

Evaluation of the Chemical Compounds and Antibacterial Properties of the Aerial Parts of Persian Heracleum Persicum Essence

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ABSTRACT

BACKGROUND AND OBJECTIVE: Emergence of resistant microbial strains due to the consumption of chemical preservatives and medications, as well as uncertainty about the consumption of foods containing synthetic preservatives, have resulted in the growing popularity of natural preservatives. This study aimed to identify the chemical compounds found in the essence of Persian hogweed (*Heracleum persicum*) and investigate the antibacterial effects of the essence of this herb on *Listeria monocytogenes* and *Escherichia coli*, the most harmful bacteria in food hygiene.

METHODS: In this experimental study, after the extraction of the essential oil of *Heracleum persicum* by Clevenger, qualitative and quantitative analyses of the constituent compounds were performed via gas chromatography-mass spectrometry (GC-MS) method. In addition, microdilution method was used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), and the last plate without turbidity was considered as MIC. By culturing the plates without turbidity on brain-heart infusion (BHI) agar, the lowest concentration of the essence to inhibit the growth was determined as MBC.

FINDINGS: In this study, the main components of the essential oil of *Heracleum persicum* were Hexyl butanoate (25.98%), Octyl 2-methylbutyrate (14.37%), Pentyl-cyclopropane (12.77%) and Octyl isobutyrate (17.82%). Moreover, MIC values for *E. coli* and *Listeria monocytogenes* were 5 and 5.2 mg/ml, respectively, and MBC values for each of the bacteria were equal to MIC.

CONCLUSION: According to the results of this study, aliphatic esters are among the main constituents of the essence of *Heracleum persicum*. Furthermore, the essential oil of this herb exerts antibacterial effects on both these bacteria, and *Listeria monocytogenes* was observed to be more sensitive to the essential oil compared to *E. coli*.

KEY WORDS: *Heracleum persicum*, Minimum inhibitory concentration (MIC), Antibacterial effect.

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Introduction

According to the statistics of the World Health Organization (WHO), diseases caused by the consumption of contaminated food and water claim the lives of 2.2 million people across the world every year, and 1.9 million of the victims are children (1). Despite the regulations regarding food hygiene, such reports emphasize the need for new and effective procedures for the maintenance and provision of food safety.

On the other hand, the emergence of resistant microbial strains due to the consumption of chemical preservatives and medications, as well as uncertainty about the consumption of foods containing synthetic preservatives, have resulted in the growing popularity of natural preservatives (1). Therefore, tendency towards the consumption of foods containing natural preservatives, also known as green foods, has increased within recent years (2, 3).

Essential oils are examples of such natural preservatives, which are extracted from certain spices and medicinal herbs and could have antimicrobial properties in addition to their flavor (4). In fact, the medical applications of essential oils are secondary to the use of their flavors and fragrances (5). Herbal essences are aromatic, oily liquids extracted from different parts of plants and are also referred to as essential oils, ethereal oils or volatile oils (6).

These essences are normally obtained through steam evaporation or water vapor condensation (1). *Heracleum persicum*, also known as Persian hogweed or Golpar, is a popular herb used in the production of food and medicine; for instance, the fruits and stems of this plant are used in the production of pickles. Furthermore, *Heracleum persicum* is known to have antiseptic, analgesic and anti-bloating properties, which have gained recognition in the Iranian traditional medicine over the past centuries (7).

There are 125 different species of *Heracleum* in the world (7), and Persian hogweed is the most important species of this pharmaceutical herb in Iran (8). The vegetation of Iran inhabits 10 different species of *Heracleum persicum*, especially in mountainous regions with an altitude of over 1500 meters in the northern part of the country (9, 10). Since the plants belonging to the genera of *Heracleum* have strong aromatic properties and are rich sources of essential oils, and given their importance in the traditional medicine and local cuisine, several researchers have investigated the chemical compounds and medical values of this plant across the world (11-16).

According to previous studies on essential oils and extracts of different species of *Heracleum*, biological features such as cytotoxicity, antimicrobial effects, immune stimulating agents and anti-seizure properties have been attributed to these herbs (17).

Moreover, aliphatic esters, alcohols and monoterpene esters (e.g. hexyl butyrate, octyl acetate and hexyl isobutyrate) have been reported to be among the major compounds in the ripe and green seeds of Persian hogweed (10, 18). The antimicrobial effects and other biological properties of essential oils are associated with the content of these essences; however, the synergistic and antagonistic agents of one of the components, which exists in minor amounts in the composition of the essential oils, might play a key role in the major functions of these oils.

Therefore, based on their unique and diverse components, different essences could exert a variety of biological effects (1). On the other hand, the compounds found in the essences extracted from different parts of plants may vary, undergoing drastic changes during different periods of growth. In addition, other factors such as climatic conditions, time of harvest, storage time, method of extraction and genetic variations of plants could affect the type and amount of the existing compounds in the essential oil of the herbs (19).

In general, reports on the chemical and antibacterial properties of *Heracleum persicum* are scarce and contradictory in most cases (20-23). Therefore, this study aimed to analyze the chemical compounds found in the essential oil of Persian *Heracleum persicum* and determine the antimicrobial properties of the aerial parts of this plant.

Methods

For this experimental study, the aerial parts of Persian hogweed were collected during the fruiting season from the mountainous regions of Sheikh Mousa, Bandpey and Babol, located in the north of Iran, in June 2014. Before the extraction of essential oil, samples were stored in shades, away from direct sunlight, and at room temperature until complete drying.

Extraction of the essential oil was carried out using Clevenger apparatus via water vapor condensation. The obtained samples were preserved in dark glass, and until the determination of the chemical compounds and antibacterial agents, were maintained in refrigerator at temperature of 2-7°C. Qualitative and quantitative analyses of *Heracleum persicum* compounds were

performed via *gas chromatography-mass spectrometry* (GC-MS) method.

In this study, gas chromatograph (Agilent Technologies-7890A) was attached to mass spectrograph (Agilent Technologies-5975C), with an HP-5MS Capillary column (30 meters height, 25.0 mm internal diameter and 25.0 mm inner layer thickness). The applied temperature was between 60-280°C with a gradual increase to 4°C; in addition, the temperature of the injection chamber was 250°F, and helium carrier gas with the rate of 2 mL/min was used as well. Parameters of ionization energy and the temperature of the ionization source were calculated to be 70 EV and 270°C, respectively.

The bacteria used in this study were *Listeria monocytogenes* (PTCC 1165) and *Escherichia coli* (PTTC 1395); these bacteria were purchased in the lyophilized form from the Iranian Research Organization for Science and Technology.

Initially, the lyophilized cultures were transferred to brain-heart infusion (BHI) broth and incubated at 37°C in two consecutive intervals for 18 hours. Following that, various amounts from the second 18-hour incubated cultured bacteria were added to covet tubes containing 5 ml of sterilized BHI broth, and a spectrophotometer was used to determine light absorption at 600 nm. Simultaneously, samples were obtained from the covet tubes, and bacterial counts were also provided; eventually, the covet tube containing 1×10^8 of bacteria per milliliter was verified (24, 25). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were calculated via microdilution technique and using 96-well microplates.

After the adjustment of density at 1×10^8 , the bacteria were diluted in BHI broth medium (1 ml of bacteria in 9 ml of BHI broth) until reaching 10^6 CFU/ml, and the essential oil was prepared in the concentration range of 0.3-8 mg/ml using solvent Dimethyl sulfoxide. Following that, 160 mL of BHI broth, 20 mL of bacteria and 20 mL of the essential oil (total: 200 mL) were poured into the 96-well microplates. Furthermore, essence control (without addition of bacteria) and bacterial control (without addition of oil) were inoculated at the last well of each plate in each row. Finally, the microplates were centrifuged at 2500 rpm for 30 seconds, and were preserved at 37°C for 24 hours.

After the interval, MIC was measured using the ocular estimate method and observation of turbidity. As

for MBC, 5 microlitres from the plates without turbidity were cultured on BHI agar medium, and the minimum saturation of the essential oil with antibacterial effects (i.e. growth inhibition in plates containing BHI agar medium) was determined as MBC. All the aforementioned tests were repeated at least 3 times (26, 27).

Result

Analysis of the Chemical Composition of *Heracleum persicum* essence via GC-MS: In this study, the analysis of *Heracleum persicum* was performed via the GC-MS method, and 99.84% of the compounds of this herb were identified; compounds with values of >0.2% are shown in table 1.

The main components of the essential oil of *Heracleum persicum* were as follows: hexyl ester (hexyl butanoate) butanoic acid (25.98%), N-octyl 2-methylbutyrate (14.37%), pentyl-cyclopropane (12.77%) and octyl isobutyrate (17.82%).

Table 1. Chemical Compounds in the Essence of *Heracleum persicum*

Compound	Inhibitory time (min)	%
Butanoic acid, butyl ester	5.618	0.7
Octanal	5.806	0.97
Isobutyl isovalerate	5.894	0.27
Hexyl acetate (Acetic acid, hexyl ester)	6.159	2.88
Isopropylbenzene	6.381	1.74
Butyl 2- methylbutyrate	6.889	1.42
Butanoic acid, 3- methylbutyl ester	7.022	1.23
Gamma-terpinene	7.342	1.31
L-linalool	8.636	0.3
β-linalool	8.746	0.34
Hexyl propionate	8.818	0.35
Hexyl butanoate	11.941	25.98
Spiro [2.5] octane	12.030	2.49
Caproaldehyde	12.140	0.28
Pentyl cyclopropane	12.505	12.77
Octyl 2- methylbutyrate	13.246	14.37
Decyl isobutyrate	596.16	2.76
Vinylcyclohexane	535.17	3.00
Octyl isobutyrate	110.18	17.82
2-(aminomethyl) butanoic acid	682.23	1.66
Angelicin	557.28	0.35
Octyl caprylate	911.28	0.24

Evaluation of the Antibacterial Properties of the Essence via Microdilution in vitro: In this study, MIC and MBC values for *E. coli* were 5 and 5 mg/ml, respectively; as for *Listeria monocytogenes*, these values were 2.5 and 2.5 mg/ml, respectively. According to the aforementioned results, although *Listeria monocytogenes* was shown to have greater sensitivity to the essence, MIC and MBC were observed to be equal in both these bacteria.

Discussion

According to the results of this study, the essential oil of *Heracleum persicum* had several antibacterial effects on the examined bacteria, and these effects were more significant on gram-positive *Listeria monocytogenes*. The antibacterial activities of essential oils are normally determined by MIC value, which has been confirmed by many researchers as an appropriate method in this regard (1).

However, the method of determination varies in different published reports, which obstacles the comparison of different papers and researches. The growing prevalence of diseases and food poisonings, as well as the subsequent economical and social issues, has resulted in the increasing number of studies regarding food safety and use of new antimicrobial compounds. Essential oils obtained from different herbs and plants are known to possess several antimicrobial agents, which could be used, instead of chemical and synthetic preservatives, to control and prevent the growth of pathogenic bacteria that are transmitted via food (1).

To date, several studies have confirmed the remarkable antimicrobial properties of essential oils against pathogenic bacteria originating from microorganisms and food spoilage (28, 29). The results of the current study were consistent with the findings of previous studies in this regard. In one study, Ghasemi Pirbalouti et al. claimed that the essence of *Heracleum lasiopetalum* Boiss could exert numerous antibacterial effects against *campylobacter coli* and *campylobacter jejuni* (22).

On the other hand, Dadfar et al. concluded that the essential oil of *Heracleum lasiopetalum* Boiss had no significant effects on *Pseudomonas aeruginosa* (23). In another study, the essential oil of *H. thomsonii* was reported to perform moderate-to-strong antibacterial functions against fungi, as well as gram-positive and gram-negative bacteria, and the main compounds found in the essence of this herb were observed to be Neryl

acetate (51.62%) and Nerol (9.87%). Moreover, a study by Dragoljub et al. was indicative of an insignificant antibacterial function by *H. sibiricum*, and MIC and MBC values against *Listeria monocytogenes* were equal to 2431.2 mg/ml and 2431.2 mg/ml, respectively (20). The strong antibacterial effect of *H. thomsonii* could be due to the noticeable amount of different compounds such as nerol (9.87%) and neryl acetate (51.62%). According to the results of the present study, gram-negative bacteria demonstrated a high resistance against the essential oil of *Heracleum persicum*, which has also been reported in case of several other essential oils and herb extracts.

This resistance is probably due to the presence of an outer membrane in gram-negative bacteria, which restricts the penetration of hydrophobic components of the oil to the lipopolysaccharide layer (1).

Antimicrobial functions of herbal essential oils may not follow a single mechanism; one of the significant features of the components of these essences is hydrophobicity, which facilitates penetration into the bacterial cell membrane, resulting in the dysfunction and increased permeability (1). In general, the presence of phenolic substances, such as eugenol and thymol, in the essential oil of plants is known to yield potent antibacterial effects (1, 30); however, the investigated essence was lacking in such components in the current study.

Heracleum persicum is native to Iran and is commonly used to flavor traditional dishes; for instance, the Bakhtiari, an ancient southwestern Persian tribe, use the fruit of this plant to flavor and maintain meat (13). Furthermore, the fruit of Persian hogweed is widely recognized as a spice in the preparation of foods, as well as dairy products such as yogurt and cheese. Since ancient times, essential oils derived from medicinal plants have been benefited from in traditional medicine due to their biological properties; evidently, these properties are associated with the constituent components of these essences. Therefore, comprehension of the specific function of essential oils mainly depends on the identification and analysis of these compounds.

To date, various metabolites have been extracted from several species of *Heracleum*; such examples are genus coumarin and flavonoids (32, 33). Moreover, octyl butyrate (36.82%), hexyl butanoate (16.08%), El-ektanol (13.62%) and octyl hexanoate (8.10%) have been reported to be the most prominent compounds found in the aerial parts of *H. sibiricum* (18).

In a study by Kulijanabhagavad et al., analysis of the essence of *H. siamicum* was indicative of the presence of chemical compounds such as octyl acetate (65.3%), parasmyn (10.35%), limonene (7.52%) and delta-2-carene (6.87%) (34). In another study by Sedaghat Brogeni et al., the main compounds of *H. lasiopetalum* Boiss essence were reported to be octanol acetate (34.48%), N-octanol (6.5%), hexanol (5.12%) and alpha-Pinene (4.82%) (35).

Moreover, in another study in this regard, the main chemical composition of the essential oil of *H. persicum* fruit was observed to be hexyl butyrate (56.50%), octyl acetate (16.50%), hexyl-2 methyl butanoate (2.20%) and hexyl butyrate (3.40%) (36). Despite some differences, it could be concluded that aliphatic esters account for the most prominent components of *Heracleum persicum* essence. In the current study, all the aerial parts of *Heracleum persicum* were analyzed,

and the major chemical compounds of the essential oil of this herb were determined as hexyl butanoate (25.98%), octyl isobutyrate (17.83%), N-octyl-2-methyl butyrate (14.37%) and pentyl-cyclopropane (12.77%). Regarding the findings of the present study on the chemical composition and antimicrobial agents of *Heracleum persicum* essence, as well as the key role of this herb in the traditional medicine and food industry, it is suggested that comprehensive research be conducted on the function of this essence in food models.

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