Bangladesh J. Bot. **45**(2): 291-296, 2016 (June)

ANTI-INFLAMMATORY ACTIVITY OF THE ESSENTIAL OILS OF *TRACHYSPERMUM AMMI* SPRAGUE SEEDS

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Key words: Trachyspermum ammi, Essential oil, Anti-inflammatory

Abstract

The present study reports the effect of application of nano-Zn oxide on chemical composition and anti-inflammatory properties of *Trachyspermum ammi* Sprague essential oil and its main compounds. The essential oil was obtained from the seeds of the plant by hydrodistillation and analysed by GC/MS. The oil was predominantly composed of γ-terpinene (33.14%). Regarding the anti-inflammatory activity the oil exhibited a potent nitric oxide scavenging effect and inhibited the expression of inducible NO synthase. These results indicate that essential oil *Trachyspermum ammi* and its main compounds might be applicable in natural medicine as well as healthy food.

Introduction

Inflammation is a complex biological response of vascular tissue to harmful stimuli caused by injury, infection, environmental agents, malignancy and cellular changes. It is a protective attempt by the body to remove the injurious stimuli as well as initiate the healing process for the tissue (Denko 1992). Inflammation is one of the body's self defense systems that is classified as part of our innate immunity. Nitric oxide (NO) is an inflammatory mediator that plays an important role in a variety of inflammatory diseases such as arthritis, inflammatory bowel diseases, and atherosclerosis (Guzik *et al.* 2003). The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world. Aromatic plants, such as those from Lamiaceae and Apiaceae families produce a large number of secondary metabolites, namely monoterpenes, sesquiterpenes and phenylpropanoids which have demonstrated several therapeutic properties, mainly antioxidant, antifungal and anti-inflammatory ones (Zuzarte *et al.* 2012). *Trachyspermum ammi* Sprague is an aromatic annual plant grown in Iran. The seeds of *T, ammi* have several therapeutic effects, including diuretic, antivomiting, analgesic, antiasthma, and antidyspnea effects. In Persian folk medicine, the fruits of *T. ammi* were used as a diuretic, anti-vomiting, carminative and antihelmentic agent (Zargari 1988). Mohagheghzadeh *et al.* (2007) showed that *T. ammi* has two chemotypes, thymol and carvacrol. The main objectives of this study were to evaluate the antiinflammatory properties of the essential oil (EO) from *T. ammi* seeds and to determine the compounds that contributed to the effects.

Materials and Methods

Seeds of *T. ammi* were sown in Jefe pot in experimental greenhouse of Ilam, Iran. Plants at flowering stage (2013-2014) were sprayed with distilled water as a control and nano-Zn oxide at 4 and 6 mM. All spray solutions were sprayed to the point of run off. The experiment was arranged

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in CRBD with three replications for each treatment. Seeds of *T. ammi* were harvested and air dried at ambient temperature in the shade. The second set included investigating the effect of essential oils on anti-inflammatory activity.

The plant was identified by Mr. Esmaili, and the voucher specimen was deposited at private herbarium of Dr. F. Esmaili (Voucher No. 121). The seeds were ground and the resulting powder was subjected to hydrodistillation for 3 hrs in an all glass Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia (2013). The essential oils were dried over anhydrous sodium sulphate and after filtration, stored at +4ºC until tested and analyzed. The GC/MS analyses were executed on a Hewlett-Packard 5973N gas chromatograph equipped with a column HP-5MS (30 m length × 0.25 mm i.d., film thickness 0.25 µm) coupled with a Hewlett–Packard 5973N mass spectrometer. The column temperature was programmed at 50ºC as an initial temperature, holding for 6 min, with 3ºC increases per minute to 240ºC, followed by a temperature enhancement of 15ºC per minute up to 300ºC and holding at the mentioned temperature for 3 min. Injector port temperature was 290ºC and helium was used as carrier gas at a flow rate of 1.5 ml/min. Ionization voltage of mass spectrometer in the EI-mode was equal to 70 eV and ionization source temperature was 250ºC. Linear retention indices for all components were determined by coinjection of the samples with a solution containing homologous series of C_8-C_{22} *n*-alkanes and comparing them and their mass spectra with those of authentic samples or with available data of the GC/MS system (WILEY 2001 data software) and Adams libraries spectra (2001).

Total phenolic contents in seeds of *T. ammi* were determined by Folin-Ciocalteu reagent (Jimoh *et al.* 2007). The total phenolic content was expressed as gallic acid equivalents (GAE) (mg/g).

Total flavonoid contents in seeds of *T. ammi* were measured as described previously (Piccolella *et al.* 2008). The total flavonoid content was calculated as rutin equivalents (mg/g).

To evaluate the anti-inflammatory potential of the oils, NO production in lipopolysaccharide (LPS)-stimulated macrophages was used. Exponentially growing macrophages (RAW 264.7 cells) were plated in 24-well microplates at a density of 2×10^5 cells per well in 400 µl of culture medium and were allowed to adhere for 24 hrs 37ºC under 5% CO_2. Cells were then treated with increasing concentrations of essential oil and pure compounds dissolved in DMSO. The final concentration of solvent in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. Cells were then stimulated with 100 µg/ml LPS and incubated at 37ºC under 5% CO_2. After 24 hrs cell-free supernatants were collected and NO was measured using the modified method of Green *et al.* (1990). Griess reagent (50 µl of 1% sulphanilamide and 50 µl of 0.1% N-1-naphtylethylene-diamine dihydrochloride in 2.5% H_3PO_4) was added in equal volume (100 µl) to cell supernatant and incubated at room temperature for 30 min. N(G)- nitro-L-arginine methyl ester (L-NAME) was used as a positive control. Absorbance was measured using an ELISA automatic microplate reader at 550 nm and the nitrite concentration was determined from a regression analysis prepared with serial dilutions of sodium nitrite (Bourgou *et al.* 2010).

The results are presented as mean ± Sd and statistically analyzed by ANOVA followed by DMRT.

Results and Discussion

Hydrodistillation showed that *T. ammi* seeds contained 1.2% (v/w) EO. Results of GC/MS analysis of the EO (Table 1) indicate that γ-terpinene was the main monoterpene hydrocarbon, with a content of 30.14%. The GC/MS analysis of *Trachyspermum ammi* oil revealed 14 compounds representing 82.49% of the total oil; γ-terpinene was the main constituent (30.92%.), followed by p-cymene (22.25%) and thymol (13.14%). The constituents of EOs of *T. ammi*

treated with nano-Zn oxide are presented in Table 1. Sixteen components were identified in untreated plants and 14 components in nano-Zn oxide treated plants (Table 1). The differences were supposed to be the effects of nano-Zn oxide on chemical composition of *T. ammi* EO. Decrease in the proportion of α-pinene, β-pinene, p-cymene and ethylene methacrylate have been

Table 1. Chemical composition of *Trachyspermum ammi* essential oil.

Components		[a]*Trachyspermum ammi* EO (%)			[b]Retention index	Identification methods
		Control (%)	Nano-Zn oxide 4 mM (%)	Nano-Zn oxide 6 mM (%)		
1	α-thujene	0.75	-	-	850	MS, RI
2	α-pinene	4.87	3.45	2.41	855	"
3	β-pinene	1.00	0.55	0.50	190	"
4	β-myrcene	1.14	0.35	0.11	920	"
5	*P*-cymene	22.25	18.24	8.54	950	"
6	β-phellendrene	4.56	_1.45	0.54	954	"
7	Limonene	1.05	1.54	2.04	960	"
8	γ–terpinene	30.14	35.47	40.35	980	"
9	4-terpineol	1.08	-	-	1.63	"
10	*Cis* limonene oxide	0.1	0.44	2.14	1085	"
11	Dodecane	-	1.12	1.50	1110	"
12	β-fenchyl alcohol	0.89	0.47	-	1126	"
13	Thymol	13.14	17.87	25.47	1208	"
14	Ethylene methacrylate	0.87	1.45	2.35	1235	"
15	Pentadecane	0.65	0.57	1.47	1264	"
16	Hexadecane	0.22	-	-	1285	"
17	Nonadecane	0.11	-	-	1293	"
18	Carvacrol	-	3.64	10.87	1306	"
Total		82.49	86.61	98.29		
Yield		1.34%	2.14%	3.11 (v/w) %		

[a]Percentage composition determined on column HP 5.[b] The retention Kovats indices were determined on HP 5 capillary column in reference to *n*-alkanes. MS = Mass Spectroscopy, RI = Retention Index.

found according to concentration of nano-Zn oxide. Some compounds such as α-thujene, 4-terpineol, hexadecane and nonadecane only detected in control (Table 1). Limonene, γ-terpinene and thymol were increased with nano-Zn oxide treatment (Table 1). The yield of the *T. ammi* oil was 1.34% in control, 2.14% (4 mM) and 3.11% (6 mM). Nano-Zn oxide significantly increased the yield of EO (Table 1). We harvested plant materials in seed stage. It might have affected the kind of oils, because it was shown that during the intensive growth period the precursor flow distributes between the cytoplasm (sites of sesquiterpene synthesis) and plastid (sites of monterpene synthesis) while after full development of the cell the majority is utilized in the plastids. Srivastava *et al.* (1999) detected 11 compounds in *T. ammi* , with carvacrol (45.2%) and p-cymene (42.0%) as the major constituents. Khajeh *et al.* (2004) showed that hydrodistilled oil of the plant contained eight main compounds, including thymol (49%), p-cymene (15.7%), γ-terpinene (30.8%) and β-pinene (2.1%), but supercritical carbon dioxide extraction (SFE) of the

EO revealed only three compounds (thymol, p-cymene and γ-terpinene), and the content of each depended on SFE conditions. Kobraee *et al.* (2011) reported that nano iron foliar application enhanced soybean yield by influencing number of seeds per plant and seed weight. Therefore, iron defficiency in soils could be a restricting factor of yield and extremely decrease crop yield quality. Application of nano-iron oxide at 0.75 g/l compared to other treatments had maximum effect on dry pod weight. It seems that the use of nano-particles causes increasing in pod and dry leaf weight and finally increases the total yield (Sheykhbaglou *et al.* 2010).

As shown in Table 2, the extraction yield of *T. ammi* ranged from lowest 66.68 ± 12 mg/g (control) to highest 101.17 ± 74 mg/g (nano-Zn oxide 6 mM). Among the three *T. ammi* extracts, *T. ammi* treated with 6 mM nano-Zn oxide showed the highest total phenolic content (300.46 ± 74 mg/g) and total flavonoid content (147.74 ± 37 mg/g). Furthermore, the total phenolic and total flavonoid contents exhibited the descending order among: *T. ammi* extract (treated with nano-Zn oxide 6 mM) > extract (treated with nano-Zn oxide 4 mM) > extract (treated with control). These results showed that the total phenolic and total flavonoid contents have an obvious variation in various concentrations.

Table 2. Extraction yields, total phenolic contents and total flavonoid contents of *Trachyspermum ammi* extracts.

	Extract	Extraction yield[a]	Total phenolic[b]	Total flavonoid[c]
1	Control	66.68 ± 12	214.07 ± 87	97.25 ± 38
2	Nano-Zn oxide 4 mM (%)	88.11 ± 87	259.07 ± 37	100.37 ± 09
3	Nano-Zn oxide 6 mM (%)	101.17 ± 74	300.46 ± 74	147.74 ± 37

The data are expressed as mean ± Sd. [a]Expressed as mg of extract per gr dry material. [b]Expressed as mg of gallic acid per gr dry extract. [c]Expressed as mg of rutin per g dry extract.

The traditional use of essential oils as anti-inflammatory agents suggests that they possess potent anti-inflammatory activity. The anti-inflammatory activity of *T. ammi Sprague* EO was evaluated on RAW 264.7 macrophages which were stimulated to induce an overproduction of NO. As presented in Table 3, the EO exhibited a strong inhibitory effect of LPS-induced NO secretion

Table 3. Effects of *Trachyspermum ammi* EO (45.0 µg/ml) and its main constituents (45.0 µM) on NO production in LPS-stimulated RAW-264.7 macrophages. Values are mean ± Sd of three replications.

Tested compounds	Control NO inhibition (%)	nano-Zn oxide 6 mM (%) NO inhibition (%)
Trachyspermum ammi EO	87.41 ± 0.11	94.35 ± 0.35
α-pinene	51.54 ± 0.34	58.45 ± 0.15
P-cymene	50.78 ± 0.45	54.35 ± 0.35
γ- terpinene	67.82 ± 0.64	73.32 ± 0.74
Thymol	67.61 ± 0.17	92.18 ± 0.98
Carvacrol	54.17 ± 0.37	69.57 ± 0.15
L-NAME	70.09 ± 0.78	70.09 ± 0.78

Results are expressed as a percentage of nitrite production by control cells maintained in culture medium.

with 94.35 ± 0.35% inhibition observed at 45.0 µM. Comparatively, the L-NAME, used as positive control inhibited NO release by 70.09 ± 0.78% (Table 3). Thymol was found to be the most active compound, inhibiting NO production by 92.18 ± 0.98% at 45.0 µM (Table 3).

Therefore, this compound may be responsible for the anti-inflammatory activity of the oil. The anti-inflammatory potential of the *T. ammi* EO or its main compound may be directly related to its scavenging ability and/or capacity to inhibit inducible NO synthase expression, the enzyme responsible for the release of high amounts of NO, during inflammatory conditions. Indeed, inflammatory mediators, such as NO have been reported to contribute to mutagenesis (Marletta 1993). This radical is an important regulator of physical homeostasis, whereas large amounts have been closely correlated with the pathophysiology of a variety of diseases and inflammations (Marletta 1993). Essential oils seem to be a good source of antioxidant and anti-inflammatory natural products. Present data indicate that the essential oil extracted from *T. ammi* exhibits potent anti-inflammatory activity, which support their use in traditional medicines. There was a good correlation between total phenol content and anti-inflammatory capacity of the extracts. In conclusion, *T. ammi* extracts appear to contain compounds with anti-inflammatory activities.

References

Adams RP 2001. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured, Carol Stream, IL. pp. 469.

Bourgou S, Pichette A, Marzouk B and Legault J 2010. Bioactivities of black cumin essential oil and its main terpenes from Tunisia. South African J. Bot. **76:** 210-216.

European Pharmacopoeia 2013. Edition 8, Maisonneuve S. A., Sainte-Ruffine.

Denko CW 1992. A role of neuropeptides in inflammation. *In:* Whicher JT, Evans SW Biochemistry of inflammation. Kluwer Pub. London. pp. 177-181.

Green SJ, Meltzer MS, Hibbs JB and Nacy CA 1990. Activated macrophages destroy intracellular Leishmania major amastigotes by an L-arginine-dependent killing mechanism. J. Immunol. **144:** 278-283.

Guzik TJ, Korbut R and Adamek-Guzik T 2003. Nitric oxide and superoxide in inflammation and immune regulation. J. Physiol. Pharmacol. **54:** 469-487.

Jimoh FO, Sofidiya MO and Afolayan AJ 2007. Antioxidant properties of the methanol extracts from the leaves of *Paullinia pinnata*. J. Med. Food. **10:** 707-711.

Khajeh M, Yamini Y, Sefidkon F and Bahramifar N 2004. Comparison of essential oil composition of *Carum copticum* obtained by supercritical carbon dioxide extraction and hydrodistillation methods. Food Chemistry **86:** 587-591.

Kobraee S, Shamsi K and Rasekhi B 2011. Effect of micronutrients application on yield and yield components of soybean. Ann. Biol. Res. **2**(2): 476-482.

Marletta MA 1993. Nitric oxide synthase structure and mechanism. J. Biol. Chem. **17:** 12231-12234.

Mohagheghzadeh A, Faridi P, and Ghasemi Y. (2007). *Carum copticum* Benth & Hook., essential oil chemotypes. Food Chem. **100:** 1217-1219.

Piccolella S, Fiorentino A, Pacifico S, D'Abrosca B, Uzzo P and Monaco P 2008. Antioxidant properties of sour cherries (*Prunus cerasus* L.): Role of colorless phytochemicals from the methanolic extract of ripe fruits. J. Agricult. and Food Chem. **56:**1928-1935.

Sheykhbaglou R, Sedghi M, Tajbakhsh Shishvan M and Seyed-Sharifi R 2010. Effect of nano iron particles on agronomic traits of soybean. Not Sciense Biological **2**(2): 112-113.

Srivastava M, Saxena A and Baby P 1999. GC-MS investigation and antimicrobial activity of the essential oil of *Carum copticum* Benth & Hook. Acta Alimentaria **28**: 291-295.

Zargari A 1988. Medicinal plants (Vol. 2). Tehran, Iran: Tehran University Publications.

Zuzarte M, Gonçalves MJ, Cruz MT, Cavaleiro C, Canhoto J, Vaz S, Pinto E and Salgueiro L 2012. Lavandula luisieri as a source of antifungal drugs. Food Chem. **135**:1505-1510.

(Manuscript received on 1 June, 2014; revised on 14 September, 2015)