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Chemical Composition of Hydrodistillated Essential Oil of *Heracleum antasiaticum* MANDEN (L.) and its Antimicrobial Activity Against Food-Borne Microorganisms

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HIGHLIGHTS

- Elemicin and apiole were identified as major components of the *Heracleum antasiaticum* essential oil.
- The oil exhibited broad-spectrum antimicrobial activity against food-borne microbes.
- An antagonistic effect was observed on probiotic bacteria such as *Lactobacillus* spp.
- Potential use of the oil as a natural food preservative was demonstrated.

Abstract

The essential oil obtained by hydrodistillation from the aerial parts of *Heracleum antasiaticum* MANDEN from Türkiye was analyzed by GC and GC-MS. Forty-five compounds were characterized, representing 92.7 % of the total components detected, and elemicin (27.8%), apiole (26.2%), *n*-octanol (4.9%), α-zingiberene (3.9%), octyl acetate (3.8%), and (E)-γ-bisabolene (3.1%) were identified as predominant components. In addition, in this study, the essential oil was tested for its antimicrobial activity against 23 different food-borne microorganisms, including 20 bacteria, 1 fungus, and 2 yeast species. The essential oil of *Heracleum antasiaticum* exhibited considerable inhibitory effects against all bacteria, fungi, and yeast species tested.

Keywords: *Heracleum antasiaticum;* essential oil; elemicin; apiole; antimicrobial activity

1. Introduction

The genus *Heracleum* L., the largest genus of the family Apiaceae, contains more than 120 species and is represented by 17 species in Türkiye. Within the species of this genus, *H. antasiaticum is a stout perennial plant*

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that reaches 50-100 cm in height and features white flowers, typically growing on the edge of forests and streams(Davis et al. 1988; Davis 1972; Duman 2000; Logacheva 2008).

Heracleum species are traditionally used as food additives, spices, and flavoring agents and show anti-inflammatory, antimicrobial, anticholinesterase, antioxidant, antiviral, cytotoxic, and anticarcinogenic effects. Earlier studies revealed that essential oils of *Heracleum* species were mainly rich in octyl acetate. However, they also contain main components such as octyl butyrate, octyl butanoate, hexyl butanoate, hexyl butyrate bornyle acetate, alpha pinene, beta pinene, elemicine, 1.8 cineole and e-anethole (Baser et al. 2000; Chu et al. 2012; Habibi et al. 2010; İşcan et al. 2003; Jagannath et al. 2012; Karimi and Ito 2012; Karuppusamy and Muthuraja 2010; Li et al. 2013; Maggi et al. 2014; Miladinović et al. 2013; Najafabadi et al. 2011; Radjabian et al. 2013; Sefidkon et al. 2002; Tkachenko 2009; Yousefirad and Karimzadeh 2008). The results of the studies mentioned here show that there is considerable variation within the genus *Heracleum*. This highlights the importance of better understanding the essential oil composition of other *Heracleum* species.

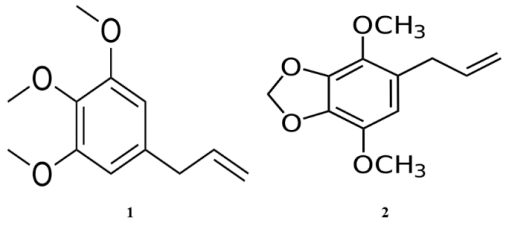

Figure 1. Chemical structures of compounds 1; elemicin, 2; apiole.

Many studies have been published on the composition and the antimicrobial properties of *Heracleum* essential oils (Akcin et al. 2013; Guleria et al. 2011; Jagannath et al. 2012; Maggi et al. 2014; Miladinović et al. 2013). These studies have emphasized the existence of marked chemical differences among the oils extracted from different species and indicated that the oils of some species of this genus had antibacterial and antifungal activity. Although a few studies (Ergene et al. 2007) have investigated *H. antasiaticum*, data on the chemical composition and antimicrobial activity of its essential oil remain limited. Therefore, this knowledge gap prompted us to identify the chemical composition of the hydrodistillated essential oil of *H. antasiaticum* growing wild in Türkiye, and to evaluate its antimicrobial activity against food-borne microorganisms.

2. Materials and Methods

2.1. Antimicrobial Activity

2.1.1. *Microbial Strains*

The essential oil was tested individually against a range of 23 microorganisms, including 20 bacteria, 1 fungus, and 2 yeast species. The microorganisms used are listed in Tables 2 and 3. Microorganisms were provided by the Food Microbiology Laboratory, Department of Food Engineering, Faculty of Agriculture, Ataturk University, Erzurum, Türkiye. The identification of the organisms used in the study was confirmed by the Microbial Identification System (Sherlock Microbial Identification System version 4.0, MIDI Inc., Newark, DE), API (BioMerieux, France), BIOLOG (MicroStation ID System, Biolog Inc., Hayward) and classical identification test from Bergey's manual of determinative bacteriology (John et al. 1994).

2.2. Experimental Design

2.2.1. Plant Material Collection and Preparation

The aerial parts of *H. antasiaticum* were collected at the flowering stage in August 2024 in the vicinity of Erzurum (at an altitude of about 2100 m), Türkiye. The taxonomic identification of plant material was confirmed by a senior plant taxonomist, Ali Kandemir, in Department of Biology, Erzincan University, Erzincan, Türkiye. Collected plant material was dried in the shade and ground in a grinder with a 2 mm mesh. The voucher specimen has been deposited at the Herbarium of the Department of Science, Erzincan University, Education Faculty, Erzincan, Türkiye.

2.2.2. *Isolation of the essential oil*

The aerial parts of air-dried plant material were subjected to hydro distillation using a Clevenger-type apparatus for 3 hours. The obtained essential oil was dried over anhydrous sodium sulphate and, after filtration, stored at +4 °C until tested and analysed.

2.3. Chemical Characterization of Essential Oil

2.3.1. GC analysis conditions

The analysis of the essential oil was performed using a Thermofinnigan Trace GC/A1300 (E.I.) equipped with an SGE/BPX5 MS capillary column (30 m x 0.25 mm i.d., 0.25 ⊚µm). Helium was the carrier gas, at a flow rate of 1 mL/min. Injector temperature was set at 220 °C. The programme used was 50-150 °C at a rate of 3 °C/min, held isothermal for 10 minutes, and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 ⊚L were injected manually and in the splitless mode. Quantitative data were obtained from the FID area percentage data.

2.3.2. *GC-MS analysis conditions*

The analyses of the essential oils were performed using a Thermofinnigan Trace GC/Trace DSQ /A1300, (E.I Quadrupole) (Thermo Finnigan, CA, USA) equipped with an SGE-BPX5 MS capillary column (Scientific Instrument Services Inc., NJ. USA) (30 m x 0.25 mm i.d., 0.25 ⊚m). For GC-MS detection an electron ionization system with ionization energy of 70 eV was used. Helium, at a flow rate of 1 mL/min, was the carrier gas. Injection and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. The programmed used was 50-150 °C at a rate of 3 °C/min, held isothermal for 10 minutes and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 ⊚L were injected manually and in the splitless mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley7N, TRLIB library data of the GC-MS system and literature data (Adams 2007; Jennings 1980) . The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature (Adams 2007).

2.4. Antimicrobial Testing Procedures

2.4.1. *Disk Diffusion Assay*

Antimicrobial tests were carried out by the disk diffusion method (Çetin et al. 2009; Murray 1995) using 100 µL of suspension containing 10^8 colony forming units (CFU)/mL of bacteria, 10^6 CFU/mL of yeast, and 10^4 spores/mL of fungi spread on nutrient agar (NA), sabouraud dextrose agar (SDA), and potato dextrose agar (PDA) medium, respectively. The disks (6 mm in diameter) impregnated with 10 µL of essential were placed on the inoculated agar. The inoculated plates were incubated at 37 °C for 24 h for mesophilic bacteria, 20 °C for 48 h for psychophysics, 30 °C for 48 h for the yeast and at room temperature for 72 h for fungi isolates (Harrigan 1998). Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay was repeated triple.

2.4.2. *MIC Agar Dilution Assay*

The agar dilution method, as described previously by (Gul et al. 2002), was used to determine the MIC values of the fungus isolate. The essential oil of *H. antasiaticum* was added aseptically to sterile molten PDA medium, containing Tween 20 (Sigma 0.5%, v/v), at the appropriate volume to produce the concentration range of 7.8-500 µg/mlL. The resulting PDA solutions were immediately poured into petri plates after vortexing. The inoculated plates were incubated at room temperature for 72 h. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. Each test was repeated at least triple.

3. Results and Discussion

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.1. Chemical composition of H. antasiaticum essential oil

In the present study, the oil isolated from aerial parts of *H. antasiaticum* was analyzed, and GC/MS analysis of the oil allowed the identification of a total of 45 compounds, which represented 92.7 % of the oil (Table 1).

Table 1. Essential oil composition of *Heracleum antasiaticum.*

RT	RI[a)]	Components	%	Identification[b)]
13.56	994	Myrcene	0.2	GC, MS, RI
13.94	999	Yomogi alcohol	0.4	MS, RI
14.56	1014	*n*-Octanal	0.6	GC, MS, RI
15.41	1034	*p*-Cymene	0.6	GC, MS, RI
15.84	1045	(*Z*)-β-Ocimene	0.2	GC, MS, RI
16.39	1055	(*E*)-β-Ocimene	0.3	GC, MS, RI
17.00	1067	γ-Terpinene	1.3	GC, MS, RI
17.88	1082	*n*-Octanol	4.9	GC, MS, RI
19.20	1106	Linalool	0.1	GC, MS, RI
19.54	1113	*n*-Nonanal	0.2	GC, MS, RI
22.13	1158	3-Methyl-2-butenyl-3-methyl butanoate	0.1	MS, RI
22.77	1172	*n*-Nonanol	0.3	GC, MS, RI
23.22	1178	Terpinen-4-ol	0.1	GC, MS, RI
24.16	1191	Methyl chavicol	0.2	GC, MS, RI
24.52	1197	Octyl acetate	3.8	GC, MS, RI
25.39	1217	(*Z*)-Carveol	0.1	GC, MS, RI
26.76	1255	(*Z*)-Anethole	0.2	GC, MS, RI
28.35	1287	(*E*)-Anethole	0.7	GC, MS, RI
28.54	1289	Thymol	0.4	GC, MS, RI
28.91	1296	Carvacrol	0.1	GC, MS, RI
30.50	1340	α-Cubebene	0.5	MS, RI
31.82	1373	α-Copaene	1.1	GC, MS, RI
32.20	1383	β-Bourbonene	0.5	MS, RI
32.37	1386	α-Isocomene	1.2	MS, RI
33.54	1413	α-Cedrene	0.1	GC, MS, RI
33.76	1419	β-Caryophyllene	0.9	GC, MS, RI
34.25	1437	(*E*)-α-Bergamotene	1.1	MS, RI
35.08	1456	(*Z*)-β-Farnesene	0.3	MS, RI
35.33	1460	α-Humulene	0.3	GC, MS, RI
36.12	1478	γ-Muurolene	tr[c)]	MS, RI
36.40	1486	Germacrene D	2.5	MS, RI
36.93	1496	α-Zingiberene	3.9	MS, RI
37.44	1508	β-Bisabolene	1.8	MS, RI
37.91	1512	γ-Cadinene	1.9	MS, RI
38.19	1518	(*E*)-γ-Bisabolene	3.1	MS, RI
38.50	1523	γ-Cuprenene	0.7	MS, RI
38.99	1530	Selina-3,7(11)-diene	2.1	MS, RI
39.80	1548	**Elemicine (1)**	27.8	MS, RI
40.99	1574	Spathulenol	0.5	MS, RI
41.21	1579	Caryophyllene oxide	0.2	GC, MS, RI
44.81	1633	Gossonorol	0.3	MS, RI
46.30	1654	Cedr-8(15)-en-9-α-ol	0.3	MS, RI
48.11	1681	**Apiole (2)**	26.2	MS, RI
56.83	1957	(*E*)-Phytol	0.5	GC, MS, RI
58.85	2000	*n*-Eicosane	0.1	GC, MS, RI
Total			**92.7**	

[a)] RI: Retention index relative to n-alkanes on a SGE-BPX5 column. [b)] MS: Tentative identification based on computer matching of the mass spectra of peaks with the Wiley7N and TRLIB libraries and published data (Shibamoto and Jennings 1980; Adams 2007); RI: comparison of retention indices based on published data (Adams 2007); GC: identification based on tR of authentic compounds on the SGE-BPX5 column. [c)] tr: Traces (less than 0.1%).

The main components of the oil were elemicin (27.8%), apiole (26.2%), *n*-octanol (4.9%), α-zingiberene (3.9), and octyl acetate (3.8%) (Table 1). Previous studies showed that octyl acetate was usually the most abundant component of *Heracleum* essential oils. However, the chemical composition of the essential oil has been noted to vary with the studied *Heracleum* species, with the main components consisting of octyl butyrate (İşcan et al.

2003; Li et al. 2013; Maggi et al. 2014), octyl butanoate (Miladinović et al. 2013), hexyl butanoate (Miladinović et al. 2013; Yousefirad and Karimzadeh 2008), hexyl butyrate (Baser et al. 2000; Karimi and Ito 2012; Radjabian et al. 2013; Tkachenko 2009; Yousefirad and Karimzadeh 2008), bornyle acetate (Jagannath et al. 2012), alpha pinene (Jagannath et al. 2012), beta pinene (Chu et al. 2012; Karuppusamy and Muthuraja 2010), 1.8 cineole (Karuppusamy and Muthuraja 2010) and e-anethole (Sefidkon et al. 2002). The essential oils from different parts of *Heracleum pastinacifolium* subsp. *incanum* were found to contain apiole, elemicine, and myristicine as major constituents, with notable variations among flower-fruit, root, and aerial part compositions (Yuca et al. 2024). Although few *Heracleum* species contained elemicine (Habibi et al. 2010; Najafabadi et al. 2011), none of the studies dealing with the essential oils of *Heracleum antasiaticum* did not report elemicine as main component. To our knowledge, this is the first study documenting elemicine as the main component in the genus *Heracleum antasiaticum*. On the other hand, apiole, another main component of our oil, was also documented in only one study within the *Heracleum* genus (Chu et al. 2012). Taken together, these findings concerning the composition of *H. antasiaticum* essential oil indicate that further research on the unexplored *Heracleum* species is needed to fully understand the extent of the essential oil variation within the genus *Heracleum*.

3.2. Antimicrobial activity

The antimicrobial activity of the essential oil of *H. antasiaticum* against the food-borne microorganisms was qualitatively and quantitatively determined by evaluating the presence of inhibition zones, zone diameter, and MIC values. In antimicrobial activity tests we used 20 bacteria, 1 filamentous fungi, and 2 yeast species (Tables 2 and 3). In general, the essential oil of *H. antasiaticum* had a broad antimicrobial spectrum and inhibited all tested microorganisms except for *S. Typhimurium*. As seen in Table 2 and 3, gram positive bacteria such as *B. cereus* and *S. aureus* that cause important issues in food industry had greater inhibition zone and MIC values than other bacteria. This supports the idea that cell wall structure is a critical determinant in the efficiency of antimicrobial substance.

Table 2. Antibacterial activities of essential oil of *Heracleum antasiaticum* against the bacterial strains tested.

Test Bacteria	Essential Oil		Antibiotic	
	DD[a]	MIC[b]	DD[c]	MIC[d]
Pathogenic and saprophytic				
Acinetobacter lwoffi BC 2819	12	62.5	18(OFX10)	7.8
Alcaligenes faecalis BC 0452	20	250	20(OFX10)	7.8
Bacillıs cereus BC 6830	32	7.8	14(SAM20)	7.8
Enterobacter cloacea BC 3213	22	125	24(KF 30)	7.8
Escherichia coli BC 1402	13	500	22(OFX10)	15.62
Escherichia coli BC 2326	9	500	26(AZM15)	7.8
Flavobacterium indologenes BC 1520	10	500	27(AZM15)	7.8
Listeria monocytogenes BC 8353	16	250	22(SAM20)	7.8
Proteus mirabilis BC 2644	11	500	28(OFX10)	125
Proteus vulgaris KÜKEM 1329	18	250	18(AMC30)	7.8
Pseudomonas fluorescens BC 7324	14	125	31(OFX10)	125
Pseudomonas pseudoalkaligenes BC3445	18	500	32(OFX10)	125
Pseudomonas putida BC 1617	15	250	16(TE30)	125
Salmonella Typhimurium RSSK 95091	-	-	12(TE30)	7.8
Staphylococcus aureus ATCC 29213	26	250	34(TE30)	7.8
Staphylococcus aureus BC 7231	28	250	22(KF30)	7.8
Staphlococcus hominis BC 2288	11	500	24(KF30)	15.62
Bifidobacterium bifidum ATCC 29521	13	500	34.1 (CC2)	7.8
Lactobacillus acidophilus BC 3219	17	250	15.2(KF30)	7.8
Lactobacillus plantarum BC 7321	14	500	20.2(NV5)	7.8

Given that the essential oil of *H. antasiaticum* had an effect on food-borne pathogens such as *E. coli*, *B. cereus*, *L. monocytogenes* and *S. aureus*, and on the bacteria such as *Pseudomonas*, *Proteus*, *Flavobacterium*, *Acinetobacter and Alcaligenes* that play a role in food spoilage, our results shows that this oil can be considered as an alternative in preserving foods and in shelf-life extension in the food industry. It is of interest to note that *H.*

antasiaticum essential oil possesses an antagonistic effect on probiotic microorganisms such as Bifidobacterium bifidum, Lactobacillus acidophilus, and *Lactobacillus plantarum* (Table 2). Therefore, the consumption of commercial products containing *H. antasiaticum* essential oil is not relevant, as it may have an adverse impact on probiotic bacteria, depending on the oil concentration. In this context, care should be taken to minimize the use of essential oils and other components containing elemicine and apiole in relatively higher concentrations, as observed in the present study, to alleviate health issues in infants and elderly people. In fact, this situation was seen in our previous study conducted on Florence fennel. In that study, we found that fennel oil in rich apiole and (E)-anethole had a similar impact against probiotic bacteria (Cetin et al. 2010).

In antimicrobial tests, we used two yeast (i.e. *Candida albicans* and *Sacharomyces cerevisiae*) and one fungi (i.e. *Aspergillus flavus*) strain. Of these, *A. flavus*, a saprophytic soil fungus that infects and contaminates preharvest and postharvest seed crops with the carcinogenic secondary metabolite aflatoxin (Amaike and Keller 2011), was influenced by *H. antasiaticum* essential oil. As observed from Table 3, the anticandidal effect of this oil was found to be higher than that of Amphotericin B, used as a standard antifungal agent. A similar effect was also observed on *Sacharomyces cerevisiae* (Table 3).

Table 3. Antibacterial activities of essential oil of *Heracleum antasiaticum* against the bacterial strains tested.

Test Bacteria	Essential Oil		Antibiotic	
	DD[a]	MIC[b]	DD[c]	MIC[d]
Yeast				
Candida albicans ATCC 1223	17	31.25	15	8
Sacharomyces cerevisiae BC 6541	15	62.5	8	62.5
Fungi				
Aspergillus flavus BC 101	12	250	17	250

[a] DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 10 µL of essential oil [b] Minimal inhibitory concentration as µg/mL. [c] DD, inhibition zone in diameter (mm) around the standard, Amphotericin B (20 µg/disk Amphotericin B) impregnated disks were used as positive reference standards antibiotic disks (Sigma). [d] Amphotericin B (µg/mL) was used as reference antibiotic in micro well dilution assay (Sigma).

So far, the antimicrobial activity of *H. antasiaticum* essential oil has not been described in the literature. However, one study related to the antimicrobial activity of ethanolic and aqueous extracts of H. antasiaticum was carried out, and it was observed that the extracts differed significantly in their activity against test microorganisms. In our present study, this antimicrobial effect of *H. antasiaticum* essential oil was probably due to the main components of this oil, elemicin and apiole (Firuzi et al. 2010; Kazemi and Rostami 2015; Pavlović et al. 2012; Tavares et al. 2008; Ušjak et al. 2017)

4. Conclusions

The essential oil exhibited a broad-spectrum antimicrobial activity, effectively inhibiting a range of food-borne pathogens and spoilage organisms, including *E. coli*, *B. cereus*, *L. monocytogenes*, *Pseudomonas spp.*, and *Aspergillus flavus*. However, its antagonistic effect on beneficial probiotic strains such as *Lactobacillus* and *Bifidobacterium* suggests that caution is needed when considering its use in products intended for human consumption.

Overall, the essential oil of *H. antasiaticum* presents promising potential as a natural preservative in the food industry. Nonetheless, further studies focusing on its toxicological safety, mechanism of action, and formulation optimization are warranted to support its practical application.

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