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Original article

Potential of *Trachyspermum ammi* (ajwain) Gel for Treatment of Facial Acne vulgaris: a Pilot Study with Skin Biophysical Profile Assessment and Red Fluorescence Photography

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Abstract

Background and objectives: Acne vulgaris is one of the most common dermatologic conditions. The available anti-acne treatments are not satisfactory and safe. In this regard, searching for new treatments, especially natural materials with reasonable side effects and satisfactory effectiveness, could be promising. The aim of the present study was to explore the safety and efficacy of a topical formulation containing *Trachyspermum ammi* (ajwain) fruits essential oil in patients with facial acne. **Methods:** The essential oil of the fruits was extracted by hydrodistillation method and formulated as a 1% gel. In this open-labeled, uncontrolled clinical trial, 20 patients with mild to moderate acne received topical ajwain gel twice daily for 8 weeks. The outcomes of acne lesion count, red fluorescence parameters and biophysical skin profiles were evaluated at baseline, 4th and 8th weeks. Any adverse reaction was recorded during the study. **Results:** All patients completed the study. Two months after treatment, the mean reduction in the total (8.2±3.36; *P*=0.000) and non-inflammatory (7.3±4.53; *p*=0.000) lesions was statistically significant. Furthermore, a significant reduction in the size and quantity of red fluorescence spots was also observed. Biophysical skin profile measurements revealed a significant reduction in erythema (*p*=0.033) and sebum (*p*=0.026) and a significant increase in pH (*p*=0.005). No serious adverse events were reported. **Conclusion:** The results of this pilot study provided a basis for the effectiveness of topical ajwain gel for the treatment of mild to moderate facial acne. Conducting further double blind clinical trials are necessary to confirm the efficacy and safety of the product.

Keywords: acne vulgaris; ajwain; clinical trial; essential oil; *Trachyspermum ammi*

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Introduction

Acne vulgaris is one of the most common causes of referring to dermatologists [1]. This disease is characterized by non-inflammatory black and white comedones and inflammatory papules, pustules, nodules and cysts on the face, neck, and the trunk [2]. The chronic nature of the disease and its appearance on face and body may lead to anxiety, depression, decreased self-esteem and in

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severe cases, suicidal thoughts in patients [3].

Acne is considered a pilosebaceous unit disorder which the following factors are involved in its pathogenesis: pilosebaceous canal obstruction due to hypercratization, increased sebum production, *Propionibacterium acne*, inflammation and oxidative stress [4].

Topical therapy is the mainstay of mild to moderate acne treatment. Retinoids and antimicrobial drugs such as benzoyl peroxide and antibiotics are the most common topical medications administered for this disease [2]. These medications target one or several pathogenetic factors via reducing follicular hyperkeratosis and anti-inflammatory, antioxidant, antimicrobial, keratolytic and sebostatic activities [5]. Increased bacterial resistance to antibiotics and skin irritation are the most common side effects of the available anti-acne treatments [6]; therefore, there is a need to develop new, safer and more effective therapies [7]. Natural medicines, by their multi-component nature and long-term use by humans, may be good candidates for this purpose [8]; however, there is not enough evidence for this claim and further studies are needed [9]. Persian medicine (PM) is one of the oldest traditional medicine systems in the world with a vast majority of experiences in treatment of diseases with medicinal herbs [10]. PM Scholars believed that skin has an important role in the secretion of waste products and any disturbance in this process may cause skin rashes and facial spots similar to acne. In traditional literatures, many kinds of topical and oral preparations are mentioned for skin cleansing in different dermatologic conditions [11].

Trachyspermum ammi L. (synonym: *Carum copticum*), commonly named ajwain and known as "Zenyan" or "Nankhah" in PM, belongs to the Apiaceae family [12]. In PM resources, topical use of ajwain fruit has been recommended for treatment of a variety of skin conditions including acne [13]. Furthermore, the anti-inflammatory [14,15], antioxidant [16-21] and antibacterial [22,23] properties of these fruits have been confirmed in pharmacologic studies. Despite traditional use and related evidences for probable anti-acne properties of ajwain fruits, no study has evaluated their safety and efficacy for treatment of acne. This pilot study aimed to evaluate the safety and efficacy of a topical formulation of ajwain essential oil for treatment of facial acne in a phase 2A clinical trial. To provide more objective results, we used fluorescence digital photography and skin biophysical parameters assessment.

Material and Methods

Ethical considerations

The study protocol was approved by the Research Ethics Committee of Tehran University of Medical Sciences (approval number IR.TUMS.REC.1394.2063 on 26/02/2016) and the trial was registered in the Iranian Registry of Clinical Trials (www.irct.ir, registration number: IRCT2016031126938N3). All patients provided written informed consent before entering the study.

Plant material

Ajwain fruits were purchased from a medicinal plant market (Tehran, Iran). They were ground in a mechanical grinder for 60 s, just before extraction of the essential oil.

Essential oil extraction

The essential oil was obtained from 500 g of ground powder of ajwain fruits by hydro-distillation method using Clevenger apparatus for 2.5 h. The obtained essential oil was yellow in color, had a pungent odor (because of thymol) and a density of 0.89 mg/mL. It was collected in a glass container and kept at refrigerator (4±1°C) and protected from light until used.

Gas chromatography analysis

The essential oil was diluted 1:100 in n-hexane (HPLC grade, Merck, Germany) before injection to Gas chromatography column. The identification of essential oil components was carried out by a GC/MS system (Agilent 6890 for Gas Chromatograph, and 5973 for Mass spectrophotometer, USA) equipped with a BPX5 column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Electron ionization (70 eV) was used for system detection. The carrier gas was helium with a flow rate of 1 mL/min. The injection room temperature was set at 250 ° C. The initial column temperature was set at 50 ° C for 1 min, which was then raised to a final temperature of 280 ° C by a rate of 5 ° C/min. The operation was done in 56 min. The identification of components was made by calculation of Kovats indices, calculated by injection of a mixture of

homologous n-alkanes (C_8-C_{25}) on GC column and further confirmation of proposed structure (by software library, Wiley nl7) with standard spectra [24]. Quantitative analysis of each compound was based on the relative area under the curve of each peak in the spectrum.

Preparation of topical gel

Based on maximum dermal use of 1.4% (w/w) for ajwain essential oil [24], a 1% (w/w) gel, was prepared by dissolving 5 g ajwain oil in hydro-alcoholic solution (containing 50 g propylene glycol, 320.8 g 96% ethanol and 119 g distilled water) and then pre-hydrated gelling agent (5 g carbomer 941) was added to this mixture under stirring (400 rpm). Finally, triethanolamine was added slowly until the final gel was formed.

Physicochemical evaluation of formulation

Physical features of the ajwain gel were assessed based on the ICH stability guideline. The prepared gel was stored at 25±2 °C and 60±5% relative humidity for 6 months. During this period, the color, odor, consistency, and homogeneity of the ajwain gel was analyzed visually.

The viscosity of gel was examined by a DV1™ digital viscometer (Brookfield, Spain) using spindle 5 at 100 rpm with the runtime of 15 seconds.

About 2 g of the gel was dissolved in 20 mL distilled water and the pH was determined by calibrated pH meter (Metrohm 827, Swiss).

Study design

This open-label uncontrolled clinical trial was conducted between December 2017 and April 2018 in Center for Research and Training in Skin Diseases and Leprosy (CRTSDL) of Tehran University of Medical Sciences, Tehran, Iran.

Inclusion and exclusion criteria

The inclusion criteria was: age of 20-58 years; mild to moderate acne affection without cystic acne; having at least 15 inflamed and 15 non-inflamed acne lesions on the face. Acne severity was determined according to the number of papules and pustules per half face: 0-5 = mild, 6-20 = moderate, 21-50 = severe and more than 50 = very severe [26]. The following conditions were considered as exclusion criteria: (1) the presence of nodulocystic acne lesion, (2) pregnancy, lactation, (3) excessive sun or UV light exposure, (4) allergy or sensitivity to product ingredients, (5) use of topical anti-acne treatments or procedures four weeks prior to the study, and (6) use of systemic anti-acne medications six months prior to the study. The dermatologist referred eligible patients to researcher.

Finally, twenty volunteers aged 20-58 years with mild to moderate facial acne from both sexes were chosen through convenience sampling by a dermatologist

Intervention

After obtaining informed consent from the patients, they were instructed to use the topical gel on their face, twice daily (morning and night) for 8 weeks.

Outcome measurement

To determine the therapeutic efficacy, clinical assessment, digital photography, fluorescence photography, and biophysical skin profile measurement were evaluated at baseline and at the week 4, and week 8 follow-up visits.

For clinical evaluations, the count of acne lesions (inflammatory, non-inflammatory, and total) was determined by a dermatologist. Additionally, Patients satisfaction from the treatment efficacy was assessed using a Visual Analog Scale (VAS) of 0 (totally unsatisfied) to 10 (totally satisfied). Local adverse effects such as erythema, itching, edema, scaling, dryness and burning/stinging were recorded during the study.

Digital photographs of the face were captured using an identical camera and photographic conditions at baseline and each follow-up session. A consent form was obtained from each individual to use their photograph for research purposes.

Fluorescence digital photography was performed using the Visiopor® PP 34 camera (Courage & Khazaka, Germany), with a narrow-band UVA light of 375 nm. Photographs were taken from the right cheek. The quantity and size (percentage of the area covered) of orange-red spots in a measured area of 6×8 mm were analyzed.

Six skin biophysical parameters (stratum corneum hydration, sebum content, trans-epidermal water loss (TEWL), erythema index, melanin and pH value) were measured using the Cutometer® dual MPA 580 (Courage-Khazaka, Germany) with the following probes: Corneometer CM 825, Sebumeter SM 815, Tewameter TM 300, Mexameter MX 18 and pH-Meter pH 908. These parameters were assessed by the same investigator on the right cheeks of the face in a laboratory with

20-25 °C (room temperature) and a relative humidity of 35-50%.

Statistical analysis

Statistical analysis was performed using SPSS 16 software (SPSS Inc., USA). The descriptive data were expressed as the mean ± SD. The paired T-test with a significance level of 0.05 was used for comparison between baseline and post-treatment values.

Results and Discussion

The main components of the oil were identified as thymol (50.17%), gamma-terpinene (16.95%) and para-cymene (27.11%). The results of this section have been presented in table 1.

The dermatologist conducted the primary screening on thirty patients with facial acne vulgaris and referred a group of twenty eligible patients (2 males and 18 females) with mild to moderate disease to the researcher. Ten patients were excluded due to acne severity and concurrent use of anti-acne treatments.

All patients recruited completed the study. All patients had moderate acne. The average age of the patients was 21.4 ± 3.4 years (range, 20-40 years). Nine patients declared a family history of acne. Twelve patients had persistent acne (began in adolescence) and rest of them had late-onset acne (first appears after 25 years old). The baseline demographic and clinical characteristics of patients have been presented in table 2.

8 weeks after treatment, a mean reduction of 8.2 ± 3.36 (p = 0.000) in total lesion count, 7.3 ± 4.53 (p = 0.000) in non-inflammatory lesion count and 0.9 ± 2.77 (p = 0.16) in inflammatory lesion count was observed. The reduction in the total and non-inflammatory lesions was statistically significant ($p \leq 0.05$). The Mean (SD) count of different lesion types has been summarized in table 3.

In fluorescence digital photography, the mean decrease in the size (0.46 ± 0.7; P = 0.009) and quantity (8.1 ± 6.6; P = 0.000) of red spots versus baseline were significant. The effect of ajwain gel on red fluorescence parameters has been depicted in figure 3.

Two months after using ajwain gel, the mean value of sebum decrease (23.05 ± 42.6; p = 0.026) and pH increase (0.48 ± 0.68; p = 0.005) were significant. Additionally, the results showed a decrease of 6.56 ± 18.99 in skin hydration and an increase of 2.66 ± 11.43 in trans-epidermal water loss (TEWL). Finally, Melanin index had an average increase of 2.17 ± 27.25 and erythema index had an average decrease of 37.71 ± 73.46 versus baseline. The value of skin biophysical parameters at baseline and follow-up visits has been shown in table 4.

At the final visit, the mean VAS score of patient satisfaction was 5.98 ± 1.14 (ranged 4-7). Ajwain gel was well tolerated and no adverse event was reported.

The clinical effectiveness of the ajwain gel has been shown in figure 1.

Acne vulgaris is a very common and chronic pilosebaceous unit disorder with a complex pathogenesis [27]. None of the available treatments for this disease are completely satisfactory and safe. Studies on natural substances with anti-acne properties could potentially help in developing efficient alternative treatments with fewer side effects [28].

Table 1. Chemical composition of *Trachyspermum ammi* essential oil, obtained by hydrodistillation method

No.	Compound	RI[1]	KI[2]	RT[3]	Percentage
1	*β*-Pinene	981	980	10.17	1.4
2	*β*-Myrcene	992	991	10.76	0.27
3	***p*-Cymene**	1031	1026	12.00	27.11
5	***γ*-Terpinene**	1063	1062	13.17	16.95
6	Terpinen-4-ol	1190	1177	24.71	0.2
9	Cinnamaldehyde	1275	1270	19.78	0.31
10	**Thymol**	1306	1290	20.457	50.17
11	Carvacrol	1312	1298	20.52	0.86
	Monoterpenes hydrocarbons				7.9
	Oxygenated monoterpenes				0.5
	Aromatic monoterpenes				90.7
	Total identified				99.1

[1]Relative index; [2]Kovats index (based on literature); [3]Retention time

Table 2. Baseline demographic and clinical characteristics of patients involved in trial

Demographic/clinical parameter Characteristic	Age (year)		Gender		Severity of Acne		
	Mean	Min/Max	Male	Female	Mild	Moderate	Severe
	21.4	20/40	2	18	0	20	0

Table 3. Mean counts of total, inflammatory and non-inflammatory lesions at baseline, 4th and 8th week after treatment and percenage reduction versus baseline

Type of lesions	Baseline (n=20)	Week 4 (n=20)		Week 8 (n=20)	
	Mean (SD)	Mean (SD)	% reduction	Mean (SD)	% reduction
Non-inflammatory	12.35 (6.4)	8.10 (5)	34	5.05 (4.09)	61
Inflammatory	3.10 (2.12)	3.00 (1.91)	3	2.20 (2.04)	29
Total	15.45 (5.62)	11.10 (5.24)	28	7.25 (4.62)	55

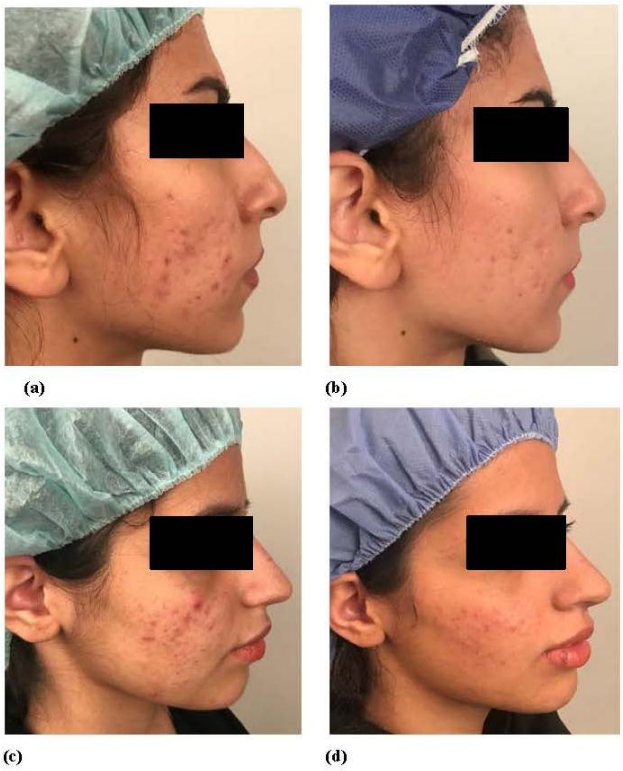

Figure 1. Digital photographs, at baseline (a & c) and 2 months (b & d) after application of 1% (w/w) ajwain gel depicts a decrease in the facial acne lesions

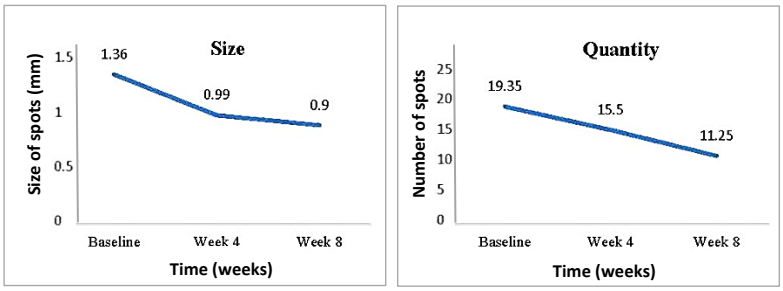

Figure 2. Mean value of red fluorescence parameters at baseline, 4th week and 8th week after treatment with *Trachyspermum. ammi* gel

Table 4. Mean value of skin biophysical parameters at baseline, 4th and 8th week after treatment

Parameters	Baseline (n=20)	Week 4 (n=20)	Week 8 (n=20)	p value*
	Maen (SD)	Maen (SD)	Maen (SD)	
Hydration	59.44 (13.56)	58.95 (11.33)	52.87 (16.86)	0.138
TEWL	20.02 (5.52)	19.41 (5.48)	22.68 (10.41)	0.311
Sebum content	74.3 (31.5)	52.15 (52.23)	51.25 (26.51)	0.026
Skin pH	6.44 (0.56)	6.56 (0.53)	6.93 (0.36)	0.005
Erythema index	411.33 (95.44)	378.75 (90.74)	373.61 (101.11)	0.033
Melanin index	179.45 (26.03)	178.84 (30.43)	181.61 (34.84)	0.726

*p_{value} of paired t-test between baseline and 8th week

In this study we investigated the clinical efficacy and safety of a topical gel made from *Trachyspermum ammi* fruits in patients with acne vulgaris. Our pilot study showed that ajwain gel was clinically efficient for treatment of mild to moderate facial acne. After two months, a statistically significant decrease was observed in the mean number of total and non-inflammatory acne lesions. Furthermore, size and quantity of red fluorescence spots, skin sebum and erythema index declined significantly after treatment. Porphyrins are metabolic products of *P. acnes* and are strongly fluorescent [29]. Significant decrease in fluorescence parameters in our study may be explained by anti-bacterial effect of ajwain essential oil.

Propionibacterium acnes is an anaerobic, gram-positive commensal bacterium that normally lives on the skin. Excessive sebum and keratinocytes derbies could plug hair follicles and make a favorable anaerobic environment for the growth of this bacterium in the pilosebaceous unit [30]. Release of porphyrins, chemotactic factors and enzymes by *P. acnes* potentiate the oxidation of sebum lipids and neutrophils accumulation at acne prone sites. All of these events result in cutaneous oxidative stress, inflammatory cascades, tissue injury and acne lesion formation [31].

Significant therapeutic response after using ajwain gel in our study may be explained by antimicrobial, anti-inflammatory and antioxidant properties of *T. ammi* fruits. Among the normal skin flora related to acne vulgaris, antimicrobial activity of *T. ammi* against *Staphylococcus aureus* [32-36] and *Staphylococcus epidermidis* [35] have been documented in several studies. *Alpha*-pinene, a minor ajwain essential oil constituent, has been found to have antimicrobial activity against *S. aureus*, *S. epidermidis* and *P. acnes* [37].

Known mechanisms for anti-inflammatory effects of *T. ammi* fruits are free radicals scavenging activity [38] and suppressing the production of nitric oxide and pro-inflammatory cytokines i.e. IL-18 and TNF-α [39,40]. These therapeutic effects are mainly attributed to major phenolic compounds of *T. ammi* named thymol and carvacrol [41].

The topical preparation of our study was well tolerated and no adverse events were reported. Acidic pH of the skin is important for keratinization, skin barrier regeneration and cutaneous antimicrobial defense [42]. Although at the end of the study, skin pH was still in acidic range, a significant rise in pH (6.44 ± 0.56 to 6.93 ± 0.36) compared with baseline was observed. Other unwanted effects of ajwain gel on skin barrier properties were mild non-significant increase in TEWL and melanin index and decrease in hydration two months after treatment.

To our knowledge, this is the first clinical trial evaluating the effect of ajwain fruit in acne vulgaris.

Tea tree oil is one of the proposed herbal treatments for acne vulgaris. In a study by Malhi et al. mean percent decrease in total lesion count after applying tea tree oil gel and face wash at week 4 and 8 were respectively 25% and 37% in comparison with 28% and 55% in our study [43],

Compared with the results of a similar study on the use of cinnamon gel, ajwain gel was more efficient in reduction of the total (55% vs.47%) and non-inflammatory (61% vs.48%) lesion counts after 8 weeks. Applying cinnamon gel caused a significant decrease in fluorescence spots size whereas ajwain gel could significantly decrease both the size and quantity of the spots. Changes of skin biophysical profile after applying ajwain and cinnamon gel was similar as follow: both preparations increased TEWL, melanin index and decreased hydration, erythema and sebum [44].

In conclusion, our results suggest that twice daily use of topical ajwain gel for two months could be safe and effective in the treatment of mild to moderate facial acne vulgaris. This is the first study about ajwain application for treatment of acne vulgaris and double-blind randomized clinical trials with larger sample size are needed to confirm these findings. In addition, further studies are warranted to elucidate the action mechanism of *T. ammi* fruits on acne pathogenic factors and its long-term effects on skin barrier properties.

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Author contributions

Mahdi Vazirian designed the study and revised the manuscript; Sima Kolahdooz prepared the manuscript and was involved in data analysis;

Ziba Talebi and Maedeh Ghovvati conducted the experiments related to pharmacognosy and pharmaceutical and clinical parts; Gholamreza kord Afshari and Mehrdad Karimi were involved in patient recruitment; Saman Ahmad Nasrollahi formulated the gel; Alireza Firooz supervised the clinical part; Aniseh Samadi was involved in data analysis.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

VAS: Visual Analog Scale; TEWL: Trans-epidermal water loss