GC/MS Analyses for Detection and Identification of Antioxidant Constituents of Carum copticum Essential Oil

M. Kazemi

Department of Horticultural Science, Faculty of Agricultural Science and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran

Corresponding author, Email: kazenimohsen85@gmail.com

Abstract

The present work was designed to study the antioxidant activity and to identify the main active components of the essential oil of Carum copticum seeds. GC/MS analyses of the essential oil showed the presence of 13 compounds. The main constituents of the oil were thymol (29.45%), p-cymene (18.32%), γ-terpinene (18.34%) and carvacrol (14%). The antioxidant and free radical scavenging activities of Carum copticum oil was evaluated by using DPPH assays. The oil exhibited a considerable dose-dependent antioxidant activity. Thymol showed clearly a higher activity (IC50=10±0.0 µg mL⁻¹) followed by Carum copticum essential oil (15.12±0.1 µg mL⁻¹). Antioxidant activity guided fractionation of the oil was carried out by the TLC-bioautography screening and fractionation resulted in the separation of the main antioxidant compound which was identified as thymol (80%).

Keywords: Carum copticum, chemical composition, antioxidant activity, DPPH assays

Introduction

Reactive oxygen and nitrogen species, ROS/RNS are continuously produced in the human body and they are controlled by endogenous enzymes (superoxide dismutase, glutathione peroxidase, catalase). Antioxidants are known as molecules capable of inhibiting oxidation process in body so preventing of forming free radicals (Sikorski, 2001). There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. Natural products have played an important role worldwide in the antioxidant activity and prevention of fungal infections and pathologies with a strong inflammatory component, with several studies referring the therapeutic properties of these metabolites (Adorjan and Buchbauer, 2010; Chin et al., 2006; Edris, 2007). Since ancient times, herbs and spices in different types of food to improve flavours are well known for their antioxidant capacities (Madsen and Bertelsen, 1995). Recent research is now directed towards finding naturally occurring antioxidants of plant origin. Among these, the antioxidant properties of many aromatic and medicinal plants have shown to be effective in retarding the process of lipid peroxidation in oils and fatty foods and have gained the interest of many research groups (Kulisic et al., 2004). For example, various herbs and spices essential oils (such as clove, oregano, rosemary, sage, and lavender) have been reported to exhibit strong antioxidant and lipid protection properties (Adorjan and Buchbauer, 2010). In recent decades, the essential oils and various extracts of plants have been of great interest as they have been the sources of natural products. Ajowan (Carum copticum) is an annual herbaceous plant belonging to the Umbelliferae family, which grows in India, Iran, and Egypt with white flowers and small, brownish seeds (Khajeh et al., 2004). During the past centuries in the Iranian traditional medicine, several
therapeutic effects including anti-vomiting, anti-asthma and anti-spasm, is postulated for *Carum Copticum* fruits (Boskabady et al., 2005). The major component of its fruit is essential oil which is composed of γ-terpinene, p-cymene, α-pinene, β-pinene, and other substances such as thymol and carvacol (Chopra, 1982). The seeds of *Carum copticum* have a therapeutic effect on some cutaneous, neural, and urinary tract disorders (Khajeh et al., 2004). The main objectives of the present study were to evaluate the antioxidant properties of the essential oil from *Carum copticum* seeds and to find out which compounds contribute to the effects.

**Materials and Methods**

**Plant Material and Oil Isolation**

The plant materials were collected from the mountains in the city of Kermanshah-Iran in 2013-2014. The *Carum copticum* seeds were ground and the resulting powder was subjected to hydro-distillation for 3 hours in an all glass Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia (1975). The obtained essential oils were dried over anhydrous sodium sulphate and after filtration, stored at +4°C until tested and analysed.

**Essential Oil Analysis**

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 50°C as an initial temperature, holding for 6 min, with 3°C increases per minute to the temperature of 240°C, followed by a temperature enhancement of 15°C per minute up to 300°C, holding at the mentioned temperature for 3 min. Injector port temperature was 290°C and helium used as carrier gas at a flow rate 1.5 mL min⁻¹. Ionization voltage of mass spectrometer in the EI-mode was equal to 70 eV and ionization source temperature was 250°C. Linear retention indices for all components were determined by coinjection of the samples with a solution containing homologous series of C8-C22 n-alkanes and comparing them and their mass spectra with those of authentic samples or with available library data of the GC/MS system (WILEY 2001 data software) and Adams libraries spectra (2001).

**Antioxidant Activity**

The efficacy of the essential oils to scavenge 2,2,0-diphenyl-1-picrylhydrazyl (DPPH) radicals was evaluated using a spectrophotometry method (Cuendet et al., 1997; Kirby and Schmidt, 1997). On basis of bleaching of the bluish-red or purple colour of DPPH solution as a reagent. Briefly, a 50 ml volume of various dilutions of each samples was mixed with 5 mL of 0.004% methanol solutions of DPPH followed by 30 min incubation at ambient temperature. Thereafter, the sample absorbance was recorded against control at 517 nm. The inhibition percentages were measured using Eq. below.

\[
\text{Inhibition} \% = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100
\]

The antioxidants activity of the test samples in concentration providing 50% inhibition, were considered as IC50 (1 g mL⁻¹).

Butylhydroxyanisole (BHA) and ascorbic acid were used as positive controls. All experiments were repeated three times and the average results and standard deviations calculated.

**Rapid Screening for Antioxidants**

For screening of antioxidant compounds in *Carum copticum* essential oil, the TLC-bioautography method was carried out (Burits and Bucar, 2000; Guleria et al., 2012). The diluted oil (1:20 in methanol) was spotted on silica gel sheets (silica gel 60 F254 TLC plates) and developed in n-hexane-ethyl acetate (9:1). Plates were sprayed with the methanolic solution of DPPH (0.2%). The active constituents were detected as yellow spots on a violet background. Only zones where their color turned from violet to yellow within the first 30 min (after spraying) were taken as positive results.

**Activity Guided Fractionation of the Essential Oil for Antioxidants**

For the isolation and identification of the active compounds in the essential oil, PTLC was performed using the conditions previously described (Guleria et
al., 2012). The regions showing DPPH scavenging activity were scrapped off then, they were eluted with chloroform. All resulting constituents were analyzed by GC/MS and also tested for their antioxidant activities.

Statistical Analysis
The quantitative data of major components of oil were statistically examined by one-way analysis of variance (ANOVA), and significant differences among groups were subsequently analyzed by Duncan’s multiple range test (P<0.05). Correlation and regression coefficients were performed using Statistical Package for the Social Sciences (SPSS).

Results and Discussion

Chemical Composition of Essential Oil
Hydrodistillation showed that Carum copticum seeds contained 4.81% (v/w) essential oil. In oil of Carum copticum, 13 compounds were identified (Table 1), representing 95.07% of total oils. The main constituents were thymol (29.45%), p-cymene (18.32%), γ-terpinene (18.34%) and carvacrol (14%). Thangam and Dhananjayan (2003) reported thymol as the main constituent (36.7%) of the Carum copticum essential oil. A previous report by Rasooli et al. (2008) indicated the major constituent of the essential oil of Carum copticum was p-cymene and in other studies, γ-terpinene was the second most abundant constituent of oil (Khajeh et al., 2004). Srivastava et al., (1999) detected 11 compounds in the plant, with carvacrol (45.2%) and p-cymene (42.0%) the major constituents. Mohagheghzadeh et al. (2007) showed that Ca Carum copticum has two chemotypes, thymol and carvacrol. Therefore, the Carum copticum oils used in the present study belonged to the thymol chemotype (Table 1). These differences in chemical compositions of the oils could be attributed to environmental effects on the plants. The high level of p-cymene and thymol in the essential oil could contribute to the valorization of Iranian Carum copticum species, since this monoterpenes is of great importance in industry as intermediate for synthesis of fragrances, pharmaceuticals and herbicides.

Table 1 Chemical compositions of Carum Copticum essential oil.

<table>
<thead>
<tr>
<th>Components</th>
<th>Carum copticum %</th>
<th>Retention Index</th>
<th>Identification Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Myrcene</td>
<td>1.88</td>
<td>523</td>
<td>MS, RI</td>
</tr>
<tr>
<td>2 Limonene</td>
<td>2</td>
<td>610</td>
<td>MS, RI, CoI</td>
</tr>
<tr>
<td>3 α-Thujene</td>
<td>1</td>
<td>932</td>
<td>MS, RI, CoI</td>
</tr>
<tr>
<td>4 α-Pinene</td>
<td>2</td>
<td>941</td>
<td>MS, RI, CoI</td>
</tr>
<tr>
<td>5 b-Pinene</td>
<td>3.11</td>
<td>948</td>
<td>MS, RI</td>
</tr>
<tr>
<td>6 Sabinene</td>
<td>1</td>
<td>981</td>
<td>MS, RI</td>
</tr>
<tr>
<td>7 α-Phyllanderene</td>
<td>2.41</td>
<td>1000</td>
<td>MS, RI</td>
</tr>
<tr>
<td>8 p-Cymene</td>
<td>18.32</td>
<td>1028</td>
<td>MS, RI, CoI</td>
</tr>
<tr>
<td>9 b-Phyllanderene</td>
<td>0.56</td>
<td>1035</td>
<td>MS, RI</td>
</tr>
<tr>
<td>10 γ- Terpinene</td>
<td>18.34</td>
<td>1060</td>
<td>MS, RI, CoI</td>
</tr>
<tr>
<td>11 Terpinene- 4 - ol</td>
<td>1</td>
<td>1177</td>
<td>MS, RI</td>
</tr>
<tr>
<td>12 Thymol</td>
<td>29.45</td>
<td>1294</td>
<td>MS, RI</td>
</tr>
<tr>
<td>13 Carvacrol</td>
<td>14</td>
<td>1306</td>
<td>MS, RI</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>95.07</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The results presented in Table 2 revealed that Carum copticum essential oil and its main constituents exhibited a remarkable activity. In particular, thymol showed clearly a higher activity (IC50 = 10±0.0 µg mL\(^{-1}\)) followed by Carum copticum essential oil (15.12±0.1 µg mL\(^{-1}\)), carvacrol (13.01±0.2 µg mL\(^{-1}\)) and γ-terpinene (15.34±0.2 µg mL\(^{-1}\)). p-Cymene (21±0.1 µg mL\(^{-1}\)) was inactive (Table 2), despite previous reports of their in vitro antioxidant activities (Ruberto and Baratta 2000). BHT and ascorbic acid as positive controls were exhibited IC50 values equal to 14.71±0.2 µg mL\(^{-1}\) and 10±0.6 µg mL\(^{-1}\), respectively. Polyphenolic compounds have been found to protect erythrocytes from oxidative stress or increase their resistance to damage caused by oxidants. They are able to act as antioxidants in a number of ways, mainly as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelating agents (Rice-Evans et al., 1996; Kaviarasan et al., 2004). Because of high antioxidant and free radical scavenging activities of Carum copticum essential oil, it was further investigated to identify its active constituents. Therefore, a preliminary screening was initially carried out using the dot-blot DPPH staining method on TLC. As the essential oil presented a significant antioxidant activity in the assays and bioautography test, it was subjected to the PTLC for isolation of the active compounds.

Components identified and their antioxidant activity relative percentages have been shown in Table 3. The major compound found in the active band were thymol (80%) and carvacrol (15%). The antioxidant activities of these volatiles in cellular assays have not been previously reported and the results could be explained by the fact that in vitro tests do not take the physiological conditions of the cell, bioavailability of the antioxidant molecule, as well as general cellular metabolism into account. Carvacrol and thymol have been reported to contribute to the in vitro antioxidant activity of essential oil (Piccaglia et al., 1993; Burits and Bucar, 2000). However, our results indicated that the antioxidant activity of the essential oil is mainly due to the action of thymol. High correlation was reported between the antioxidant capacity and total phenol and flavonoids contents of plants (Silva et al., 2007; Tawaha et al., 2007). Besides antioxidant capacity, phenolic compounds exhibit a wide range of biological activities, including anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic, immune-stimulating agents, antiallergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antithrombotic, anti-stress, anti-hyperglycemia, cardio protective and vasodilatory effects (Balasundram et al., 2006). It is well known that plant phenolics, in general, are the highly effective free radical scavenging and antioxidants.

## Conclusions

Results of the study show that Carum copticum oil and one of its main compounds, thymol, is a source of antioxidant for the food, cosmetic and pharmaceutical industries. Because bioautography screening and separation of antioxidant compounds led to the identification of thymol as the major antioxidant constituent of the oil.

## References


Manuscript received 23 July 2014, accepted 19 August 2014