashes containing antibacterial monoterpenes such as thymol, eucalyptol, menthol and borneol.^[2]

In 1998 Sigmund Socransky grouped *P. gingivalis*, *T. denticola* and *T. forsythia* as red complex bacteria due to its high association with periodontal disease. *P. gingivalis* is a potent periopathogen with a number of virulence factors (fimbriae, lipopolysaccharide, gingipains etc) that result in the destructive periodontal disease directly or indirectly by altering the host immune response.^[3] *A. actinomycetemcomitans* is also considered as a strong causative agent in periodontitis, that affects mainly the young individuals resulting in localised aggressive

periodontitis. It also produces many virulence factors that include (leukotoxin, lipopolysaccharide, heat shock proteins, collagenase etc., contributing to periodontal diseases.^[4]

Nowadays, interest in natural medicinal products is found to be increasing because of the high rate of adverse effects and resistance of microorganisms to the conventional drugs. Whereas a number of natural medicinal plants are proved to have the same therapeutic effect as that of conventional drugs and have less side effects and no drug resistance seen with it.

Genus *Trachyspermum* belongs to family *Apiaceae* which has two species; *Trachyspermum ammi* and *Trachyspermum roxburghianum*. Ajwain seeds are medicinally very useful and their therapeutic uses include antifungal, antioxidant, antinociceptive, cytotoxic activities and digestive properties. Many studies have been done on ajwain seeds and it is proved that *Trachyspermum ammi* possess antimicrobial activity against many microbial pathogens like *S. aureus*, *Escherichia coli*, *P. aeruginosa* etc. which is due to the presence of thymol in *T. ammi* which is potent enough to inhibit the growth of pathogenic microorganisms present in the oral epithelium also.^[5] Therefore, the present study was aimed to determine minimum inhibitory concentration and minimum bactericidal concentration of *Trachyspermum ammi* (ajwain) seeds against *A. actinomycetemcomitans* and *P. gingivalis*.

MATERIALS AND METHODS

• Extract Preparation

The dried seeds of *Trachyspermum ammi* was collected and authenticated from KAHER Shri B M Kankanwadi Ayurveda Mahavidayala Belagavi. The extract preparation was done in the KLE's Dr. Prabhakar Kore Basic Science Research Center. Collected seeds were powdered and soaked in ethanol and kept in shaker for 3 days for proper mixing. After that the solvent was filtered using Whatman No.1 filter paper and kept for evaporation for 5 days. The stock solution was prepared, mixing 1ml of prepared extract and 9ml of Dimethyl sulfoxide (DMSO) and working solution was prepared, mixing 1ml of stock solution and 9ml of water. (Figure 1 and 2)

• Inoculum Preparation

Preparation of inoculum was carried out in BHI broth. Standard colonies of same morphological type were selected from an agar culture plate. Each colony was scooped using sterile loop and transferred into a small bottle containing 4-5ml BHI broth followed by incubation at 37°C for 2 days.

• Determination of MIC and MBC

96 well plates were taken and 10 wells were selected from each plate. 100µl of broth was added to first 8 wells and 100 µl of extract was added to the first well and serially diluted till the last well. 10µl of inoculum was added to all 10 well and kept for incubation in McIntosh and Fildes anaerobic jar for 2 days. After 48 hours rezachurin dye was added and waited for colour change from blue/violet to slight pink/magenta. MBC was done in agar plate using streaking method in triplicate. Three agar plates were taken for each pathogen and were divided into 8 wells. The loop was dipped in the wells of 96 well plate and was streaked in each section of the agar plate. The same procedure was followed for both *A. actinomycetemcomitans* and *P. gingivalis*, in triplicate (Figure 3, 4 and 5). After streaking, the agar plates were kept for incubation in McIntosh and Fildes anaerobic jar for 2 days and the readings were recorded.

RESULTS

Minimum inhibitory concentration of *A. actinomycetemcomitans* was found to be 12.5mg, 12.5mg and 25mg in Plate 1, Plate 2 and Plate 3 respectively and average was calculated to be 16.66mg. Minimum inhibitory concentration of *P. gingivalis* was found to be 12.5mg, 12.5mg and 25mg in Plate 1, Plate 2 and Plate 3 respectively and average was calculated to be 16.66mg. (Table 1). The minimum bactericidal concentration of *A. actinomycetemcomitans* was found to be 25mg, 25mg and 50mg in Plate 1, Plate 2 and Plate 3 respectively and average was calculated to be 33.33mg. The minimum bactericidal concentration of *P. gingivalis* was found to be 25mg, 25mg and 50mg in Plate 1, Plate 2 and Plate 3 respectively and average was calculated to be 33.33mg. (Table 2).

The results obtained were subjected for statistical analysis. Mann Whitney U test was used to assess the antibacterial activity of *Trachyspermum ammi* (ajwain) seeds against *A. actinomycetemcomitans* and *P. gingivalis*. It was seen that there was no significant

difference in the p value between the two microorganisms. (p value was found to be 1.00 and 1.00 for MIC and MBC) viz. (Table 3)

DISCUSSION

Periodontitis is an infection-driven inflammatory disease of the tooth supporting tissues or the periodontium wherein the genetics, environmental factors and behavioural factors have a role in the progression of the disease, exposing the susceptible individuals to its initiation, because of which it is considered multifactorial in origin.^[6]

Scaling and root planning is considered as the basic and gold standard non-surgical treatment for gingival and periodontal infections.^[7] But, for a long time, use of chemical therapeutic agents along with the mechanical debridement is considered as a treatment of choice with improved results. Commonly used therapeutic agents such as Chlorhexidine, is chemically derived and is considered as the gold standard therapeutic agent used for plaque control.^[8] But certain adverse effects have been reported with the long-term use of chlorhexidine such as staining of teeth, altered taste sensation, mucosal irritation, parotid swelling and enhanced calculus formation. These factors triggered the need for the development of an alternative agent which is equally effective and help in minimizing all the adverse effects seen with the existing agents.^[8]

Herbal medicine is an upcoming alternative and is considered as the most acceptable form of therapy by many researchers because of its diverse medicinal properties with fewer side effects. It is as old as human civilization and it has been used in various traditional systems such as Ayurveda, Siddha, European, Tibetan and Unani. About 75-80% of cases in developing countries are using this traditional way for primary health care as it is more acceptable and compatible with the human body with less side effects and cost effectiveness.^[9] Because of these reasons, plants with medicinal properties and therapeutic uses are gaining popularity among the researchers to explore the different uses of plants and their compounds responsible for specific properties. *T. ammi* is such a medicinal plant, belongs to family *Apiaceae*, which is a very old and well known Ayurvedic spice. It is also commonly known as ajwain, Bishops's weed, Yamini etc.^[10] It is an aromatic spice having close resemblance to the flavor of thyme and is considered as a principal

source of thymol. *T. ammi* is proved to have various therapeutic uses such as antibacterial, antifungal, antioxidant, anti-inflammatory, hypotensive, analgesic etc.^[10] Considering this fact, present study aimed to evaluate antibacterial activity of *Trachyspermum ammi* against the most important periodontal pathogens, *A. actinomycetemcomitans* and *P. gingivalis* (red complex bacteria) which are associated with periodontal destruction.

In our study, minimum inhibitory concentration and minimum bactericidal concentration of ethanolic extract of ajwain seeds against *A. actinomycetemcomitans* was assessed and it was found to be 16.66mg and 33.33mg respectively. MIC and MBC of ethanolic extract of ajwain seeds against *P. gingivalis* was found to be 16.66mg and 33.33mg respectively.

This was in accordance with the study done by Dadpe et al to determine the antibacterial efficacy of *Trachyspermum ammi* oil at different concentrations against five oral bacteria; *S. mutans*, *S. oralis*, *Lactobacillus acidophilus*, *Lactobacillus fermentum* and *C. albicans*. The results of this study revealed that *T. ammi* oil moderately inhibits bacterial growth with mean MIC of 250, 125, 250, 125, and 250 µg/ml, respectively. Mean MBC for *T. ammi* oil obtained was 18.60 ± 0.65 , 11.60 ± 1.14 , 14.10 ± 0.55 , 11.50 ± 0.61 , and 15.10 ± 0.74 mm. The possible explanation for the antibacterial property of ajwain can be due to the presence of its main ingredient's thymol and carvacrol. Thymol has the potential to kill bacteria which are resistant to even prevalent third generation antibiotics and multidrug-resistance microbial pathogens. It can also damage membrane integrity by changing pH hemostasis and equilibrium of inorganic ions. This leads to intracellular adenosine triphosphate leakage, loss of cytoplasmic material and potassium, thereby altering cell surface morphology through disturbed bacterial cell metabolism.^[11]

CONCLUSION

In this study it was found that *T. ammi* is an effective antibacterial agent against *A. actinomycetemcomitans* and *P. gingivalis*. *T. ammi* oil is available naturally and abundantly which is nonirritating and nontoxic to the human body. Also, it is cheaper than other artificially formulated mouthrinses. Hence *T. ammi* oil can be used in the future as an effective antiplaque agent due to its antimicrobial profile against oral plaque-forming

bacteria after exploring its biological and chemical profile in detail.

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Table 1. Minimum Inhibitory Concentration of *A.a* and *P.g*

Sr.No.	Samples	Minimum inhibitory concentration (MIC) in mg			
		<i>A.a. comitans</i>	Average	<i>P.gingivalis</i>	Average
1	<i>Trachyspermum ammi</i> extract.	12.5	16.66	12.5	16.66
2		12.5		12.5	
3		25		25	

A.a – *Aggregatibacter actinomycetemcomitans*, *P.g* – *Porphyromonas gingivalis*

Table 2: Minimum Bactericidal Concentration of *A.a* and *P.g*

Sr.No.	Samples	Minimum bactericidal concentration (MBC) in mg			
		<i>A.a. comitans</i>	Average	<i>P.gingivalis</i>	Average
1	<i>Trachyspermum ammi</i> extract.	25		25	
2		25	33.33	25	33.33
3		50		50	

A.a – *Aggregatibacter actinomycetemcomitans*, *P.g* – *Porphyromonas gingivalis*

Table 3: Results of statistical analysis

MIC			MBC				
A.a		P.g	A.a		P.g		
Mean Rank	Exact Sig. [2*(1-tailed Sig.)]	Mean Rank	Exact Sig. [2*(1-tailed Sig.)]	Mean Rank	Exact Sig. [2*(1-tailed Sig.)]	Mean Rank	Exact Sig. [2*(1-tailed Sig.)]
3.50	1.000	3.50	1.000	3.50	1.000	3.50	1.000



Figure 1 : Extract kept in shaker

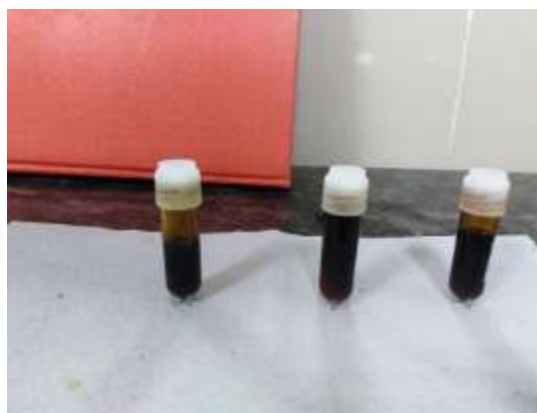


Figure 2 : Extract preparation done



Figure 3 : MIC determination of *A.a* and *P.g*



Figure 4 : MBC determination of *P. gingivalis*



Figure 5: MBC determination of *A. actinomycetemcomitans*