

***Dedicated to Professor Florin Dan Irimie on the
Occasion of His 65th Anniversary***

COMPARATIVE STUDY ON ESSENTIAL OILS OF SELECTED APIACEOUS SEEDS CULTIVATED IN TRANSYLVANIA

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ABSTRACT. A comparative study for analysis of essential oils obtained from plants grown in Transylvania, plants belonging to *Apiaceae* family, was achieved. Five types of plants seeds from *Coriandrum sativum* L. (coriander), *Anethum graveolens* L. (dill), *Pimpinella anisum* L. (anise), *Carum carvi* L. (caraway) and *Foeniculum vulgare* L. (fennel) were hydrodistilled to yield essential oils. The chemical composition of the obtained essential oils was determined; also the antioxidant, antimicrobial and tyrosinase inhibitory activities were investigated. The major constituent of coriander essential oil were linalool (73%), for dill (52.7%) and caraway (67.55%) was carvone and for anise (95.2%) and fennel (89.64%) was trans-anethole. The anise seeds essential oils provide the highest antioxidant activity while the coriander essential was the lowest. The fennel essential oils proved to be a potent tyrosinase inhibitor. All studied essential oils exhibited considerable antimicrobial activity, but coriander essential oil proved to have a very high antibacterial activity on gram-negative bacteria *E. Coli* and *Klebsiella pneumonia*.

Keywords: *essential oils, antioxidant activity, tyrosinase inhibitors, antimicrobial activity, GC/MS*

INTRODUCTION

Because of growing population concern about using unhealthy synthetic additives, consumers are increasing interests in ingredients from natural sources [1,2]. Essential oils are complex, combination of volatile compounds

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from natural sources, with potent medicinal beneficial properties which make them perfect ingredients in cosmetic, pharmaceutical and food industries. Presently, it is mandatory, to investigate plants essential oils compositions and biological activities for understanding and fasten the use of essential oils in practical application. Based on several studies, many essential oils, constituents of plants, herbs, spices, seeds are an excellent source of bioactive compounds with powerful antioxidant and antimicrobial activities [3].

Apiaceae (or *Umbelliferae*) are a family of mostly aromatic plants, spread in regions with a temperate climate used as culinary herbs and spices. Due to the high concentration of secondary metabolites such as essential oils, in seeds and herbs, the apiaceae are used as flavouring agents and also for medical purposes and are known as nutraceutical plants [4].

For our study we have chosen from this class, five aromatic plants, coriander (*Coriandrum sativum*), dill (*Anethum graveolens*), fennel (*Foeniculum vulgare*), caraway (*Carum carvi*) and anise (*Pimpinella anisum*) due to the fact that there are indigenously available raw materials/oilseeds, they have a high concentrations of essential oils, are very aromatic and intensely used in our cuisine for their flavour, so are good potential candidates as food additives. It has been reported that essential oils from apiaceous fruits have some medicinal properties [5,6]. Coriander seeds essential oil proved to be antimicrobial and has insecticidal effects [7]. Dill essential oil is used for antispasmodic effects [8]. It has been reported that fennel oil essential have antifungal, antioxidant and hypoglycemic properties [9] while caraway essential is antimicrobial, antioxidant and have cytotoxic activities [10]. Anise seed essential oil can be used as antibacterial, antifungic, antiviral, and antispasmodic agents [11].

Due to the influence of climatic conditions, growth region, on qualitative composition and biological activities of plants, in this paper we propose the investigation of antioxidant, antimicrobial and antityrosinase activity of essential oils extracted from fruits of *Coriandrum sativum* L. (coriander), *Anethum graveolens* L. (dill), *Pimpinella anisum* L. (anise), *Carum carvi* L. (caraway) and *Foeniculum vulgare* L. (fennel), cultivated in Transylvania, Romania. Also, identification and comparison of isolated essential oils chemical components by GC/MS was investigated.

RESULTS AND DISCUSSION

The aim of this work was to achieve a comparative study for analysis of coriander, dill, fennel, caraway and anise seeds by comparing the chemical composition, the antioxidant, antimicrobial activity and tyrosinase inhibitory activities of the obtained essential oils. The essential oils were obtained by

hydrodistillation of selected seeds plant using Clevenger apparatus. The seeds used were collected from plants from Botanical Garden of Târgu Mures, Transylvania, Romania.

The essential oils yield and the chemical composition of the essential oil analyzed by GC–MS

The yields of the obtained essential oils are provided in table 1. Comparing the obtained essential oils from selected *Apiaceae*, the highest yield was obtained for Anise seeds (2.4 ± 0.2 (%v/w)), followed by Caraway seeds (2.1 ± 0.1 (%v/w)). The lowest essential oils yield was obtained in case of Coriander seeds (0.9 ± 0.1 (%v/w)). The obtained dill seeds essential oil yields 1.2 ± 0.1 (%v/w) and fennel seeds essential oil yield is 1.7 ± 0.2 (%v/w).

There are some reports about the influence of many factors like geographic growth region, type of cultivar, climatic conditions, stage of plant maturity, soil fertilizer on the yield and on chemical composition of *Apiaceae* seeds essential oils from different countries [12,13,14].

The reported coriander seeds essential oils yields, cultivated in different countries, is between 0.3-1.2% [15,16], but also higher yields were reported [6], 1.9-3%, even in a different geographical area in Romania (2%) [17].

The reported dill seeds essential oil yield gave similar yields [6,18,19] with our results, but also really high yields were reported [20].

The fennel seeds essential oil gave similar yields with yields reported in literature (1.6-2%) [6,21], but also difference in yield comparative with plants from others geographical regions were reported (2.81-7.1%) [22,23,24].

Caraway essential oil obtained yield was higher than some literature results (0.48-1.41)[25], but also comparable with other results obtained from literature⁶. The literature reported yields for anise seed essential oils vary from 1.4% to 6% [6,24,26,27].

Table 1. Essential oils yields

Yield(%v/w)	Essential oil
0.9±0.1	Coriander
1.2±0.1	Dill
1.7±0.2	Fennel
2.1±0.1	Caraway
2.4±0.2	Anise

The main components of the essential oils obtained from five plants from *Apiaceae* family plants are presented in Table 2. Identification of the constituents was based on comparison with mass fragmentation pattern and spectral comparison using NIST and Wiley mass spectra libraries of standards.

The major constituents of coriander essential oil obtained were linalool (73%), camphor (6.7%), p-cymene (6.02%), α -pinen (4.57%) and limonene (1.8%). In dill essential oil, carvone (52.7%) was the main constituent identified, followed by dillapiole (22.05%) and limonene (15.89%). In fennel essential oil the main components were identified as trans-anethole (89.64%), limonene (7.37%), fenchone (1.73%), estragole (1.26%). Caraway essential oil composition had as a main components carvone (67.55%) and limonene (26.06%). In addition, various minor components were identified. Anise essential oil contain trans-anethole (95.2%) and p-anisealdehyde (4.8%). It is known that anise flavor it is very similar with fennel flavor, the high concentration of trans-anethole found in both analyzed essential oils proved that the smell and taste can be very similar.

Table 2. Chemical composition of the obtained essential oils analysed by GC-MS

Component	M	Rt	Coriander %	Dill %	Fennel %	Caraway %	Anise %
alpha-pinene	136	6.0	4.57	<0.1	<0.1	<0.1	-
camphene	136	6.3	1.53	-	<0.1	-	-
sabinene	136	6.7	1.12	<0.1	<0.1	<0.1	-
beta-pinene	136	6.82	1.03	-	-	-	-
p-cymene	136	7.63	6.02	<0.1	<0.1	-	-
γ -terpinene	136	7.68	<0.1	<0.1	<0.1	<0.1	-
limonene	136	7.72	1.7	15.29	7.27	26.06	-
cis-linalool oxide	170	8.36	1.88	-	-	-	-
trans-linalool oxide	170	8.62	1.45	-	-	-	-
linalool	154	8.85	73	-	-	-	-
fenchone	152	9.28	-	-	1.33	-	-
cis, limonene oxide	152	9.46	-	<0.1	-	0.3	-
trans,limonene oxide	152	9.53	-	-	-	0.19	-
camphor	152	9.77	6.7	-	-	-	-
cis-dihydrocarvone	152	10.54	-	4.53	-	2.49	-
trans-dihydrocarvone	152	10.64	-	4.23	-	-	-
dihydrocarveol	154	10.83	-	<0.1	-	0.6	-
cis-carveol	152	10.86	-	<0.1	-	0.32	-
cis-dihydrocarveol	154	11.05	-	-	-	1.52	-
trans-anethole	148	11.16	-	-	89.64	-	95.1
carvone	150	11.33	<0.1	52.7	-	67.5	-
carvone oxide, cis	166	11.72	-	-	-	3.43	-
estragole	148	12.35	-	-	1.26	-	-
geranyl acetate	198	12.77	0.8	-	-	-	-
p-anisealdehyde	136	14.33	-	-	<0.1	-	4.8
caryophyllene oxide	200	15.77	-	-	-	0.72	-
cis-isopiolle	222	16.22	-	-	-	0.12	-
dillapiole	222	16.77	-	22.05	-	-	-

The main components, in all analyzed essential oils, were similar with literature data [21,28,29,30,31]. Differences appear in compounds relative concentration. This variation in composition might be connected with climatic conditions, geographic position of the growth region also with plant stage of development and metabolism. The volatile compounds classes percentages are provided in Table 3.

Table 3. Compound classes classification in studied essential oils

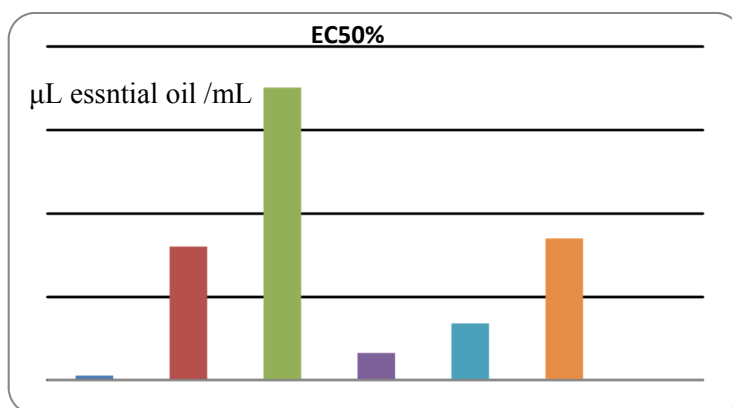
Component	Coriander	Dill	Fennel	Carawa	Anise
	%	%	%	y %	%
Monoterpene hydrocarbons	16.07	15.89	7.37	26.06	-
Oxygenated monoterpenes	83.93	83.61	92.63	76.77	100
Sesquiterpene hydrocarbons	-	-	-	0.72	-

Antioxidant activity

Antioxidant activities of essential oils extracted from five plants from *Apiaceae* family plants were investigated using DPPH scavenging method. DPPH percent scavenging activities of volatile oils were measured at different concentrations between 0.01 and 0.25 mg/ml. EC50% is a parameter used for quantification of antioxidant activity and is giving by the amount of plant extract used for decreasing the initial DPPH concentration by 50%. A lower EC50% indicates a higher antioxidant activity. All tested essential oil present antioxidant and free radical scavenging activities. Comparing the antioxidant activity, measuring EC50%, we can conclude that anise seeds volatile oils provide the highest antioxidant activity comparable with vitamin E's activity used as standard. Fennel essential oils, which major component is also trans-anethole, exhibit also a high antioxidant activity. The results obtained were comparable with literature data [22]. Dill and caraway essential oil have a similar medium persistent antioxidant activity. Coriander seeds essential oil proved to have a weak antioxidant potential, comparative with the other obtained essential oils. The small antioxidant activity of essential oil from Coriander seeds was also found in literature data from plants grown on different geographic regions [15]. The results obtained in this study, showed that high antioxidant activity may be correlated with the presence of anethole, carvone and limonene in essential oils, but further research has to be made.

Table 4. Antioxidant capacity parameters

Sample	EC50%($\mu\text{L}/\text{mL}$)	Equiv. $\mu\text{L Vit E}/\text{mL}$ essential oil
Vitamine E	2.68 \pm 0.13	-
Dill (<i>Anethum graveolens</i>)	80.28 \pm 0.52	73.09 \pm 6.13
Coriander (<i>Coriandrum sativum</i>)	175.45 \pm 0.43	57.35 \pm 7.9
Anise (<i>Pimpinella anisum</i>)	16.31 \pm 0.45	125.63 \pm 6.40
Fennel (<i>Foeniculum vulgare</i>)	34.06 \pm 0.25	99.68 \pm 2.39
Caraway (<i>Carum carvi</i>)	85.19 \pm 0.27	62.25 \pm 4.26

**Figure 1.** Effective concentration of studied essential oils (EC50%)

Tyrosinase inhibitory activity

Tyrosinase is an important enzyme involved in melanin biosynthesis [32] in animals and enzymatic browning in fruits [33]. Single compounds and plants extracts with anti-tyrosinasic inhibitory activity can be used as whitening agents in cosmetics or as additives in food industry to prevent fruits enzymatic browning phenomenon [34]. Tyrosinase inhibitory activity assay was followed spectrophotometrically using L-tyrosine as the substrate. Anti-tyrosinase inhibitory activity of studied essential oils was evaluated. All studied essential oils showed an inhibitory activity, the reaction rate of standard reaction was lowered with different percents 93% (fennel essential oil), 78%(caraway essential oil), 69%(anise essential oil), 61%(dill essential oil), 46% (coriander essential oil). The studied essential oil proved to be potent tyrosinase inhibitors, which make them good candidates for cosmetic use.

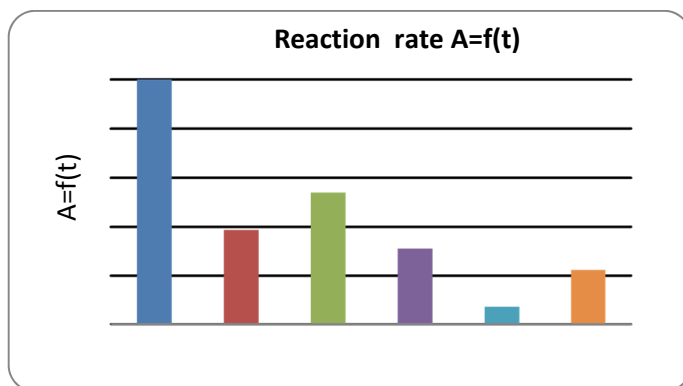


Figure 2. Comparison of reaction rates for tyrosinase enzyme inhibitory activity of studied essential oils

Antimicrobial activity

The antimicrobial activity of the obtained essential oils was examined against a panel of six microorganisms by the application of agar disc diffusion method. The data expressed as diameter of growth inhibition zone (mm). The results are illustrated in Table 5. All samples of volatile oils exhibited considerable antimicrobial activity. Coriander essential oil proved to have a very high antimicrobial activity on gram-negative bacteria *E. Coli* and *Klebsiella pneumonia* and a high antifungal activity on *Candida albicans*, fennel essential oil has a high antimicrobial activity on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, while caraway essential oils proved to be more effective on *Bacillus cereus* and on *Pseudomonas aeruginosa*. Dill essential oil proves to have the higher activity of all studied essential oils on *Staphylococcus aureus* and a high activity on *Candida albicans*. Anise essential oils proved to have the smallest inhibition zones from all studied essential oils, but still active on all studied microorganisms. The standard used proved to be not effective on gram-negative bacteria *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *E Coli*, while our studied essential oils exhibited all antibacterial activity. The antimicrobial activity of our studied essential oils is in agreement with other literature results [6,15,22,29,31] differences in activity appear due to the geographical and climatical grown region. The high revealed antimicrobial activity of our essential oils, make them an important source of new therapeutic and antimicrobial preperates.

Table 5. Antimicrobial activity of studied essential oils

Microbial species	Inhibition diameter (mm)					
	Anise	Fennel	Coriander	Caraway	Dill	M CFR30
<i>Staphylococcus aureus</i>	10±1	13±1	12±1	10±1	15±1	26±1
<i>Bacillus cereus</i>	7±1	10±1	9±1	12±1	8±1	15±1
<i>E. coli</i>	9±1	8±1	23±1	10±1	9±1	-
<i>Klebsiella pneumoniae</i>	7±1	9±1	13±1	9±1	9±1	-
<i>Pseudomonas aeruginosa</i>	8±1	12±1	7±1	9±1	12±1	-
<i>Candida albicans</i>	9±1	17±1	15±1	6±1	15±1	17±1

CONCLUSIONS

A comparative study for analysis of five plants from *Apiaceae* family was achieved. The essential oils seeds were compared in terms of chemical composition, antioxidant, antimicrobial and tyrosinase inhibitors activities. The indigenously available *Apiaceae* plants fruits proved to have a high concentration of essential oil. The chemical composition of the obtained essential oils showed us that major constituents were similar with data obtained from other essential oils obtained from plants cultivated in different geographic regions. Differences still appear in compounds relative concentration. This variation in composition might be connected with climatic conditions, geographic position of the growth region also with plant stage of development and metabolism. The anise seeds essential oils provide the highest antioxidant activity, followed by fennel and caraway. Fennel essential oils proved to be a very potent inhibitor of tyrosinase activity, which make it a very good candidate as a whitening agent for cosmetic use. Coriander essential oil proved to have a very high antimicrobial activity on gram-negative bacteria.

All studied essential oils exhibited considerable antioxidant, antimicrobial and antityrosinase activity, proving that essential oils can be a good indigenous resources for natural food additives, new pharmaceutical prepartes and also natural additives in cosmetic industry.

EXPERIMENTAL SECTION

MATERIAL AND METHODS

Five plants from *Apiaceae* family plants, *Coriandrum sativum* L. (coriander), *Anethum graveolens* L. (dill), *Pimpinella anisum* L. (anise), *Carum carvi* L. (caraway) and *Foeniculum vulgare* L. (fennel), were procured from Botanical Garden of Târgu Mures, Transylvania, Romania. The fruits were dried and grounded. All reactive and standards were purchased from Merck (Darmstadt, Germany). Antioxidant and antityrosinasic activities were determined using a Varian Cary 50 Spectrophotometer in a kinetic mode.

GC-MS apparatus

A Trace DSQ Thermo Finnigan quadrupole mass spectrometer coupled with a Trace GC was used. The Rtx-5MS capillary column, 30m x 0.25mm, 0.25 μ m film thickness was used in a temperature program from 50°C, 2 min, then 8°C /min to 250°C, with 30°C /min at 310°C (10min) for essential oils compounds analysis. Helium was used as carrier gas at a flow rate of 1 mL/min. 1 μ L of each sample was injected into the GC-MS using the split mode (10:1) using a TriPlus autosampler(Proanalysis, Bucharest, Romania). The mass spectrometer was operated in EI mode at 70 eV, emission current was 100 μ A and mass spectra mass range 50-500 a.m.u. Transfer line temperature was set at 250°C, injector at 250°C and ion source at 250°C.

EXTRACTION PROCEDURE FOR ESSENTIAL OILS

100 g of dried and grounded coriander, dill, fennel, caraway and anise seeds were submitted to hydrodistillation in a Clevenger type apparatus for 4h. The collected essential oils were dried over anhydrous Na₂SO₄. For the GC-MS analysis samples were diluted 100 μ L/mL in ethanol.

DETERMINATION OF ANTIOXIDANT ACTIVITY

For determination of antioxidant activity, DPPH antioxidant assay was used. Samples essential oils were used to decolorize an ethanolic solution of 40 μ M DPPH. The monitoring of DPPH reduction was followed at 517nm. The percentage of DPPH scavenging activity is expressed using following formula: $DPPH_{inhibition}\% = [(A_i - A_t) / A_i] \times 100$. For determination of effective concentration (EC₅₀), different concentration of essential oils 10, 20, 40, 80, 160 μ L/mL were used. The EC₅₀ was determined by plotting the DPPH_{inhibition}% against used extract concentration. Vitamine E was used as standard for the calibration curve and was plotted at 2, 4, 6, 8, and 10 μ L/mL, prepared in ethanol.

DETERMINATION OF TYROSINASE INHIBITION ACTIVITY

For determination of antytyrosinasic activity of essential oils, a spectrophotometric method was used. To 897 μL sodium phosphate buffer solution (20mM, pH= 6.8), 4 μL L-tyrosine water solution (4mM) and 3 μL phosphate buffer solution of tyrosinase (≥ 0.2 unit/mg solid in 1 μL) 5 μL of ethanol diluted essential oil sample (100 $\mu\text{L}/\text{mL}$) was added. The enzymatic reaction was followed at 475nm for 15 minutes. The reaction rates were calculated from regression curve slope.

DETERMINATION OF ANTIMICROBIAL ACTIVITY

The antimicrobial activity of the essential oils were tested using Agar Diffusion Test (ADT). For testing the antibacterial activity of the obtained essential oils, five species of bacteria were chosen, two gram-positive bacteria *Staphylococcus aureus*, *Bacillus cereus*, three gram-negative species *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* and a fungi specie *Candida albicans*. The discs (6 mm in diameter) were impregnated with 5 μL essential oils placed on inoculated agar. As standard antibiotic the Cefadroxil (M CFR30), 30 $\mu\text{g}/\text{disc}$ was used. Antimicrobial activity was evaluated by measuring the inhibition zone.

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