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SHORT COMMUNICATION

# Evaluating Antibacterial Activity of *In Vitro* Culture of Ajwain (*Trachyspermum copticum*) Extract and Comparison with Seed Extract and Essential Oils

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**Abstract:** *Trachyspermum copticum* (Apiaceae) is an annual plant which grows in Iran. The fruits of *T. copticum* (Ajwain) traditionally were used as diuretic, carminative, and antihelmentic. Some biological effects of Ajwain such as antiviral, antifungal and antioxidant activities have been confirmed. The objective of the present investigation was to evaluate the antibacterial activity of extracts of callus and seed and essential oil of Ajwain against some bacterial strains (*Pseudomonas viridiflava, Pseudomonas syringae* pv. syringae and *Escherichia coli*). The extracts and essential oil were prepared and the antibacterial activity was evaluated via growth inhibitory zone assay using disc diffusion agar technique. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was measured by micro broth dilution assay. The results revealed no significant effect for callus extract, however, the effect of seed extract and essential oil on tested bacterial strains was statistically significant. The greatest impact was observed for essential oil and inhibition halo diameter was reported 28.5 mm for *P. syringae* pv. syringae, MIC and MBC were measured 1.56 and 3.12% v/v, respectively.

**Keywords:** antibacterial activity, *Trachyspermum copticum*, callus

## INTRODUCTION

Application of plant-derived biocides in agriculture have been more popular during the past thanks to their low health risk and feasibility. In recent years much attention has been given to non-chemical systems for seed treatment to protect them against many plant pathogens [1]. During the last decade, development of antibiotic resistance as well as undesirable side effects of some drugs have led to the search for new antimicrobial agents. Many researchers have shown that plants have antimicrobial activity and other biological effects [2].

*Trachyspermum copticum* (Apiaceae) is an annual plant which grows in Iran and known as 'Zenyan' (Esfahan, Yazd and Khorasan) [3]. Evaluation of therapeutic effects of *T. copticum* has been the subject of several studies in recent years. Some biological effects of Ajwain such as antiviral [4], antifungal [5], and antioxidant activity [6] have been confirmed. Plant extracts and essential oils show antifungal activity against a wide range of fungi [7]. In addition, methanol extract of this plant was reported to have antibacterial activity against multi-drug resistant *salmonella typhi* [8]. In recent years the production of secondary metabolites through plant cell culture, as an important approach in cell culture studies, has greatly interested. So that in some cases the amount of secondary

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metabolites in vitro culture is much more than in the whole plant and sometimes cultivated cells produce metabolites that is not produced in primary plant [9,10]. Establishment of cell cultures of medicinal plants produce antibacterial compounds in vitro [11], e.g. antibacterial activity of suspension culture cell extract of *Ricinus communis* is evaluated and proven [12] also inhibitory effects of methanol extract of callus and *Cleome rosea* cells were assessed on 19 different bacteria [13].

According to numerous reports about the antimicrobial properties of extracts derived from cell cultures of different plants [14], the present study aimed to evaluate the antibacterial effect of methanol extract of two *in vitro T. copticum* culture broth micro-dilution and disk diffusion method on three bacteria (*Pseudomonas viridiflava, Pseudomonas syringae* pv. syringae and *Escherichia coli*) and its comparison with essential oil seed extract.

## MATERIALS AND METHODS

Plant samples of Ajwain were collected from Department of Agronomy and Plant Breeding, Aburaihan Campus, University of Tehran, Iran and pathogens were collected from Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT), Sari Agricultural Sciences and Natural Resources University, Iran.

### Germination of Seeds

Seeds of *T. copticum* were surface-sterilized by sub-merging in ethanol (70%, v/v) for 3 min followed by continuous agitation in 5% commercial sodium hypo-chlorite for 20 min and rinsing three times with sterile distilled water. These seeds were then germinated on ½ MS medium (pH 5.8) supplemented with sucrose (1.5%, w/v) [15]. All cultures were kept in growth chambers at (25±2)°C under a 16/8 h (light/dark) photoperiod at a photon flux rate of 60 µmol/m$^2$/s provided by cool daylight fluorescent lamps.

### Preparation and maintenance of callus tissue

Cotyledon explants of two weeks old in vitro germinated seedlings were transferred to MS agar medium (Sigma), containing 1.98 mg/l 2,4-dichlorophenoxy acetic acid (Sigma) and 0.48 mg/l kinetin (Sigma) and sucrose (3% w/v). These were kept in growth chambers at (25±2)°C under a 16/8 h (light/dark). Calli were maintained by sub-culturing every 4 weeks [16].

### Preparation of extracts

Powder of dried calli and plant seeds were filled separately in the thimble and extracted successively with methanol using a soxhlet extractor for 48 h. The extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extracts was weighed and preserved in airtight bottles until further use. 1 g of each solvent residue which was dissolved in 10 ml of respective solvents were used as the test extracts for antimicrobial activity assay [17,18].

### Essential oil isolation

The powder of seeds was subjected to hydro-distillation for 3 hours in an all glass clevenger-type apparatus according to the method recommended by the European Pharmacopoeia. The extracted oil samples were dried over anhydrous sodium sulphate and were stored in sealed vials at 4°C.

### Antibacterial activity by micro broth dilution assay

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of samples were determined by micro broth dilution assay. Extract and essential oil were prepared with DMSO 10% at concentrations 1.56-100 mg/ml consecutively. 100 µl of these dilutions were prepared in a 96-well microtitre plate. Muller hinton broth were used as broth media [19]. The bacterial suspensions (*P. viridiflava, P. syringae* pv. syringae and *E. coli*) were diluted ($10^6$ cfu/ml) and then 100 µl was added to each well and incubated at 35°C. MICs were defined as the lowest concentration of compound that inhibit bacteria after 24 h, respectively. MBC values were the first well that showing no growth on solid media.

### Antibacterial activity by disc diffusion assay

Antimicrobial tests were carried out by disc-diffusion method [20]. 50 µl of each bacterial suspesion ($10^6$ cfu/ml) were separately spread on the nutrient agar solid plates with a sterile swab. Thereafter sterile whatman™ filter discs (6 mm diameter) were placed in plates and 20 µl of each methanol extracts (200 and 400 mg/ml) and essential oil (10 and 100% v/v) were poured on the paper discs. Methanol and tetracycline served as control. The inoculated plates were incubated at 37°C for 24 h and the inhibitory zone were measured in millimeter (mm). Each treatment consists of three repeats [21].

### Statistical analysis

The results were analyzed with SAS software V.9.0 and Duncan test was used for comparison of means.

## RESULTS

The maximum amount of MBC and MIC were observed for extract callus and the lowest were for seeds essential oil. The MIC values of callus and seed extract and essential oil of Ajwain against different bacteria were in the ranges of 50, 12.5-25 and 1.56-3.12 mg/ml, and the MBC values were in the ranges of 100, 25-50 and 3.12-6.25 mg/ml respectively. However, the MIC and MBC values of tetracycline were in the ranges of 25 and 12.5 mg/ml. *P. syringae* pv. syringae were the most sensitive bacteria (Table 1).

**Table 1.** The antibacterial activity of Ajwain by micro broth dilution assay (mg/ml).

| | *E. coli* | | *P. viridiflava* | | *P. syringae* | |
|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC |
| Callus extract | 50$^{a}$ | 100 | 50$^{a}$ | 100 | 50$^{a}$ | 100 |
| Seed extract | 12.5$^{c}$ | 25 | 25$^{b}$ | 50 | 12.5$^{c}$ | 25 |
| Seed essential oil | 3.12$^{d}$ | 6.25 | 3.12$^{d}$ | 6.25 | 1.56$^{e}$ | 3.12 |
| Tetracycline | 12.5$^{c}$ | 25 | 12.5$^{c}$ | 25 | 12.5$^{c}$ | 25 |

In all rows means MIC with the same letters are not significantly different. (P > 0.05)

**Table 2.** Inhibitory zone activity (in millimeter) of Ajwain against bacteria.

| Samples | *E. coli* | *P. viridiflava* | *P. syringae* |
|---|---|---|---|
| Callus extract (200mg/ml) | 7.8$^{f}$ | 7$^{f}$ | 7$^{f}$ |
| Callus extract (400mg/ml) | 9$^{e}$ | 8$^{f}$ | 8.7$^{f}$ |
| Seed extract (200mg/ml) | 11$^{e}$ | 12$^{e}$ | 12$^{e}$ |
| Seed extract (400mg/ml) | 14.5$^{d}$ | 13.9$^{d}$ | 15.3$^{d}$ |
| Seed essential oil (10% v/v) | 17$^{cd}$ | 14.2$^{d}$ | 15$^{d}$ |
| Seed essential oil (100% v/v) | 29$^{a}$ | 25$^{a}$ | 28.5$^{a}$ |
| Tetracycline | 17.5$^{cd}$ | 18.7$^{b}$ | 18$^{b}$ |

Means with the same letters are not significantly different. (P > 0.05)

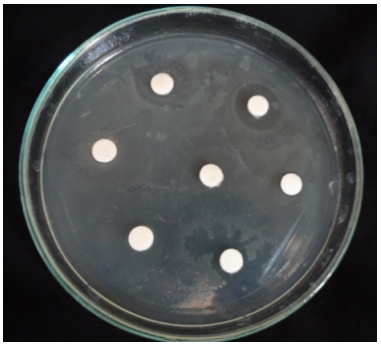

**Figure 1.** Inhibitory effect of *T. copticum* extracts on the growth of *P. syringae* pv. syringae.

The results showed significant inhibitory effect on the growth of bacteria. inhibition growth halo of bacteria of callus extract and seed extract and essential oil of the seeds were equivalent to 7-9 and 11-15 and 15-29 mm. The results of mean diameter of inhibition zone by disk diffusion method are presented in Table 2. The figure of inhibition zone by disk diffusion method of samples on the growth of *P. syringae* pv. syringae are showed in figure 1.

## DISCUSSION

Some researchers have reported the relationship between the chemical structure of important compounds in Ajwain with antibacterial effect on plant and compounds such as thymol, carvacrol, Gamatrpynn, cymene have been reported to have strong antibacterial effect [6, 22]. In a study examined Ajwain essential oil for antibacterial activity against canker of pome fruits (*P. syringae* pv. syringae). In this study inhibition growth halo of bacterial canker of pome fruits was equivalent to 20.2 mm. In the study inhibitor essential oil and extract halo of seed of Ajwain in conjunction with bacteria was calculated 15.3 and 28.5 mm. In a study examined *T. copticum* essential oil for antibacterial activity against a number of bacteria. In this study inhibition halo diameter was reported 21 mm for *E. coli* bacteria, the MIC and MBC were reported 0.031 and 0.062 % v/v respectively. It was concluded that Ajwain, due to monoterpenes compounds can be used as a natural antibacterial compound [23]. In the present study oil effects of Ajwain on pathogenic bacteria of the food was examined and the minimum inhibitory concentration was 5.4 to 12.5 µg/ml [24]. In this study minimum inhibitory concentration for essential oil of *T. copticum* on bacteria such as *E. coli* was in the range of 0.03 to 0.5 mg/ml, respectively [25]. In this

study minimum inhibitory concentration of Ajwain essential oil against bacteria *E. coli* and *Pseudomonas syringae* pv. syringae was determined less than 3.12 and 1.56 mg/ml. Researchers carried out a study on antibacterial and antifungal effect of ethanol extract of the herb, cumin on *E. coli, C. albicans, S. aureus,* and their results showed that the extract has significant effects on optional bacteria and fungi [26]. The effect of water extract, ethanol and acetone from 6 herbs against *E. coli, S. abony, S. typhimurium, S. aureus* was studied and the effects of plant extracts of Apiaceae family was more in comparison with other plants [27]. The results of this study showed that in the three sample the essential oil of Ajwain in inhibiting the growth of bacteria was effective. Since our country has favorable conditions for agriculture and allows production of medicinal plants at relatively low cost, there is the possibility of using them in fight against plant pathogens, along with other control methods that can prevent the indiscriminate use of antibiotics and pesticides. Several studies were conducted on the effects of antifungal [28], antiviral [29] and antibacterial [12], cell culture extracts of plants. The study which was conducted in 2012, antibacterial activity of methanol extracts of *cleome rosea* callus was confirmed but these effects compared to organs of the plant extract was less that correspond with the above results, these differences can be attributed to differences in tissue differentiation [13].

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# ارزیابی فعالیت ضد باکتریایی عصاره کشت این ویترو زنیان (*Trachyspermum copticum*) و مقایسه با عصاره بذر و اسانس

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## چکیده

زنیان (*Trachyspermum copticum*) گیاهی یکساله، از خانواده اپیاسه بوده که در ایران می‌روید. میوه‌های این گیاه بطور سنتی بعنوان داروی مدر، بادشکن و ضد انگل استفاده می‌شود. برخی اثرات بیولوژیکی زنیان مانند فعالیت‌های ضد ویروسی، ضد قارچی و آنتی‌اکسیدانی اثبات شده است. هدف از تحقیق حاضر ارزیابی فعالیت ضد باکتریایی عصاره‌های کالوس، بذر و همچنین اسانس بذر این گیاه بر علیه استرین‌های سودوموناس ویریدیفلاوا، سودوموناس سیرینجی و اشریشیا کلی بود. بعد از تهیه عصاره و اسانس، فعالیت ضد باکتریایی بر اساس هاله ممانعت‌کنندگی از رشد با استفاده از روش انتشار دیسک بررسی شد و حداقل غلظت بازدارندگی (MIC) و حداقل غلظت باکتری کشی (MBC) با استفاده از تکنیک رقیق‌سازی در حجم کم تعیین شد. نتایج بدست آمده تاثیر معنی‌دار عصاره بذر و اسانس زنیان در ممانعت از رشد باکتری‌های مورد آزمون را نشان داد، در حالیکه عصاره کالوس تاثیر معنی‌داری نداشت. بیشترین تاثیر توسط اسانس بر باکتری سودوموناس سیرینجی مشاهده شد. قطر هاله بازدارندگی رشد برای این باکتری ۲۸/۵ میلی متر و MIC و MBC به ترتیب ۱/۵۶ و ۳/۱۲ درصد تعیین شد.

**کلمات کلیدی:** فعالیت ضد باکتریایی، زنیان، کالوس