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PHARMACOGNOSTIC STUDY ON *HERACLEUM ASPERUM* M.BIEB. (*APİACEAE*) SPECIES FROM ISMAYILLI DISTRICT OF AZERBAIJAN

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Essential oil was extracted from the fruits of *Heracleum asperum* M.Bieb. using the hydrodistillation method. The chemical composition, antibacterial and antifungal activities, as well as the macro- and micromorphological characteristics of the fruits and roots, were investigated. Among the identified monoterpenoids, cis-Verbenol, acetate (37.98%), α-Terpinolene (6.82%), and Terpinen-4-ol (1.51%) were the predominant constituents of the essential oil. The essential oil extracted from the fruit of the Heracleum plant has no effect on the encapsulated bacterium *Klebsiella pneumoniae* and blue-green pus-forming bacteria, which are naturally resistant to most antibiotics. But the essential oil affected *Staphylococcus aureus* and *Candida albicans* cultures, significantly reducing their growth. The macro- and micromorphological properties of the plant were studied. Essential oil glands and resins were found in the intersections of the root sockets. The fruits consist of mericarp parts and are straw yellow in colour.

Keywords: essential oil, gas chromatography-mass spectrometry, hydrodistillation, microbiology, morphology

INTRODUCTION

The genus *Heracleum* belongs to the Apiaceae family and consists of perennial herbaceous plants distributed across the Northern Hemisphere, including Europe, Asia, and the Americas, as well as the southern Himalayas and southern India. These plants typically grow to heights ranging from 50 cm to 1 m. Globally, approximately 70 species of this genus have been identified, with 25 species occurring in the Caucasus region and 7-8 species in Azerbaijan [Ivanovich, 1955]. Azerbaijan is particularly notable for its abundant raw material reserves of species within this genus [Ibadullayeva, 2005]. Several bioactive compounds have been isolated from Heracleum species, including the psoralen-based methoxyfurocoumarin furomethoxyheracline [Kurbanova et al., 2012], as well as coumarins such as pimpinellin, ostrutol, bergapten, psoralen, umbelliferone, and auraptene [Imanli et al., 2016]. Essential oils have also been extracted from their aerial parts which exhibited antioxidant, antitumor, and cytotoxic activities [Gökalp, 2003; Omidreza et al., 2010]. Coumarin derivatives in particular have garnered significant attention from chemists and biologists pharmacologists due to their diverse bioactive properties, including anticoagulant and analgesic effect, tumor treatment potential, coronary artery dilation, and photosensitizing capabilities [Abishov, 2003].

This study aimed at analysing the chemical composition and microbiological activity of the essential oil from *Heracleum asperum* fruits, as well as to examine the macromorphological and micromorphological features of its fruits and roots.

MATERIAL AND METHODS

Study material. The plant material (fruit) was collected on August 10, 2023, from the Girdimanchay area near Lahij village in Ismayilli district. The plant material was identified at the Institute of Botany, MSERA.

Hydrodistillation method. Essential oil (EO)

was extracted from 250 g dried of collected and dried fruits using the hydrodistillation method. The hydrodistillation process was carried out for 3 hours. The yield of essential oil was 0.6%. The essential oil was dissolved in chloroform, dehydrated with anhydrous sodium sulphate, and stored at 4°C in a dark, protected environment.

GC-MS analysis The chemical composition of the EO was analyzed using gas chromatography-mass spectrometry (GC-MS). The analysis was performed with an Agilent technologies 6890N Network CG System, 5975 inert Mass Selective Detector mass spectrometric chromatograph. Split/Splitless, injection-Split, Inlet pressure 60.608 kPa, Split-100, Low Mass-40, High Mass-400, Threshold 150 was used as a detector. A 30-meter quartz capillary column, "HP-5MS 5% Methyl Siloxane" (0.25 mm internal diameter, 0.25 μm stationary phase thickness), was employed. The tests were conducted under a temperature programming mode from 50°C to 280°C at a rate of 15°C/min. Column temperature regime was: initial column temperature 50°C, 2 min. constant; temperature increase up to 280°C, 10 min; vacuum HiVac 3.38 e-005. Chloroform was used as the solvent for sample dilution, with helium as the carrier gas at a flow rate of 1 mL/min. Identification of components was achieved using the NIST mass spectrometric library. The total analysis time was 30 minutes. The resulting GC-MS chromatogram and corresponding data are presented in figure and table 1.

Antimicrobial activity. EO was evaluated against several microorganisms, including *Candida albicans, Bacillus anthracoides,* methicillin-susceptible *Staphylococcus aureus* (MSSA), and gram-negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae*. The bacterial strains were cultured in Müller-Hinton medium at 37°C for 24 hours, while the *C. albicans* strain was grown in dextrose-Sabouraud medium for the same duration. Cell suspensions with a concentration of 10^8 cells per ml were prepared from the cultures using McFarland standard 0.5 as a reference.

The antimicrobial activity of essential oils was assessed qualitatively using the disk diffusion method. Filter paper discs, 6 mm in diameter, were impregnated with the essential oils and placed on the surface of nutrient media inoculated with the test microorganisms. The Petri dishes were incubated horizontally at 37°C for 24 hours. After incubation, the diameters of the inhibition zones – areas where microbial growth was absent – around the essential oil-impregnated discs were measured and recorded in millimetres.

Study of morphological characteristics Established methods for micromorphological identification of fruits were used [Kerimov et. al. 1999]. For the microscopic analysis, a BIOLAM-C microscope, a MBC-1 binocular, and a L74WIDE Samsung camera were used. The dried fruits were softened in a mixture of ethyl alcohol 95% and glycerin (1:1), and longitudinal and transverse sections were studied to detect and determine the type of containers.

RESULTS AND DISCUSSIONS

Chemical content of the essential oil obtained from *H. asperum* fruits was analyzed using GC-MS. (Fig. 1, Tab. 1)

As can be seen from Table 1, 31 components were identified in the composition of essential oil. The essential oil consists of 52.82% monoterpenoids. Among these components, cis-verbenol acetate 37.98%, α-Terpinolene 6.82%, Terpinen-4-ol 1.54% are monoterpenoids. The main advantage in terms of percentage compared to other components is the monoterpenoid cis-verbenol acetate, which is 37.98%.

The essential oil exhibited significant inhibitory effects on the growth of *Staphylococcus aureus* and *Candida albicans* cultures. However, *Bacillus anthracoides* was resistant to its effects. Additionally, the essential oil showed no activity against encapsulated bacteria such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, which are inherently resistant to most antibiotics.

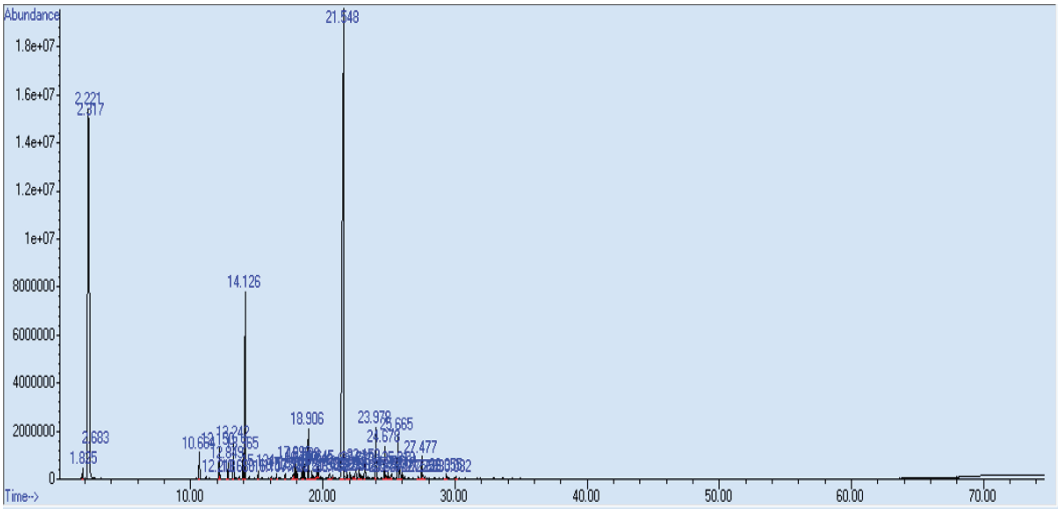

Figure 1. GC-MS chromatogram of the essential oil of *Heracleum asperum* fruits

Table 1. Chemical composition of essential oil of *Heracleum asperum* fruits

№	Compound	RT	Peak part	Formula
1	alpha-Pinene	10.664	0.79	$C_{10}H_{16}$
2	cis-sabinene	12.150	0.88	$C_{10}H_{16}$
3	beta-Pinene	12.218	0.10	$C_{10}H_{16}$
4	beta-Myrcene	12.849	0.49	$C_{10}H_{16}$
5	alpha-Phellandrene	13.242	1.04	$C_{10}H_{16}$
6	alpha-Terpinene	13.680	0.09	$C_{10}H_{16}$
7	p-Cymene	13.965	0.83	$C_{10}H_{14}$
8	α-Terpinolene	14.126	6.82	C_6H_{10}
9	gamma-Terpinene	15.134	0.23	$C_{10}H_{16}$
11	Linalool	16.503	0.14	$C_{10}H_{18}O$
12	cis-Verbenol	17.783	0.15	$C_{10}H_{16}O$
13	trans-Verbenol	17.896	0.49	$C_{10}H_{16}O$
14	p-Mentha-1,5-dien-8-ol	18.588	0.44	$C_{10}H_{16}O$
15	Terpinen-4-ol	18.906	1.51	$C_{10}H_{18}O$
16	Estragole	19.548	0.33	$C_{10}H_{12}O$
17	Citronellol	20.506	0.15	$C_{10}H_{20}O$
18	Benzaldehyde	20.765	0.11	C_7H_6O
19	cis-Verbenol, acetate	21.548	37.98	$C_{12}H_{18}O_2$
20	Phellandral	21.816	0.19	$C_{10}H_{16}O$
21	Myrteynl acetate	23.159	0.40	$C_{12}H_{18}O_2$
22	Geranyl isobutyrate	24.678	0.10	$C_{14}H_{24}O_2$
23	Cyclohexane	24.946	0.21	$C_{15}H_{24}$
24	Cyclodecene	25.137	0.10	$C_{10}H_{18}$
25	Methyleugenol	25.223	0.16	$C_{11}H_{14}O_2$
26	alpha-Maaliene	25.818	0.32	$C_{15}H_{24}$
27	gamma-Elemene	26.001	0.20	$C_{15}H_{24}$
28	Germacrene D	27.225	0.10	$C_{15}H_{24}$
29	Naphthalene	27.477	0.78	$C_{10}H_8$
30	1H-Cyclopropeazulene	27.739	0.08	$C_{15}H_{24}$
31	Spathulenol	30.082	0.12	$C_{15}H_{24}O$

Terpenoid groups	numeral	%
Monoterpenoids	22	52.82
Sesquiterpenoids	6	1.6
Other substance	3	0.42

In contrast, *Escherichia coli* was sensitive to the essential oil. Therefore, the essential oil showed potential for use against *S. aureus* and *C. albicans*. Study on the antibacterial effect of several species of the genus *Heracleum* was conducted earlier [Tkachenko et al., 1995]. Authors treated essential oils of the species against only two species (*Staphylococcus aureus, Staphylococcus saprophyticus*). However, in this work, experiments were conducted against six microbial strains (Tab. 2, Fig. 2).

Study morphological characteristics. Established methods for micromorphological identification of fruits were applied [Kerimov et. al., 1999]. For the microscopic analysis, a BIOLAM-C microscope, a MBC-1 binocular, and a L74WIDE Samsung camera were used.

The dried fruits were softened in a mixture of ethyl alcohol 95 % and glycerin (1:1), and longitudinal and transverse sections were studied to detect and determine the type of containers.

The roots are substantial in size, featuring a brown, wrinkled exterior and a white interior when viewed in cross-section. Sections of the roots reveal pores filled with essential oil and resin, which are prominently visible in the cross-sectional view (Fig. 3). The fruits are composed of two equal mericarps, which are elliptical or obovate in shape with a straw-colored exterior (Fig. 4). The dorsal ribs are straight and thread-like, featuring wing-like, thickened edges. Excretory ribs are spaced apart. The epidermal cells are randomly arranged, isodiametric in

Table 2. Antimicrobial activity of essential oil extracted from *Heracleum asperum* fruit

Microorganisms	*Essential oil from Heracleum asperum fruits*
	Diameter of the inhibited growth zone, mm
Klebsiella pneumoniae	No effect
Staphylococcus aureus	12
Bacillus anthracoides	No effect
Escherichia coli	8
Candida albicans	14
Pseudomonas aeruginosa	No effect

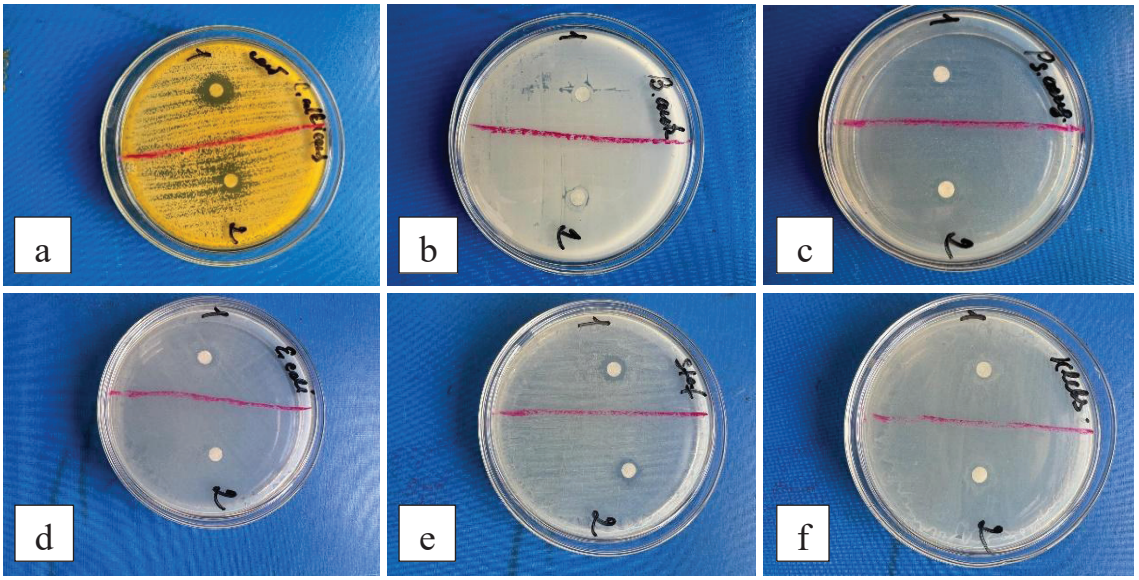

Figure 2. Effect of essential oil on microbial strains: a) *Candida albicans*; b) *Bacillus anthracoides*; c) *Pseudomonas aeruginosa*; d) *Escherichia coli*; e) *Staphylococcus aureus*; f) *Klebsiella pneumoniae*

shape, and have straight or are flat or grooved-hilly (Fig. 5).

The chemical composition of EO from H. asperum fruits has been studied. Compared to other literature sources [Tkachenko et al., 1995] several microbial culture strains were used in the study of antimicrobial activity. The effect of the essential oil on *S. aureus* and *C. albicans* cultures was more effective than on other strains and significantly reduced their growth.

For the first time, we have studied the morphological and anatomical characteristics of the roots and fruits of the Heracleum asperum plant. The cavities visible in the cross-section of the roots are filled with essential oil and resin.

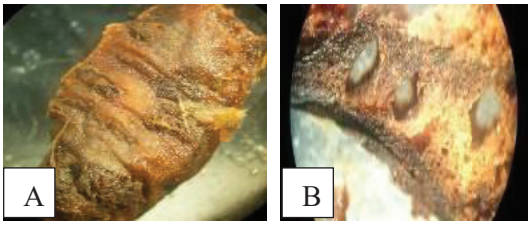

Figure 3. Root of *Heracleum asperum:* A- root surface; B - root cross-section.

Figure 4. *Heracleum asperum* fruit: left dorsoventral, right ventral side

Figure 5. The epidermis of *Heracleum asperum* fruit

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Фармакогностическое исследование *Heracleum asperum* M.Bieb. (*Apiaceae*) из Исмаиллинского района Азербайджана

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Из плодов растения борщевик жёсткий методом гидродистилляции получено эфирное масло, определен компонентный состав, бактериологическое и противогрибковое действие, а также макро- и микроморфологические характеристики плодов и корней. Основной монотерпеноидов преобладают цис-вербенол, ацетат 37.98%, α-терпинолен 6.82%, терпинен-4-ол 1.54%. Эфирное масло, полученное из плодов растения *Heracleum asperum*, не влияет на капсульную бактерию *Klebsiella pneumoniae* и сине-зеленую гнойную палочку, которая по своей природе устойчива к большинству антибиотиков. Но эфирное масло подействовало на культуры *Staphylococcus aureus* и *Candida albicans* и существенно замедлило их развитие. Изучены макро- и микроморфологические характеристики растения. В местах пересечения корневых лунок обнаружены железы эфирного масла и смолы. Плоды состоят из частей мерикарпиев и имеют соломенно-желтый цвет.

Ключевые слова: эфирное масло, газовая хроматография-масс-спектрометрия, гидродистилляция, микробиология, морфология

Azərbaycanın İsmayıllı rayonunda yayılan *Heracleum asperum* M.Bieb. (*Apiaceae*) növünün farmakoqnostik tədqiqi

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Sərt baldırğan bitkisinin meyvələrindən hidrodistilyasiya üsulu ilə efir yağı alınaraq komponent tərkibi, bakterioloji və göbələk əleyhinə təsirləri, eləcə də meyvə və köklərin makro və mikromorfolji xüsusiyətləri müəyyən olunmuşdur. Efir yağının komponent tərkibində əsas üstünlüyü monoterpenoidlərdən sis-verbenol asetat 37,98 %, α- Terpinolen 6,82%, Terpinen-4-ol 1,54% üstünlük təşkil edir. *Heracleum asperum* bitkisinin

meyvəsindən alınmış efir yağı kapsullu bakteriya olan *Klebsiella pneumoniae* və əksər antibiotiklərə təbii rezistentliyi olan göy-yaşıl irin çöplərinə təsir etmir. Lakin efir yağı *Staphylococcus aureus* və *Candida albicans* kulturalarına təsir edərək, onların inkişafını kifayət qədər azaltmışdır. Bitkinin makro və mikromorfoloji xüsusiyyətləri öyrənilmişdir. Köklərin yuvacıqlarının kəsişmələrində efir yağı vəzicikləri və qətranlar aşkar edilmişdir. Meyvələr merikarp hissələrdən ibarət olub, sarı-samanı rənglidirlər.

Açar sözlər: efir yağı, qaz-xromatoqrafiyalı-kütlə spektrometriya, hidrodistilyasiya, mikrobiologiya, morfologiya