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**ORIGINAL ARTICLE**

# Characterization of Chemical Composition and Antioxidant Properties of *Trachyspermum ammi* Seed as a Potential Medicinal Plant

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**KEYWORDS**

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**ABSTRACT:** In this study, the essential oil obtained by hydrodistillation of the seed of *Trachyspermum ammi* (Umbelliferae), growing wild in Sabzevar, Khorasan Razavi province (Iran), were analyzed by GC and GC/MS. The yield of total volatiles was 2.3% (w/w). Forty four compounds representing 91.6% of the aerial parts oil were identified. The main components of the oil were Hexadecanoic acid (27.5%), ethyl linoleate (8.5%), 6-methyl-α-ionone (8.0%), isobutyl phthalate (5.8%), α-cadinol (4.7%), germacrene D (4.3%) and δ-cadinene (3.5%). The oil was rich in nonterpenoids (56.0%). The total flavonoid content of different extracts of the plant was found to be in the range 53.2-164.5 mg/g while the maximum amount concern to methanol extract. The antioxidant activity of the extracts was also measured by radical scavenging activity of antioxidants against free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method. Results showed that $IC_{50}$ of the extracts of *Trachyspermum ammi* seed are higher than the standard synthetic antioxidants, BHT, ascorbic acid and gallic acid.

## INTRODUCTION

All plants synthesize and accumulate secondary metabolites such as alkaloids, sterols, terpenes, flavonoids, saponins, glycosides, volatile oils, etc., many of which have medicinal value. Since ancient times, nearly all cultures have used medicinal plants for the treatment of a variety of maladies and diseases.

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Furthermore, practitioners in many of these cultures often use the same or similar plants, which suggest that the active components of these plants have been found to be generally effective [1]. A plant used traditionally in Iran is *Trachyspermum ammi* L. (=*Trachyspermum copticum*=*Carum copticum*) [2]. As a part of on-going work on the medicinal plants of Iran, we decided to investigate the oil and antioxidant properties from *Trachyspermum ammi* seed.

*Trachyspermum ammi* (Umbelliferae) is an annual herb growing wild or planted in Iran [3, 4]. Trachyspermum ammi, commonly referred as bishop's weed, bullwort, omum plant and ajowan caraway (English names). Some biological effects of ajowan such as antiviral [5], anti-inflammatory [6], antifungal [7], analgesic [8] and antioxidant activity [9] have been reported.

In this study, the hydrodistilled volatile oil from seeds of *Trachyspermum ammi* from Davarzan, Khorasan province (Iran), was studied by GC and GC/MS. Total phenolic content and radical scavenging capacity were used to determination of antioxidant activity of different extracts of the plant's seed.

Antioxidants have already been found in plant materials and supplements. Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic ones [10]. The use of natural antioxidantsfrom plants does not induce side effects, while synthetic antioxidants were found to have genotoxic effect [11]. Therefore, the investigations of biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are numerous. The basic aim of the research was to determine the total phenolic content andradical scavenging capacity in various extracts of the species *Trachyspermum ammi seed* using spectrophotometric methods.

## MATERIALES AND METHODS

### *Chemicals*

Solvents (methanol, chloroform, ethyl acetate) were purchased from Merck (Darmstadt-Germany) products. Standards of phenolic acids (gallic acid) and 2, 2-dyphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co., St Louis, MO, USA. The Folin-Ciocalteu's phenol reagent and 3-tert-butyl-4-hydroxyanisole (BHA) were from FlukaChemie AG, Buchs, Switzerland. All other solvents and chemicalswere of analytical grade.

### *Plant Material*

The plant material was collected in July 2013 from Davarzan in Khorasan Province, Iran. A voucher specimen has been deposited in the herbarium of Research Center of Natural Resources, Sabzevar, Iran. The collected plant material was air-dried in darkness at room temperature (20 ºC). Seeds of dried plant were separated from plant and stored in tight-seal dark containers until needed.

### *Essential oil isolation*

Air-dried seeds of *Trachyspermum ammi* (100 g) were subjected to hydrodistillation in a Clevenger-type apparatus for three hours to produce oils. The yield of total volatiles was 2.3% (w/w). The oils were dried over anhydrous sodium sulfate and stored in sealed vials at 4 °C before analysis.

### *GC analysis*

GC analysis was performed using a Shimadzu GC-9A gas chromatograph, equipped with a HP-5MS fused silica column (30 m×0.25 mm i.d., film thickness 0.25 µm). The oven temperature was held at 50 °C for five minutes and then programmed to 250 °C at a rate of 3 °C/min. The injector and detector (FID) temperatures were 290°C. Helium was used as carrier gas with a linear velocity of 32 cm/s.

***GC/MS analysis***

GC/MS analysis was carried out on a Hewlett-packard 6890 gas chromatograph fitted with a fused silica HP-5MS capillary column (30m×0.25 mm; film thickness 0.32 µm). The oven temperature was programmed from 60 to 220 °C at 6 °C/min. Helium was used as carrier gas at a flow rate of 1 mL/min. The chromatograph was coupled to a Hewlett-Packard 5973 mass selective detector with an ionization voltage of 70 eV.

***Qualitative and quantitative analyses***

Constituents of the volatile oils were identified by comparison of their retention indices relative to C9-C21 n-alkanes and of their mass spectral fragmentation pattern with those reported in the literature [12] and stored in a MS library (Wiley 275). The quantification of the components was performed on the basis of their GC peak area data from the HP-5MS column separation.

***Preparation of the extracts***

The conventional maceration method was used for preparing the extracts. Dried and powdered aerial parts of Trachyspermum ammi (50 g) extracted by 400 mL of solvents. This process replicated trice with the same volume of fresh solvent. Three solvents having different polarities (methanol, ethyl acetate, chloroform) were used. An overhead stirrer mixed the materials for 24 h. All the mixtures were filtered through Whatman paper No. 41. The solvents were removed below 40°C using a rotary evaporator (Heidolph, Germany) and stored at 4 °C for further use.

***Determination of Total Phenolic Contents***

The total phenolic content of extract was determined spectrophotometrically by Folin-Ciocalteu method according to the procedure reported by Singleton et al. [13] with some modifications. Briefly, 500 µl of (methanol, ethyl acetate, chloroform) extracts solution, 1500 µl distilled water and 500 µl of 1:10 Folin-Ciocalteu reagent were mixed for 1 minute. Then 5 minutes later, 1000 µl of sodium carbonate (5.0%) were added and shaken. After two hours in the dark of incubation at room temperature, the absorbance at 760 nm was measured by a UV-Visible spectrophotometer, (Unico UV-2100, China). The total phenolic concentration was calculated from gallic acid (GA) calibration curve (5-100 mg/L). Total phenolic content were expressed as gallic acid equivalents (GAE)/g of extracts averaged from three replicates.

***DPPH Radical Scavenging Activity Assay***

The ability of the plant extract to scavenge DPPH free radicals was assessed by the standard method Mensor et al. [14]. Briefly, 10 µL of the extracts was added to 2.5 mL of a 0.004% solution of DPPH in methanol. The stock solution of extracts were prepared in concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 20, 40, 60 and 80 $\mu$ g/ml. Diluted solutions (1 ml each) were mixed with 1 ml of methanolic solution of DPPH in concentration of 0.004%. After 30 minincubation in darkness at room temperature (23 $^{o}$C), the absorbance was recorded at 517 nm. Ascorbic acid, gallic acid and BHT (20, 40, 60, 80 µg/mL) were used as positive references.Control contained all the reagents except the extract. Percentage of scavenged DPPH wascalculated using equation 1. The data were presented as mean values ± standard deviation (n = 3).

DDPH scavenging activity = 100 × (Ac – As)/Ac (1)

where Ac is the absorbance of the control and As is the absorbance of the sample. $IC_{50}$ values calculated denote the concentration of the sample required to decrease the absorbance at 517 nm by 50%.

## RESULTS AND DISCUSSION

As a part of on-going work on the chemical analysis of oils obtained from the wild plants of Iran, we decided to re-investigate the oils of this specific plant. Hydrodistilled volatile oils from the crushed dry seeds of *Trachyspermum ammi* (Umbelliferae) from Davarzan (Iran), was studied by GC and GC/MS. The air-dried

aerial parts of the plant yielded 2.3% (w/w) oil. The oil was clear and yellowish. Forty four components were identified in the seed's oil that contained 91.6% of the compounds. Table 1 lists formulas, percentages, and retention indices of identified compounds in the oil. As can be seen, the main components are Hexadecanoic acid (27.5%), ethyl linoleate (8.5%), 6-methyl-α-ionone (8.0%), isobutyl phthalate (5.8%), α-cadinol (4.7%), germacrene D (4.3%) and δ-cadinene (3.5%).

In this study, GC and GC/MS analysis method revealed several nonterpenoid hydrocarbons (NH), monoterpenoid hydrocarbons (MH), sesquiterpenoid hydrocarbons (SH), oxygenated sesquiterpenes (OS), oxygenated monoterpenes (OM),diterpene hydrocarbon (DH) and oxygenated diterpene (OD) in the oil from the seed of *Trachyspermum ammi*. Onemonoterpene hydrocarbons (0.1%), five oxygenated monoterpene (8.8%), thirteen sesquiterpene hydrocarbons (13.4%), six oxygenated sesquiterpene (10.4%), seventeen nonterpene hydrocarbons (56.0%), one diterpene hydrocarbon (2.5%) andone oxygenated diterpene (0.4%) were detected in this oil. The data lead to a rank order of constituent groups: NH>SH>OS>OM>DH>OD>MHfor the seed oil. The main components in this oil were Hexadecanoic acid (27.5%), ethyl linoleate (8.5%), 6-methyl-α-ionone (8.0%), isobutyl phthalate (5.8%), α-cadinol (4.7%), germacrene D (4.3%) and δ-cadinene (3.5%).

**Table 1**. Constituents of the essential oils from seed of Trachyspermumammiobtained by hydrodistillation[a]

| No. | compound | Formula | Percentage | RRI[b] | Class |
|---|---|---|---|---|---|
| **1** | Limonene | $C_{10}H_{16}$ | 0.1 | 1031 | MH[c] |
| **2** | 4-Terpineol | $C_{10}H_{18}O$ | 0.1 | 1179 | OM[d] |
| **3** | Fenchyl acetate | $C_{12}H_{20}O_2$ | 0.4 | 1223 | OM |
| **4** | Anethole | $C_{10}H_{12}O$ | 0.1 | 1285 | OM |
| **5** | α-Copaene | $C_{15}H_{24}$ | 0.2 | 1364 | SH[e] |
| **6** | β- Bourbonene | $C_{15}H_{24}$ | 0.8 | 1385 | SH |
| **7** | β-Cubebene | $C_{15}H_{24}$ | 0.1 | 1390 | SH |
| **8** | Tetradecane | $C_{14}H_{30}$ | 0.1 | 1400 | NH[f] |
| **9** | β-Caryophyllene | $C_{15}H_{24}$ | 0.3 | 1418 | SH |
| **10** | α-Guaiene | $C_{15}H_{24}$ | 0.1 | 1439 | SH |
| **11** | Aromadendrene | $C_{15}H_{24}$ | 0.6 | 1442 | SH |
| **12** | α-Humulene | $C_{15}H_{24}$ | 2.0 | 1452 | SH |
| **13** | (E)-β-Farnesene | $C_{15}H_{24}$ | 0.1 | 1457 | SH |
| **14** | Germacrene D | $C_{15}H_{24}$ | 4.3 | 1480 | SH |
| **15** | β-Ionone | $C_{13}H_{20}O$ | 0.2 | 1488 | OM |
| **16** | Bicyclogermacrene | $C_{15}H_{24}$ | 0.7 | 1500 | SH |
| **17** | γ-Cadinene | $C_{15}H_{24}$ | 0.6 | 1515 | SH |
| **18** | 6-Methyl-α-ionone | $C_{14}H_{22}O$ | 8.0 | 1518 | OM |
| **19** | δ-Cadinene | $C_{15}H_{24}$ | 3.5 | 1522 | SH |
| **20** | Cadina-1, 4-diene | $C_{15}H_{24}$ | 0.1 | 1533 | SH |
| **21** | Germacrene D-4-ol | $C_{15}H_{26}O$ | 0.6 | 1574 | OS[g] |
| **22** | Spathulenol | $C_{15}H_{24}O$ | 1.5 | 1578 | OS |
| **23** | Caryophyllene oxide | $C_{15}H_{24}O$ | 0.5 | 1583 | OS |
| **24** | Humulene epoxide II | $C_{15}H_{24}O$ | 1.7 | 1608 | OS |
| **25** | τ-Muurolol | $C_{15}H_{26}O$ | 1.4 | 1643 | OS |
| **26** | α-Cadinol | $C_{15}H_{26}O$ | 4.7 | 1656 | OS |
| **27** | Tetradecanoic acid | $C_{14}H_{28}O_2$ | 1.8 | 1760 | NH |
| **28** | Octadecane | $C_{18}H_{38}$ | 0.4 | 1800 | NH |

Continue of Table 1

| | | | | | |
|---|---|---|---|---|---|
| **29** | 6, 10, 14-Trimethyl-2-Pentadecanone, | $C_{18}H_{36}O$ | 1.7 | 1848 | NH |
| **30** | 2-Hydroxy-Cyclopentadecanone | $C_{15}H_{28}O_2$ | 0.4 | 1853 | NH |
| **31** | Pentadecanoic acid | $C_{15}H_{30}O_2$ | 0.3 | 1867 | NH |
| **32** | Isobutyl phthalate | $C_{16}H_{22}O_4$ | 5.8 | 1877 | NH |
| **33** | Cyclohexadecane | $C_{16}H_{32}$ | 0.3 | 1883 | NH |
| **34** | 16-methyl-Oxacyclohexadecan-2-one, | $C_{16}H_{30}O_2$ | 0.3 | 1943 | NH |
| **35** | Sandaracopimara-8(14), 15-diene | $C_{20}H_{32}$ | 2.5 | 1969 | DH[h] |
| **36** | di-Butylphthalate | $C_{16}H_{22}O_4$ | 0.9 | 1973 | NH |
| **37** | Hexadecanoic acid | $C_{16}H_{32}O_2$ | 27.5 | 1977 | NH |
| **38** | Eicosane | $C_{20}H_{42}$ | 2.0 | 2000 | NH |
| **39** | Heptadecanoic acid | $C_{17}H_{34}O_2$ | 0.4 | 2065 | NH |
| **40** | Methyl linoleate | $C_{19}H_{34}O_2$ | 0.6 | 2084 | NH |
| **41** | Phytol | $C_{20}H_{40}O$ | 0.4 | 2111 | OD[i] |
| **42** | (Z, Z)-9,12-Octadecadienoic acid | $C_{18}H_{32}O_2$ | 2.7 | 2136 | NH |
| **43** | Ethyl linoleate | $C_{20}H_{36}O_2$ | 8.5 | 2164 | NH |
| **44** | Octadecanoic acid | $C_{18}H_{36}O_2$ | 2.3 | 2172 | NH |
| | Total identified | | | 91.6 | |

[a]The compounds have been arranged according to their retention indices on an HP-5 MS capillary column

[b]Kovatz retention indices given in the literature

[c]Monoterpene hydrocarbons

[d]Oxygenated monoterpene

[e]Sesquiterpene hydrocarbons

[f]Nonterpene hydrocarbons

[g]Oxygenated sesquiterpene

[h]Diterpene hydrocarbon

[i]Oxygenatedditerpene

### *Antioxidant activity*

Methanol, ethyl acetate and chloroform extracts were preparedto examine the total phenolic content and antioxidant activity. The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of Gallic acid equivalent (the standard curve equation: $Y=0.0086x + 0.0175$, $r^2 = 0.999$). The values obtained for the concentration of total phenols areexpressed as mg of GA/g of dry extract (Table 2).

The total phenolic contents in the examined extracts ranged from 53.2 to 164.5 mgGA/g of dry extract.

The total phenolic contents in plant extracts of the species *Trachyspermum ammi* depends on the type of extract, i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extractsobtained using polar solvents for the extraction [15].

**Table 2.** Total phenolic contents in the plant extracts expressed in terms of gallic acid equivalent (mg of GA/g of extract)

| Extract | Absorbance | mg of GA/g of extract [1] |
|---|---|---|
| **Chloroform** | 1.37 | 82.0±1.6 |
| **Ethyl acetate** | 1.75 | 53.2±2.8 |
| **Methanol** | 1.51 | 164.5±1.3 |

[1] Each value is the average of three analyses ± standard deviation.

The antioxidant activity of different seed extracts from *Trachyspermum ammi* was determined using a methanol solution of DPPH reagent. DPPH is very stable free radical. Unlike invitro generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has theadvantage of being unaffected by certain side reactions, such as metal ion chelation and enzymeinhibition. A freshly prepared DPPH solution exhibits a deep purple color with an absorptionmaximum at 517 nm. This purple color generally fades when antioxidant moleculesquench DPPH free radicals (i.e. by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them into a colorless-bleached product (i.e. 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resultingin a decrease in absorbance at 517 nm band [16]. The antioxidant activity of five different extracts from the species *Trachyspermum ammi* is expressed in terms of percentage of inhibition (%) and $IC_{50}$ values (µg/ml) (Table 3). Parallel toexamination of the antioxidant activity of plant extracts, the values for three standard compoundswere obtained and compared to the values of the antioxidant activity. The standardsubstances were BHT, Gallic acid and ascorbic acid.

The examination of antioxidant activities of plant extracts from *Trachyspermum ammi* showed different values. The obtained values varied from 9.0% to 103.8%. The largest capacityto neutralize DPPH radicals were found for methanol extract, which neutralized 50% offree radicals at the concentration of 74.5µg/ml. A moderate activity was found for chloroform extract. The minutest capacity to inhibit DPPH radicals was determined for ethyl acetate. In comparison to $IC_{50}$ values of BHT, ascorbic acid and Gallic acid, methanol extract from *Trachyspermum ammi* manifested the moderate capacity for neutralization of DPPH radicals.

The extraction of antioxidant substances of different chemical structure was achieved using solvents of different polarity. Numerous investigations of qualitative composition of plant extracts revealed the presence of high concentrations of phenols in the extracts obtained using polar solvents .The extracts that perform the highest antioxidant activity (Table 3) have the highest concentration of phenols (Table 2). Between the values for the concentrationof phenolic compounds (Table 2) and antioxidant activity of different plant extracts of *Trachyspermum ammi* has been proved a significant linear correlation. Methanol extract from *Trachyspermum ammi* have high concentration of total phenols (Table 2) and DPPH scavenging capacity (Table 3), which is in correlation with intense antioxidant activity of these extract.

**Table 3.** Antioxidant (DPPH scavenging) activity of investigated seed extracts and synthetic antioxidants presented as percentage of DPPH radicals inhibition and $IC_{50}$ values ( $\mu$ g/ml).

| | DPPH | | | | |
|---|---|---|---|---|---|
| **Extract** | **20 ppm** | **40 ppm** | **60 ppm** | **80 ppm** | **$IC_{50}$** |
| **Chloroform** | 12.3±2.3 | 27.0±2.1 | 38.2±1.5 | 47.1±2.1 | 84.6±3.4 |
| **Ethyl acetate** | 9.0±2.5 | 16.4±2.4 | 24.8±1. 6 | 38.4±5.7 | 103.8±4.3 |
| **Methanol** | 17.5±1.2 | 23.1±2.6 | 32.4±2.3 | 59.0±4.7 | 74.5±4.9 |
| **Synthetic antioxidant** | 20 ppm | 40 ppm | 60 ppm | 80 ppm | IC50 |
| **BHT** | 76.0±4.8 | 93.8±0.8 | 94.6±1.1 | 96.7±0.9 | 14.9±0.9 |
| **Ascorbic acid** | 34.2±5.2 | 51.8±2.4 | 67.2±0.6 | 85.5±1.7 | 40.3±1.1 |
| **Gallic acid** | 82.8±1.6 | 91.0±1.9 | 91.5±2.6 | 92.6±0.9 | 7.9±1.2 |

## CONCLUSIONS

The chemical composition of the essential oil of seed from *Trachyspermum ammi* (Umbelliferae) growing in Davarzan, northeast of Iran, was investigated. This study showed considerable amounts of Hexadecanoic acid (27.5%), ethyl linoleate (8.5%), 6-methyl-α-ionone (8.0%), isobutyl phthalate (5.8%), α-cadinol (4.7%), germacrene D (4.3%) and δ-cadinene (3.5%). These results demonstrated that the chemical composition of the essential oil of the same species can change depending on a variety of conditions, including climate, time of collection, and the ground composition of the sampling area besides of growth stages of plant. Also results of our study suggest the great value of the species *Trachyspermum ammi*f or use in pharmacy and phytotherapy. Based on this information, it could be concluded that this plant isnatural sources of antioxidant substances of high importance. It is noticed that the highest concentration of phenolic compounds in the extracts were obtained using solvents of high polarity; the methanol extract manifested greater power of extraction for phenolic compounds from *Trachyspermum ammi*. The high contents of phenolic compounds and the values of antioxidant activity indicated that these compounds contribute to the strong antioxidant activity.Further studies of this plant species should be directed to carry out in vivo studies of itsmedicinal active components in order to prepare natural pharmaceutical products of highvalue.

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