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Original Research

Evaluation of Antimicrobial Activity of *Trachyspermum ammi* (L.) Sprague Essential Oil and Its Active Constituent, Thymol, against Vaginal Pathogens

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Abstract

Vaginal infections are one of the major reasons women visit a gynecologist. Increased resistance to conventional antibiotics is one of the main factors mitigating the development of new antimicrobial agents, especially those of natural origin. In traditional Persian medicine, *Trachyspermum ammi* has been claimed to clear vagina from excessive discharge. Therefore, in this study, the antimicrobial activity of Ajwain essential oil was evaluated against some vaginal pathogens. The essential oil of ajwain was picked up and the minimum inhibitory and bactericidal concentrations (MIC and MBC) were revealed. The most frequently detected microorganisms involved in genital infections including *Candida* spp., *Gardnerella vaginalis*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Lactobacillus acidophilus* were considered. Evaluation of the essential oil of *Trichomonas vaginalis* was done by calculation of percent of growth inhibition. The essential oil showed a remarkable activity against the studied bacteria and fungi with MIC at a range of 0.0315 - 0.5 mg/ml and MBC at a range of 0.125 - 4 mg /ml. The highest inhibition and bactericidal activity was observed in *S. agalactiae* and *G. vaginalis*. 100% inhibition of *T. vaginalis* growth was shown at a concentration of 2000 µg/ml after 48 h by essential oil. The antimicrobial activity of the essential oil was more than that of thymol. Supposedly essential oil of *Trachyspermum ammi* fruit could inhibit vaginal pathogens growth .Further preclinical and clinical studies are required to confirm the efficacy of this natural agent in vaginitis.

Keywords: Ajwain; Medicinal plant; Thymol; Vaginitis; Trichomoniasis; Vaginal candidiasis

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Introduction

Vaginal infections may lead to pain, discomfort, dissatisfaction with sexual relationships and absence from school or work [1]. Symptoms such as exudation, itching and smell can cause distress and embarrassment. Vaginitis can cause several gynaecologic complications, such as pelvic inflammatory disease (PID), postoperative infections, cervicitis, vulvitis, positive urine cultures in neonates and neonates respiratory tract infection, preterm labour and chorioamnionitis [2-7]. In the United States, it is estimated that about 10 million hospital visits per year are for vaginal symptoms. One of the 3 infections: bacterial vaginosis (BV), trichomoniasis or vulvovaginal candidiasis (VVC) can cause vaginitis [8]. Overgrowth of anaerobic bacteria as *Gardnerella vaginalis* with diminution in *Lactobacillus* populations in the vagina lead to BV [9,10]. Prevalence of BV was 23 and 38% in Waves 1 (2005–2006) and 2 (2010–2011), and increased with age in United States [11]. Aerobic vaginitis (AV) inhibits the activity of lactobacilli by various aerobic bacteria and is provoked after unsuccessful treatment for BV [12-15].

Prevalence of VC in the UAE is increasing and the frequency of drug resistance to *Candida glabrata, Candida tropicalis* and *Candida krusei* is high [16-19]. The quality of life of young women is acutely influenced by RVVC [20]. Furthermore, continual treatments might induce drug resistance [21-24]. The most common non-viral sexually transmitted infection (STI) is trichomoniasis with almost 248 million new infections with *Trichomonas vaginalis* (TV) per year [25]. Approximately 60 to 80% of women with BV are coinfected with TV. Coexistence of BV and Candida species is 20 to 30% [26].

Azole-drug (metronidazole and tinidazole), nystatin and clindamycin are the standard treatments for vaginal infections [8,27,28]. Inadequate treatment success (about 50%) and increased recurrence rate (50%) within 6–12 months after drug discontinuation cause displeasure in most women [22-24]. Furthermore, allergic reaction and resistance to azole drugs are other problems encountered in the management of vaginitis [27-29]. The prevalence of resistance among VVC patients treated with fluconazole and itraconazole was more than 40% [30].

Medicinal plants are assumed as an influential source for discovering new drugs for various disorders including vaginal infections [31]. lately, great efforts to select and determin the mechanisms of plant compounds against vaginal infections have been carried out [32-34].

In Persian medicine, the fruit of *Trachyspermum ammi* has been claimed to eliminate excessive discharge of vagina and cervix and used for the treatment of vaginal infections [35]. The purpose of this study is to assess the antimicrobial activity of *T. ammi* essential oil as well as its active compound, thymol, against some vaginal pathogens.

Experimental

Plant material

Trachyspermum ammi was acquired from herbal market in Tehran and a voucher number (PMP-736) was alloted at the herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences (Tehran, Iran).

Preparation of essential oil of T. ammi

The essential oil of *T. ammi* was procured by hydrodistillation for 3 h.

GC-MS analysis of essential oil of T. ammi

An Agilent gas chromatograph 6890 plus (Agilent Technologies, Palo Alto, CA, USA) equipped with a 5973 quadrupole mass spectrometer was used for GC–MS analysis. The column used was the BPX5 5% phenyl column (30 m, 0.25 mm i.d., 0.25 µm film thickness; SGE, Scantec, Stockholm, Sweden).

Antimicrobial activity

Antimicrobial activity of the essential oil and thymol, were manifested against *Staphylococcus aureus* ATCC 25923, *Streptococcus agalactiae* PTCC 1768, *Escherichia coli* ATCC 25922, *Lactobacillus acidophilus* PTCC 1643, *Gardnerella vaginalis* ATCC 49145 and two pathogenic yeast: *Candida albicans* ATCC 10231 and *Candida krusei* PTCC 5295.

Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC) were determined by broth micro-dilution susceptibility method with using 96 well trays according to Standard protocol of Clinical Laboratory and Standards Institute (CLSI) with some modifications. Each experiment was conducted in triplicate. The inoculants of the microbial strains were adjusted to 0.5 McFarland standard turbidity sterile with normal saline and more diluted (1:100 for bacteria and 1:1000 for yeasts) just before addition to the wells containing a favorite content of diluted samples in Mueller-Hinton broth medium. Samples were assessed in a concentration range of 0.03 to 64 mg/ml. The results were recorded after incubation of inoculated trays for 22 h at 37°C. For determination of MBC, 100 µl of wells with no visible growth was cultured onto proper agar containing media and results were recorded after 24 h incubation at 37°C [36].

Anti-T. vaginalis activity

For checking the anti-*T. vaginalis* activity of the essential oil and thymol, trophozoites were cultured in TYM media at 24-well plates (5×105 cell/well) as triplicate and double blind. Metronidazole as positive control (50 µg/ml) and different concentrations (125, 250, 500, 1000 and 2000 µg/ml) of *T. ammi* essential oil and thymol were added to each well separately. The number of parasites in each well plate was counted after 24 and 48 h by trypan blue staining. In the negative control group, trophozoites were cultured in TYM media as triplicate without any drug.

The essential oil of *T. ammi* and thymol anti-*T. vaginalis* efficacy was evaluated by calculation of percent of growth inhibition (GI%) [37-41]. The IC_{50} was calculated using Prism 5 software.

Results

GC-MS analysis

The main components of the essential oil of T. ammi were thymol, ρ-cymene and γ-terpinene (Table1).

Table 1: Chemical constituents of the essential oils of *Trachyspermum ammi*

No	Rt	RI	Area% GCMS	Compound
1	4.03	921.0227	0.47	α-Thujene
2	4.17	928.9773	0.12	α-Pinene
3	4.85	967.6136	0.02	Sabinene
4	4.94	972.7273	2.15	β-Pinene
5	5.14	984.0909	0.3	β-Myrcene
6	5.7	1012.5	0.08	α-Terpinene
7	5.96	1024.107	22.06	p-Cymene
8	6.01	1026.339	0.5	Limonene
9	6.72	1058.036	26.78	γ-Terpinene
10	6.84	1063.393	0.02	cis-Sabinene hydrate
11	7.31	1084.375	0.01	α-Terpinolene
12	7.54	1094.643	0.01	Linalool
13	7.59	1096.875	0.04	trans-Sabinene hydrate
14	9.38	1170.782	0.07	Isogeranial
15	9.53	1176.955	0.07	4-Terpineol
16	10	1196.296	0.01	α-Terpineol
17	12.75	1299.254	47.05	Thymol
18	12.86	1303.529	0.22	Carvacrol

Antimicrobial activity

The results of this research showed that the essential oil showed that essential oils work well against the bacteria and fungi studied with MIC at a range of 0.0315 to 0.5 mg ml^{-1} and MBC at a range of 0.125 to 4 mg ml^{-1}. The highest inhibition and bactericidal activity, respectively were observed on the *Streptococcus agalactiae* and *Gardnerella vaginalis*. For thymol, the MIC was from 0.0625 to 2 mg ml^{-1} (Table 2).

Table 2. Anti-microbial activity of *Trachyspermum ammi* essential oil

Microorganism	Essential oil		Thymol		Standard antimicrobial*	
	MIC mg/ml	MBC or MFC mg/ml	MIC mg/ml	MBC or MFC mg/ml	MIC µg/ml	MBC or MFC µg/ml
Escherichia coli ATCC 25922	0.5	1	0.0625	0.0625	2	8
Staphylococcus aureus ATCC25923	0.0625	4	0.5	1	0.06	0.125
Streptococcus agalactiae	0.0312	1	0.0625	0.25	0.03	0.06
Candida Albicans ATCC10231	0.5	1	2	1	31.25	31.25
Candida krusei PTCC5295	0.0625	0.5	0.0625	0.25	7.81	7.81
Lactobacillus acidophilus PTCC1643	0.5	0.5	2	4	4	8
*Gardnerella vaginali-s*ATCC 49145	0.06	0.125	0.125	0.25	>500	>500

* Cefixime for *E. coli*, *S. aureus* and *S. agalactiae*; Clindamycin for *L.acidophilus* and *G. vaginalis*; Nystatin for *C. krusei and C. Albicans*

Anti-T. vaginalis activity

As regards *T. ammi* essential oil and thymol treated culture, there were 91% inhibition of the TV growth after 24 h incubation at a concentration of 2000 and 4000 µg/ml respectinely. (Tables 3 and 4).

The IC_{50} of essential oil of *T. ammi* and thymol after 24 and 48 h is shown in Figure 1.

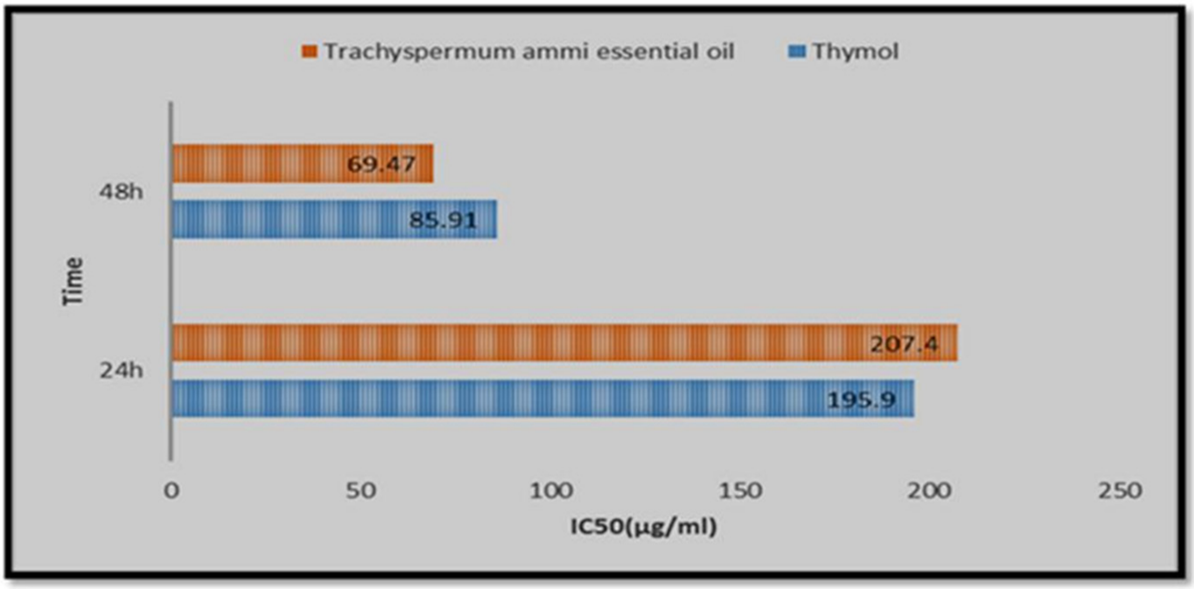

Figure 1: IC_{50} essential oil of *Trachyspermum ammi* and thymol after 24 and 48 h

Discussion

A large number of antibiotics have been discovered from plant sources and have an influential role in the treatment of infectious diseases.

The current findings showed that *T. ammi* essential oil contained thymol (47.05%), ρ-cymene (22.06) and γ-terpinene (26.78) as the main components similar to that of previous investigations.

Thymol seems that is active against *C. albicans* [42]. Based on this study, thymol and essential oil had almost the same effects on Candida spp.

In another research, Ajowan essential oil had a good inhibitory effect on Fluconazole-susceptible and resistant *C. albicans* growth. Although, the FLU-susceptible group was more irritable, the two groups did not have a significant difference. Therefore, these essential oils can reduce resistance to azoles [43].

As shown in this study, *T. ammi* essential oil has a good effect on inhibition of the growth of *Gardnerella vaginalis* with MIC = 0.06 mg/ml, while MIC for *Lactobacillus acidophilus* was 0.5 mg/ml. Based on this study, essential oils have a better effect than thymol and clindamycin.

G. vaginalis often override during BV and is supposed to create a biofilm [44,45]. Adhered *G. vaginalis* will start to form a biofilm by repositing lactobacilli [46]. Treatment failure and recurrent disease are common in therapy for BV [47]. Therefore, it seems more studies on *T. ammi* essential oil can have significant effects on biofilms of *G. vaginalis*.

The prevalence of diagnosed AV varies from 5 to 10.5%. *Streptococcus* spp., *S. aureus* and coagulase-negative staphylococci and *E. coli* were the most common pathogen identified. Carvacrol and thymol, significantly inhibited uropathogenic *E. coli* (UPEC) biofilm formation via reduction of fimbriae production and the swarming motility of UPEC. Additionally, carvacrol and thymol considerably decreased the hemagglutinating strength of UPEC [48]. Based on another study, thymol and carvacrol mixtures can be associated with changes in host colon flora (increasing *Lactobacillus crispatus* and *Lactobacillus agilis* and on the other hand reducing *Lactobacillus salivarius* and *Lactobasillus johnsonii*), decrease inintestinal ulcers and decrease in mortality rate in chickens infected with this bacteria [49]. Positive effects of ρ-cymene have been proven with increase in carvacrol antimicrobial activity with reduction in the activity of O157; H7 *E. coli* and *Vibrio cholerae* in juices and foods [50,51]. ρ-Cymene have synergistic effects with carvacrol, 4-terpineol and nicin, and therefore, reduce the concentration of antimicrobials in the pharmaceutical, food and cosmetic industries [52].

In the present study, the effect of bactericidal activity of thymol on Gram negative bacteria is greater than that of essential oil. In the case of Gram-positive bacteria, the bactericidal effect of the essential oil is greater than that of thymol alone.

Hassanshahian et al .showed that resistant *E .coli* and *S. aureus* were sensitive to the essential oil at a concentration of 100 ppm [53]. High dose of thymol inhibits Gram-positive more than negative bacteria. It seems that phenolic compounds interfere with cell membrane, change pH and ions homeostasis [43].

These results show that thymol is not exclusively responsible for anti-trichomonas activity of essential oil and this activity may be enhanced by other constituents of essential oil via their synergistic activity or enhance penetration of thymol into TV.

It seems that *T. ammi* essential oil is a potent inhibitor of the growth of all vaginal pathogens including bacterial, fungal and parasitic pathogens *in vitro* and thus can be assumed as a promising medicine for the management of all types of vaginal infection including BV, VVC and trichomoniasis. Also, other components of extract may increase the effect of thymol on *T. vaginalis*. Further investigations are needed to confirm the efficacy and mechanism of action of *T. ammi* essential oil in vaginitis.

Table 3. Percent of growth inhibition after addition of different concentrations of *T. ammi* essential oil on *Trichomonas vaginalis* twice

Concentration	24h	48h
Negative control	-	-
Positive control (50µg/ml)	100%	100%
125µg/ml	41%	65%
250µg/ml	52%	76%
500µg/ml	64%	85%
1000µg/ml	80%	92%
2000µg/ml	91%	100%

Table 4. Percent of growth inhibition after addition of different concentrations of thymol on *Trichomonas vaginalis* twice

Concentration	24h	48h
Negative control	-	-
Positive control (50µg/ml)	100%	100%
125µg/ml	44%	65%
250µg/ml	58%	73%
500µg/ml	67%	79%
1000µg/ml	77%	87%
2000µg/ml	88%	93%
4000µg/ml	91%	97%

Conflict of Interest

None.

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