Preliminary evaluation of antimicrobial activity of cream formulated with essential oil of *Trachyuspermum ammi*

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Abstract: In our present research we studied physiochemical properties and antimicrobial activity of *T. ammi* (*Trachyuspermum ammi)* seeds. The seeds yielded 2.86% essential oil. Refractive index, specific gravity, acid value, and easter values were 1.496, 0.9212, 1.12 and 2.80 respectively. GLC was used to determine the composition of oil. The major component of oil was Thymol (55.308%). Antimicrobial activity of the oil was carried out against four species of bacteria and fungs. The oil was used to formulate a cream and the pH, physiochemical stability, phase separation and dermal irritation of cream were investigated. The cream was examined for healing wound in rabbits in comparison with Iodine tincture.

Keywords: *Trachyuspermum ammi,* essential oil, wound healing activity, dermal irritation test.

INTRODUCTION

Plants have been used as medicines since centuries and recently they are used in the formation of various medicines, drugs and food (Bakhru, 1998). Pharmacological properties of essential oils are well known for their possible antimicrobial properties (Leal-Cardoso & Fonteles, 1999). Moreover, these oils are also believed to be useful against breathing and topical infections, as well as in cold and fever (Nakamura *et al*., 1999). Infectious diseases constitute prominent problem in the developing countries since the microbes amplify their resistance due to indiscriminant use of antibiotics (Davies, 1994).

Trachyspermum ammi also known as Ajowan belongs to the family Apiaceae (Bentely and Trimen, 1999). In folklore medicine it is believed to have stimulant and energizer properties. Its essential oil, in addition to pleasant flavor, is considered to be very useful in aroma therapy (Frag *et al*., 1990). It is claimed to cure digestive gases and diarrhea (Bentely and Trimen, 1999). The essential oil of Ajowan is reported to show very good efficacy against microbes (Singh *et al*., 1997; Diwivedi and Singh, 1999)

Healing of wounds is a complex biological process consisting of a number of steps involving coagulation, inflammation, proliferation and remodeling involving activity of blood cells, cytokines and growth factors (Clark, 1991; Eming *et al*., 2007).

Experimental

The fresh seeds of *Trachyspermum ammi* were purchased from the local market of Lahore and were identified from the botany department of Punjab University. The seeds were cleaned from the impurities and crushed with the help of pastel and mortar in order to expose the oil glands. The extraction was carried out by steam distillation using reverse Dean Stark steam distillation method (Sattar, 1989) and anhydrous sodium sulfate was used to remove moisture content. The oil was preserved in yellowish-brown bottle and stored at 4°C.

Physicochemical investigations

Specific gravity of the essential oil of *Trachuspermum ammi* was determined by specific gravity bottle at 35°C. Solubility and refractive index was determined by (IUPAC, 1979). Acid value and ester value were determined by methods described elsewhere (Williams, 1966).

Equipment

The refractive index of the oil was measured on the Abbe's Refractometer Atago 3T. pH was determined by Mettler Toledo seven multi pH meter. GLC was used to analyze the components of essential oil and 0.2 µl of the oil was injected in the chromatograph and run on GC-14A equipped with Flame Ionization Detector with SE 30 Packed glass column having the column temperature of 70°C/2 min-5°C/min-200°C and Nitrogen as a carrier gas. Shimadzu Chromatopac C-R 4A detector at 250°C with charter speed of 3cm/min was used. The detection of various components was made by comparison of their retention time with standard chemical and by co-injection technique (Grob Jr, 1984). Gas chromatogram of *Trachuspermum ammi* is given in fig. 1.

Antimicrobial Activity

Mould (*Aspergillus oryzae, Aspergillus niger, Penicillium digitatum*, Mucor), yeast (*Candida utilus*) and bacteria (*E. coli, Lactobacillus acidophilus, Staphylococcus*

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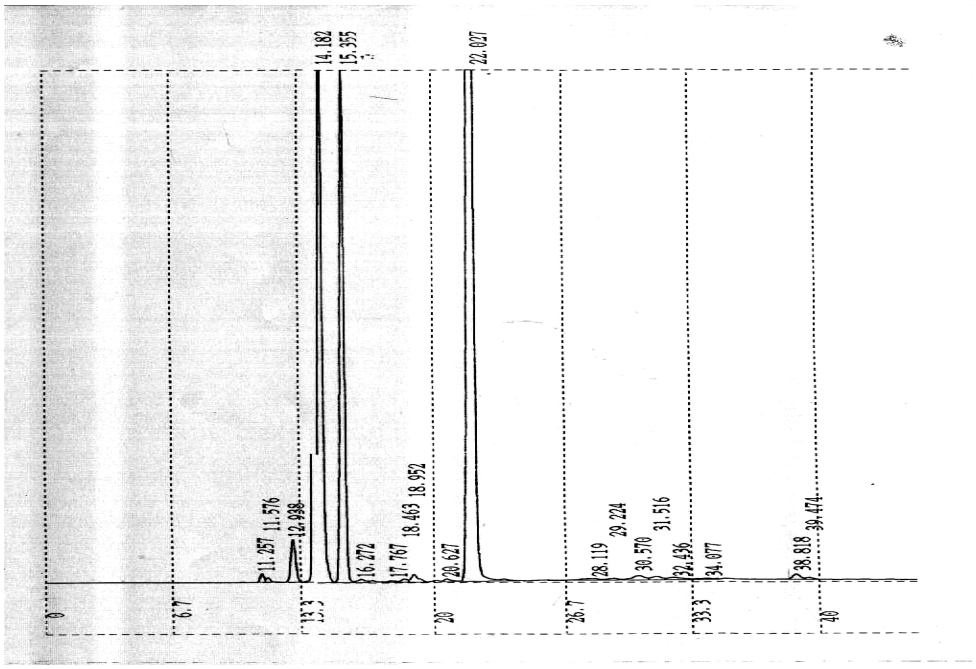

Fig. 1: Gas Liquid Chromatogram of the *essential oil of Trachyspermum ammi* seeds

aureus, Micrococcus luteus) were selected for evaluation of the antimicrobial activity. Disc method was opted for antimicrobial assay (Berghe *et al*, 1991). Lacto bacillus acidophilus were inoculated on MRS (De Man's Rogosa and Sharpe) broth, *Escherichia coli* were inoculated on N.A. (nutrient agar) while mould (*Aspergillus Niger, Aspergillus oryzae, Penicilium digitatum*) were inoculated on Potato Dextrose Agar (P.D.A). Sterile 4 mm paper discs were impregnated with essential oil of *T. ammi* and placed on the newly seeded bacterial lawns. One control plate for each bacterium was also prepared. After 24 hours; the inhibition zones were measured with the help of a scale to the nearest mm.

Formulations

Cream

Cream was prepared with Phase A (40% Emulsifying wax, 1% Cetyl alcohol, 1% Bee's wax, 1.3% Lanolin at 75°C), phase B(100mL Distilled Water and 0.15% preservative (Methyl Parahydroxy Benzoate) and Phase C (6% Glycerine and 5% essential oil of *Trachyspermum ammi*). The ingredients of phase A were mixed and heated at 75°C. The ingredients of phase B were mixed separately. The mixture of phase B was mixed with phase A and stirred well at room temperature till the complete mixing. The ingredients of phase C were mixed with mixture of phase A and B and stirred well till the complete mixing.

Physiochemical stability

Physiochemical stability of the prepared cream was determined according to World Health Organization method (Casagrande *et al*., 2007; Singh, 1999). The cream was stored at 4°C, 30°C with 60% relative humidity and 40°C with 70% relative humidity for six months (Casagrande *et al*., 2007; Singh, 1999) and than observed for their stability.

PH Value

Cream was mixed with deionized water in 1:2 and pH was determined at room temperature (Anchisi *et al*., 2001; Di Mambro *et al*., 2007).

Phase separation

Phase separation was visually observed after the sample was centrifuged at 1660 revolutions per minutes for about 30min at room temperature (Anchisi *et al*., 2001).

Animals

Healthy rabbits were purchased and kept in polypropylene cages in controlled temperature of 25±1°C and 50±2% relative humidity. The study was carried out in according to the international animal ethics and approved from laboratory animal ethics committee.

Dermal irritation test

Occluded dermal irritation test method was adapted to determine the degree of skin irritation in rabbits (Auletta, 1995). Healthy rabbits of same age and weight were divided into three groups. Group 1 was left untreated, group 2 was treated with *T. ammi* cream and group 3 was treated with market available Iodine tincture. Tincture was composed of 25gm of Iodine and Potassium Iodide. 25ml of water and 90% alcohol was added to produce 1000ml tincture. After light anesthesia the hair was removed from dorsal body area of the rabbits. After 24 hours thin layer of *T. ammi* cream and market available Iodine tincture were applied evenly and covered. After 24 hours rabbits

were observed for the presence of erythema and edema as described by Draize dermal irritation scoring system (Draize, 1959).

Excision Wound Testing Of Formulation

Biological assay of Cream

Cream was evaluated biologically using excision wound model. Healthy rabbits of same age and weight were divided into three groups. Group 1 was left untreated, group 2 was treated with T ammi cream and group 3 was treated with Iodine tincture.

Table 1: Chemical composition of essential oil of *Trachyspermum ammi* (seeds) by GLC analysis

Peak #	Retention time	Compound	Concentration %
1	11.257	α- thujene	0.2893 %
2	11.576	α- pinene	0.1509 %
3	12.938	Myrcene	1.7638 %
4	14.182	Limonene	20.5294 %
5	15.355	α- terpinene	18.9218 %
6	16.272	P – cymene	0.185 %
8	17.767	Unidentified	0.0998%
9	18.4 63	Δ-carene	0.1561 %
10	18.952	Unidentified	0.4417 %
12	20.627	Unidentified	0.1453 %
13	22.027	Thymol	55.308 %
15	28.119	α- terpinenol	0.2404 %
16	29.224	Carveol acetate	0.1462 %
17	30.57	Carvone	0.441 %
18	31.516	Carveol	0.2813 %
19	32.436	Unidentified	0.2972 %
20	34.077	Unidentified	0.1081 %
22	38.818	Bergeptene	0.3705 %
23	39.474	Unidentified	0.1243

Table 2: Antimicrobial activity of essential oil of *T. ammi*

	Test Organisms	Inhibition zones in mm ±SDV
Test bacterias	*Lactobacillus acidophilus*	0.00 ± 0.0
	Escherichia coli	14.3± 0.031
	Micrococcus luteus	05.2± 0.031
	Staphylococcus aureus	09.2± 0.043
Test fungus	*Aspergillus oryzae*	14.4± 0.037
	Aspergillus niger	12.2± 0.037
	Penicillium digitatum	06.4± 0.033
	Mucor	09.3± 0.041

Wound Infliction

A standard wound of 15mm diameter (176.625mm$^{2)}$ was formed with the help of a round seal according to Morton JJP and Malone's method (Morton *et al.*, 1972) under light ether anesthesia.

Wound Contraction

The wound area contraction was traced with a transparent paper and thereafter measured with 1mm^2 graph sheet until epithelization and complete scar area was recorded. Wound contraction in percentage was determined by $(A_0 - A_t)/A_0 \times 100$. Where A_0 is the original wound area and A_t is the area of wound at specific time period after wound infliction.

RESULTS

Physical properties

The *T. Ammi* seed yielded 2.86% essential oil and it had yellowish brown colour with pungent smell. The observed value of refractive index was 1.496 at 25^{o}C. The specific gravity acid values and Easter value were 0.9212, 1.12 and 2.80; respectively. The oil was found to be soluble in 40% and 80% alcohol.

Gas Chromatogram

The gas chromatographic analysis revealed the presence of monoterpenes hydrocarbons and oxygenated fraction. The identified components of essential oil were α-thujene (0.2983 %). α- pinene (0.1509 %), p-cymene (0.185%), myrcene (1.7638), Limonene (20.5294%), α-Terpinene (18.9218%), Δ careen (0.1561%), carveol acetate (0.1462%), carvone (0.441%), Thymol (55.308%), α-Terpineol (0.2404%), carveol (0.2813%) and bergeptene (0.3705%).

Physiochemical stability

The pH value of cream showed a little change with in the pH range of 5.68 to 5.62 in accelerated storage conditions (40 ^{0}C/70% relative humidity). It showed stability to centrifugation test and no phase separation was observed.

Dermal irritation test

Neither edema nor erythema was observed in all groups

Wound contraction

The Iodine tincture group showed 100% wound contraction on the 15th day. The control group indicated only 96.57% wound healing. On the other hand; T ammi cream group indicated 99.68% of contraction in the wound.

DISCUSSION

The major component of the essential oil of *Trachyspermum ammi* was Thymol (55.308%) and it was found consistent with the reported value (Malhotra and Vijay, 2004). Due to higher content of thymol, the essential oil is used in various pharmaceuticals preparations and local material media for treatment of stomach ailments. The next major component was α-Terpinene (18.9218%); a monoterpene hydrocarbon. The oxygenated portion of the essential oil constituted

Table 3: Wound healing activity in rabbits

Group	Wound diameter (mm) ± SDV (% Wound contraction)				
	3rd Day	6th Day	9th Day	12th Day	15th Day
Control	13.74 ±0.10 (16.09)	10.70±0.21 (49.12)	7.5±0.25 (75.00)	3.88±0.07 (90.51)	2.78±0.16 (96.57)
Iodine Tincture	13.13±0.094 (23.34)	9.83±0.11 (57.02)	5.78±0.18 (85.13)	2.08±0.18 (98.07)	0.02±0.03 (100.0)
T ammi cream	13.58±0.12 (17.99)	10.13±0.25 (54.36)	6.63±0.28 (80.44)	3.32±0.32 (95.11)	0.85±0.30 (99.68)

56.4169% of the essential oil containing thymol, α-Terpineol, linalool, carveol, carvone, carveol acetate; which may recommend the use of essential oil in perfumery as flavoring agent and in aromatherapy.

Absence of erythema and edema indicates that cream does not cause irritation. Cream shows good wound healing activity as compared to control group.

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