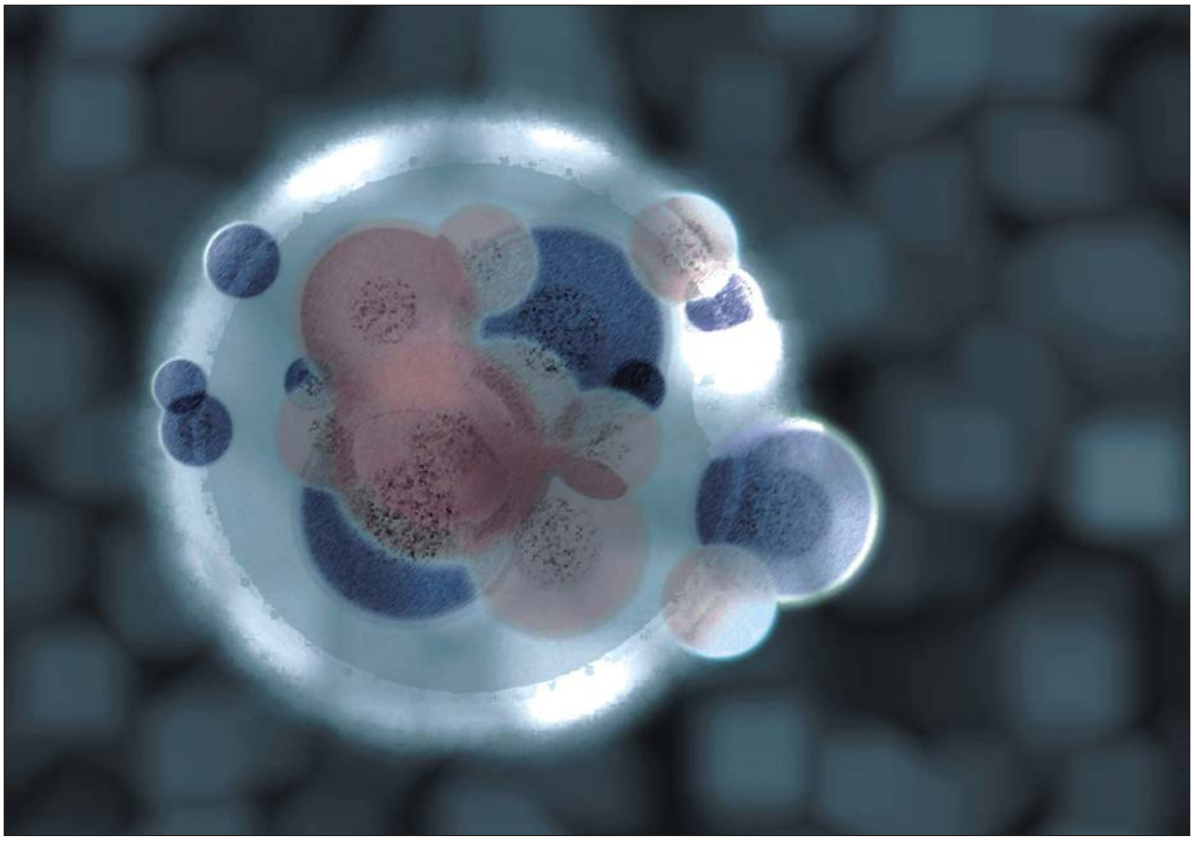

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Research Article

Evaluation of Essential Oil Composition, Antioxidant and Antimicrobial Properties of *Chaerophyllum crinitum* Boiss (Apiaceae) from Turkey: A Traditional Medicinal Herb

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Abstract

To determine essential oil composition, antioxidant and antimicrobial features of the aerial parts of *Chaerophyllum crinitum* Boiss. The obtained oil of the plant analysed by GC and GC/MS. The analysis has led to the identification of 64 components comprising 85.5% of the oil. In addition, DPPH assay and disc diffusion method were used to evaluate antioxidant and antimicrobial properties, respectively. α-terpinolene (20.3%), β-cubebene (9.3%), α-terpineol (7.2%) and limonene (5.8%) were dedected as major components. Moreover, *C. crinitum* exhibited remerkable antimicrobial effect against most of used microorganisms. But it was inactive in the DPPH assay. The chemical composition, antioxidant and antimicrobial effects of the essential oil of aerial parts of *C. crinitum* were reported for the first time and obtained results show that it may be a good candidate for the future studies.

Key words: *Chaerophyllum crinitum*, essential oil, α-terpinolene, antioxidant, antimicrobial

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Apiaceae (Umbelliferae) usually known as carrot or parsley family is a member of mostly aromatic plants with hollow stems (Simpson, 2006). Apiaceae is the 8th largest family in Turkey and the family comprising about 446 genus and 3540 species among which 33% are endemic in Turkey (Davis *et al*., 1988; Guner *et al*., 2000; Ozhatay *et al*., 2009). This family is rich in secondary metabolites and embodies numerous genera of high economic and medicinal value yielding flavonoids, terpens, coumarins and essential oils. It is well-known that origination of essential oils and oleoresins are characteristic properties of the family (Yilmaz and Tekin, 2013). The genus Chaerophyllum is the largest genus in the subtribe Scandicinae, belonging to Apiaceae family, comprised of about 110 species which includes annual and perennial herbal plants widely distributed in temperate and subtemperate zones of Asia, Africa and Europe. The genus is also represented in the Flora of Turkey by 15 species of which four are endemic (Guner *et al*., 2000).

These plants are also used in traditional medicine in a lot of countries, fresh leaves and stems are occationally added to salads, while tea made of dried leaves and roots are used as herbal remedy to soothe sore throat, cough and allergies (Shafaghat, 2013). Preceding phytochemical investigations of *Chaerophyllum* species have revealed the presence of secondary plant metabolites like lignans, phenylpropanoids and polyacetylenes, phenolic acids and related compounds flavonoid glycosides as reported by Mikaya *et al*. (1981), Dall'Acqua *et al*. (2004) and Gonnet (1986). Now a days, there is an increasing interest in usage of plant extracts and essential oils. Particularly, the antimicrobial and antioxidant activities of solvent extracts and essential oils as well as their potential anticancer activity have been investigated in recent date (Mimica-Dukic *et al*., 2004; Sylvestre *et al*., 2005).

As far as we know that there is no study about biological activity of *Chaerophyllum criniticum*'s essential oil. With this aim, we did not study only antioxidant properties but also antimicrobial features. Moreover, chemical composition of essential oil was determined in this study.

MATERIALS AND METHODS

Plant material: *Chaerophyllum crinitum* was collected from the rocky and plain areas with an altitude of 1,400-1,500 m in Nurs village, Bitlis in 2013. The taxonomic identification of the plant materials was confirmed by Dr. Hayta. Voucher specimens were kept at the Fırat University Herbarium (FUH-4822).

Extraction of the essential oils: Air-dried aerial parts of the plant samples (100 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h to yield.

Gas Chromatography (GC) analysis: The essential oil was analyzed using HP 6890 GC equipped with an FID detector and an HP-5 MS column (30 m×0.25 mm i.d., film tickness 0.25 µm capillary column). The column and analysis conditions were the same as in GC-MS. The percentage composition of the essential oil was computed based on the GC-FID peak areas without correction factors.

Gas Chromatography/Mass Spectrometry (GC-MS) analysis: The oil was analyzed by GC-MS, using a Hewlett Packard system (HP-Agilent 5973 N GC-MS system with 6890 GC) at the Plant Products and Biotechnology Res. Lab. (BUBAL) of Fırat University. The HP-5 MS column (30 m×0.25 mm i.d., film thickness 0.25 µm) was used with Helium as the carrier gas. Injector temperature and split flow were 250°C and 1 mL min^{-1}, respectively. The GC oven temperature was kept at 70°C for 2 min and programmed to 150°C at a rate of 10°C min^{-1}. Then constant was kept at 150°C for 15 min to 240°C at a rate of 5°C min^{-1}. Alkanes were used as reference points in the calculation of the Relative Retention Indices (RRI). The MS was taken at 70 eV with a mass range of 35-425. Component identification was carried out using spectrometric electronic libraries (Wiley, NIST). The identified constituents of the essential oil were listed in Table 1.

Antioxidant activity

DPPH assay: Different aliquots (1.5-40 µL) samples of pure essential oil of *Chaerophyllum crinitum* were mixed with 0.4 mL 0.5 mM DPPH in etanol. Final volume adjusted up to 2 mL with etanol. Mixtures were vorteksed and left 30 min at room temperature in dark. Ethanol was used as blank. One milliliter 0.5 mM DPPH diluted in 4 mL of ethanol (1:4), respectively and used as control. Absorbaces were determined at 517 nm (Cuendet *et al*., 1997). Inhibition percentages (I%) was calculated as follows:

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, I: Inhibition value, $A_{control}$: Absorbance of control, A_{sample}: Absorbance of sample.

Antimicrobial activity

Microbial strains: The essential oil of *C. crinitum* was tested against a set of ten microorganisms include; *Entorococcus*

Table 1: Constituents of the essential oil *Chaerophyllum crinitum*

Compounds	RRI	*Cheropyhllum crinitum*
Hexenal	935	0.1
Trans-2,4-heptatienal	1007	0.2
α-thujene	1016	0.1
α-pinene	1021	0.1
Camphene	1034	0.1
Sabinene	1052	0.3
β-pinene	1056	1.1
β-myrcene	1065	0.3
α-phellandrene	1077	0.1
[+]-4-carene	1084	0.1
p-cymene	1090	5.0
dl-limonene	1097	5.8
Cis-ocimene	1098	0.3
γ-terpinene	1116	3.3
Cyclohexane,1,3-dimethyl-2-methylene	1124	0.1
α-terpinolene	1136	20.3
Benzene-1-methy-4	1139	0.7
Phenol-4-methyl	1145	0.1
1,3,8-p-menthatriene	1156	0.3
D-fenchyl alchol	1161	0.8
Z-p-mentha-2,8-dienol	1163	0.1
Cyclobutene,bis(1-methylethylidene)	1171	0.4
8-oxaspiro[5.2]-oct-2-ene	1177	3.8
Trans-2-decalone	1188	0.1
Pinocarvone	1192	0.2
Borneol	1199	0.2
3-cyclohexen-1-ol	1203	4.3
Ethanone	1207	0.5
α-terpineol	1214	7.2
Cylohexane,5-methyl-3(1 metyhlethenyl)	1216	0.4
Fenchyl acetate	1229	3.4
2-cylohexan-1-one	1247	0.2
Chrysanthenyl acetate	1251	0.1
Carvacrol	1257	0.1
Bornyl acetate	1280	1.1
Thymol	1286	0.1
Phenol,2-ethyl-4,5-dimethyl	1293	0.1
Bicyclobutylidiene	1301	0.1
1,3,6-heptatriene,2,5,6-trimethyl	1322	0.1
Safranal	1327	0.1
Silane, trimethylfenyl	1331	0.1
Cis-ocimene	1336	0.1
α-longipinen	1343	0.1
α-cubebene	1358	0.2
α-copaene	1360	0.1
Caryophyllene	1391	0.2
β-gurjunene	1394	0.1
β-cubebene	1401	9.3
Trans-β-farnesene	1416	1.9
Germacren D	1435	0.3
Bicylogermacrene	1445	0.1
β-bisabolene	1450	0.2
Bergamotol	1462	4.1
α-calacorone	1472	0.9
δ-3-carene	1475	0.2
α-farnesene	1488	0.1
Spathulenol	1493	0.8
Caryophyllene oxide	1496	0.8
Camphene	1501	0.2
p-cymol	1505	1.0
Bergamotone α-Z	1510	2.5
Cedrol	1537	0.1
Apiol	1545	0.2
Tricosane	1889	0.1
	Total	85.5

*RRI: Relative retention index

faecium (ATCC 700229), *Candida tropicalis* (ATCC 13803), *Citrobacter freundii* (ATCC 8090), *Escherichia coli* (ATCC 35218), *Proteus mirabilis* (ATCC 7002), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633), *Burkholderia cepacia* (ATCC 25608), *Staphlococcus aureus* (ATCC 33862) and *Acinetobacter baumannii* (RSKK 02026). The used microorganisms are commersial standart strains. In this study, ATCC (American Type Culture Collection) numbers of strains are clear and there is no necessity to add isolates.

Disc diffusion assay: Antimicrobial activity of essential oil of *Chaerophyllum crinitum* was dedected with disc diffusion method (NCCLS., 1997). The microorganisms were cultured in brain heart infusion broth at 37°C for 24 h and standardized to number 0.5 of the McFarland Nephelometer that according to the order of 10^8 CFU mL^{-1} (Barry and Thornsberry, 1985). Hundred microliters of prepared cultures were spread on the surface of mueller-hinton agar. Twenty five microliters pure essential oil of the plant was impregnanted to 6 mm steril discs and placed on the culture medium (Barry and Thornsberry, 1985). Streptomiycine standart discs were used as positive control. The samples were inoculated at 37°C for 24 h and inhibition zone diameters were dedected.

RESULTS

In this study, chemical composition of essential oil of *C. crinitum* was investigated by GC and GC-MS. The composition, percentage and retention indices of components of the oil were listed in Table 1. The essential oil yield is 0.2 (v/w) of *C. crinitum*. Sixty four constituents were comprised the 85.5% of the total oil. The main constituents were determined as α-terpinolene (20.3%), β-cubebene (9.3%), α-terpineol (7.2%), limonene (5.8%), p-cymene (5.0%) and 3-cyclohexen-1-ol (4.3%).

In addition, antimicrobial activity of the essential oil was dedected with disc diffusion method and it showed remarkable antibiotic effect against most of used

Table 2: Antimicrobial activities of *Chaerophyllum crinitum*

	Inhibition zone diameters (mm)									
Plant	EF	EnF	BC	SA	AB	BS	CF	EC	PM	CT
Chaerophyllum crinitum	7	7	8	10	16	-	-	-	-	11
Positive Control[a]	33	23	27	21	38	19	25	38	19	25

EF: *E. faecalis*, EnF: *Entorococcus faecium*, BC: *B. cepacia*, SA: *S. aureus*, AB: *A. baumannii*, BS: *B. spizemi*, CF: *C. freundii*, EC: *E. coli*, PM: *P. mirabilis*, CT: *C. tropicalis* [a]: Streptomiycine

microorganisms. The inhbition zone diameters presented in Table 2. On the other hand, the essantial oil of the plant did not exhibit antioxidant effect. It's inhibition percentage was 18%.

DISCUSSION

Chaerophyllum crinitum has a high level of monoterpene groups as reported by Nematollahi *et al*. (2005) that chemical compositions of the essential oil of *C. crinitum* and *C. macropodum*. At the end of the study 98.5% (28 components) and 84.3% (11 components) were dedected from the oils of *C. macropodum* and *C. crinitum*, respectively. In the study, α-pinene (23.0%), β-pinene (17.3%), fenchyl acetate (13.8%), α-ocimene (8.6%), β-ocimene (6.5%), limonene (6.3%), myrcene (5.5%) and three oxygenated monoterpenes (2.4%) were determined as major components in *C. macropodum* oil. Besides, β-ocimene (50.5%), β-phellandrene (8.8%), p-cymene (7.1%) and γ-terpinene (6.5%) were determined as major components of *C. crinitum* oil (Nematollahi *et al*., 2005). In another study, the essential oil of *C. macrospermum* from Iran and they found β-ocimene (55.9%), terpinolene (9.8%) and α-pinene (7.5%) as major components (Sefidkon and Abdoli, 2005). Similarly, Mamedova (1994) reported that 33 and 28 components of the oil obtained from flowers and leaves-stems of *C. macrospermum*. In the work 1,8-cineole (7.2 and 1.4%), linalool (6.7 and 2.1%), δ-3-carene (4.4 and 5.0%), α-terpineol (4.7 and 1.5%) and eugenol (1.0 and 9.3%) were identified as major constituents when oxygenated monoterpenes were found as predominant constituents. The rate of monoterpene hydrocarbons of the essential oil of *C. villosum* leaf which collected from India-Uttarakhand is (91.34%) and γ-terpinene (74.93%), is the single major component. Furthermore, the antioxidant capacity of the essential oil was examined using an *in vitro* radical scavenging activity test (Joshi, 2013a). Chemical analysis of root essential oil of *Chaerophyllum villosum* from Uttarakhand, led to the identification of 31 constituents accounting for 91.49% of the total oil. The carvacrol methyl ether (31.12%), myristicin (19.06%), thymol methyl ether (18.60%), γ-terpinene (11.69%), were the principle components. The studies have shown that the essential oil composition of leaf and root was totally different. In another report, γ-terpinene and p-cymene were found as the major constituents of leaf oil and carvacrol methyl ether and thymol methyl ether were noticed as the major constituents in rhizome essential oil of *C. villosum* (Joshi, 2013b).

In the present work, antioxidant activity of essantial oil of *C. crinitum* was dedected with DPPH method. Essential oil of the aerial parts of *C. crinitum* did not show antioxidant activity (18%). In accordance with our study, essential oils from another *Chaerophyllum* species were also reported inactive in the DPPH assay (Ebrahimabadi *et al*., 2010). However, in a recent study, Joshi (2014) stated that the chelating power activity of essential oil of *C. villosum* and it was found as a good natural antioxidant.

On the other hand, the essential oil obtained from aerial parts of *C. crinitum* were tested against a set of ten microorganisms to estimate its antimicrobial activity. *Chaerophyllum crinitum* exhibited significant antibiotic activity against most of used microorganism. Results presented in Table 2. In parallel to findings by Kurkcuoglu *et al*. (2006) that essantial oils of *C. libanoticum* and *C. byzantinum* demonstrated antifungal and antimicrobial effect on some bacterial strains. Besides, Shafaghat (2009) indicated that flower, leaf and stem oils of *C. macropodum* showed antimicrobial acvtivity. Moreover, Ebrahimabadi *et al*. (2010) stated that the essential oils of *C. macropodum* exhibited considerable antibiotic activity against several microorganisms include *Candida albicans*. Furthermore, Lakusic *et al*. (2009) indicated that strong antimicrobial effects of *C. aureum* L. oil were found on gram-positive and gram negative bacteria. As a conclusion, according to our data *C. crinitum* may be a good candidate to use in several areas as a natural antimicrobial agent but it is need to further studies.

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