International Journal of
Health Systems and Medical Sciences

ISSN: 2833-7433
Volume 2 | No 3 | March -2023

Chemical Composition and Antimicrobial Activity of the Essential Oil of the Plant Phlomoides Isochila

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Abstract: For the first time, the chemical composition of the volatile components of the aerial parts of the Phlomoides isochila plant growing in the Republic of Uzbekistan was studied. The main components of the essential oil were β-Thujone (29.76%), Camphor (18.03%) and 1,8-Cineol (12.12%). In vitro studies of antibacterial and antifungal activity have shown that Ph. isochila exhibits a significant antibacterial effect against gram-positive bacteria Bacillus subtilis (15.04±0.10) and Staphylococcus aureus (10.08±0.12).

Keywords: Phlomoides isochila, essential oil, antimicrobial activity.

Introduction. *Phlomoides isochila* (desert equal-lipped) densely pubescent perennial up to 40-50 cm high. Leaves entire, oval or rounded, bluntly serrated. Inflorescence of contiguous 4-6-flowered whorls. Bracts subulate, 9-15 mm long. The calyx is broadly funnel-shaped, 30-40 mm in diameter, with a membranous limb and short, wide, sharp teeth. Corolla light yellow, 30-35 mm long. From the plains to the lower belt of mountains. Central Asia [1].

Ph. isochila is an aromatic plant with a specific smell. So far, the chemical composition has not been studied. This paper presents the results of a chemical analysis of essential oils from the aerial parts of *Ph. isochila* obtained by chromato-mass spectrometry (GC-MS).

Materials and methods. Aerial parts of *Ph. isochila* were collected in the summer of 2020 in Namangan at an altitude of 1400 m. Taxonomic identification was carried out by the Institute of Plant and Animal Gene Pool of the Academy of Sciences of the Republic of Uzbekistan.

Obtaining essential oil. The essential oil was obtained from the above-ground part of the air-dry raw material of the plant by hydrodistillation on a Clevenger apparatus for 2 h using dichloromethane as a trap [3].

GC-MS analysis. The analysis was carried out on an Agilent 5975C inert MSD/7890A GC chromato-mass spectrometer. The separation of the essential oil components was carried out on an Agilent HP-INNOWax quartz capillary column (30m×250μm×0.25μm) in the temperature regime: 50°C (1 min) - 4°C/min up to 220°C (6 min) - 15°C/min to 250°C (15 min). The volume of the introduced sample was 1.0 μl, the flow rate of the mobile phase (H2) was 1.1 ml/min. The temperature of the evaporator is 220°C, the temperature of the ion sources is 230°C. The ionization of molecules was carried out by the electron impact method (70 eV). EI-MS spectra were obtained in the m/z range 10-550 a.m.u. Components were identified based on a comparison of the characteristics of the mass spectra with data from electronic libraries (Wiley Registry of Mass Spectral Data-9th Ed., NIST Mass Spectral Library, 2011) and a comparison of the retention indices

(RI) of the compounds, determined with respect to the retention time of a mixture of n-alkanes (C9-C28), as well as comparison of their mass spectral fragmentation with those described in the literature [2, 3]. The quantitative content of the components of essential oils was calculated from the areas of chromatographic peaks.

Antimicrobial activity. *In vitro* antibacterial and antifungal activity of the samples was determined by the agar disc diffusion method [4-6], using the following strains of microorganisms as test cultures: gramm-positive bacteria - *Bacillus subtilis* (RKMUz - 5), *Staphylococcus aureus* (ATCC 25923), gramm-negative bacteria - *Escherichia coli* (RKMUz - 221), *Pseudomonas aeruginosa* (ATCC 27879), and the opportunistic fungus *Candida albicans* (RKMUz - 247). Ampicillin, ceftriaxone and fluconazole were used as positive controls. 20 µl of test materials were applied to sterile paper discs and deposited on the surface of the inoculated agar plates. Plates with bacteria were incubated at 37°C for 24 h, and with fungi for 48 h at 29°C. After the incubation time, the zone of inhibition (including disc diameter) was measured and averaged (triple repetition).

Results and discussion. This study represents the first screening of *Ph. isochila* growing in the Republic of Uzbekistan and the study of their antimicrobial activity. To obtain essential oil from aerial parts, 70 g of air-dry raw materials were placed in a working flask with a capacity of 500 ml, and filled with distilled water (200 ml). The distillate was distilled for 2 hours using a Clevenger apparatus. The resulting distillate was extracted with dichloromethane, the essential oil extract was dried over anhydrous sodium sulfate. The yield of essential oil was 0.5% based on air-dry raw materials.

The composition of the isolated volatile substances was determined by GC-MS, the results of the analysis are presented in **table 1.**

As can be seen from Table 1, in the composition of volatile substances Ph. isochila, 62 components were identified, accounting for 93.0% of the total components of the oil. The main components of the essential oil are - Camphene (1.76%), Leden (2.96%), Caryophyllene (3.19%), *α*-Humulene (4.75%), *α*-Thujone (9.63%), 1,8-Cineol (12.12%), Camphor (18.03%), as well as *β*-Thujone (29.76%) (Table 1).

Table 1.Component composition of essential oils *Ph. isochila*

№	Compound Name	RT	RI	%
1	*β*-Pinene	2.747	1055	0.01
2	Camphene	2.857	1064	**1.76**
3	Hexanon-3	3.263	1099	0.78
4	*α*-Pinene	3.386	1106	1.73
5	*δ*-Terpinene	3.583	1116	0.16
6	1-Butanol	3.927	1135	0.01
7	Cyclohexene	4.345	1157	0.95
8	Benzene	4.585	1170	0.11
9	5-Chloropentane acid	4.721	1177	0.01
10	*D*- Limonene	5.034	1193	1.20
11	**1,8**-Eucalyptol	5.286	1202	**12.12**
12	Ocimene	5.809	1213	0.09
13	Prenitol	5.969	1215	0.43
14	Sikloheksan	6.172	1223	0.03
15	*p*-Cymol	6.516	1226	0.35
16	*α*-Terpinolene	6.817	1231	0.35
17	Hexen-3-ol-1	9.529	1282	0.01
18	***β*-Thujone**	10.795	1414	**29.76**
19	***α*-Thujone**	11.207	1428	**9.63**
20	Octene-3	11.564	1440	0.17
21	Cyclohexadiene-1,4	11.908	1452	0.29
22	Cadinadien-1.4	12.757	1482	0.01

23	**Camphor**	13.150	1495	**18.03**
24	*α*-Gurjunen	13.464	1506	0.06
25	Bicyclo[3.1.1]heptanone-3	13.747	1516	0.10
26	Limonene	14.023	1525	0.02
27	3-Karen	14.189	1530	0.23
28	Cyclohexene	14.380	1537	0.44
29	*p*-Menthatriene-1,3,8	14.571	1543	0.07
30	Mentadiene-1,8	14.657	1546	0.02
31	Acetic acid	14.946	1556	0.84
32	**Caryophyllene**	15.395	1571	**3.19**
33	2-Karen	15.640	1580	1.04
34	Azulene	15.819	1586	0.06
35	Naftalen	16.606	1613	0.07
36	***α*-Humulene**	17.411	1642	**4.75**
37	Isotherpinolene	17.786	1655	0.07
38	Octatriene-1,3,6	17.928	1660	0.23
39	Viridifloren	18.075	1665	0.20
40	Isoborneol	18.346	1675	1.80
41	4-(1,5-Dimethyl-4-hexenylidene)-1-cyclohexene	18.604	1684	0.12
42	Elemen	18.758	1690	0.05
43	Gurjunen	19.253	1699	0.13
44	Cadinen	19.754	1726	0.09
45	Cycloheptene	20.246	1744	0.07
46	Cyclopentane	22.004	1804	0.08
47	Cymenen	22.373	1810	0.07
48	Prenyten	22.785	1818	0.02
49	Aromadendren	25.343	1864	0.25
50	Neocloven	25.983	1876	0.36
51	Longifolene	26.192	1880	0.07
52	Azulene	26.493	1885	0.02
53	Selinen	26.770	1890	0.46
54	Pyrithione	27.040	1895	0.74
55	Ocimene	27.981	2005	0.16
56	**Leden**	28.276	2007	**2.96**
57	Germakren	28.424	2008	0.04
58	Isolongifolin	29.254	2014	0.14
59	Cobaltocene	29.875	2019	0.03
60	Dehydroaromadendren	30.619	2024	0.04
61	Phenol	31.811	2033	0.12
62	Tujopsen	33.226	2044	1.18

The antibacterial and antifungal activity of the essential oil was evaluated by a modified agar disc diffusion method [4-6]. The results are presented in table 2. Screening results showed that all test microorganisms are sensitive to the action of the essential oil from (Table 2). At the same time, the greatest antibacterial activity is manifested in relation to the gramm-positive bacterium *Bacillus subtilis* (15.04±0.10).

Table 2.The antibacterial and antifungal activity of *Ph. isochila*

Samples	Inhibition zone diameter (mm, ± SD, P≤0.05)				
	Gramm-positive bacteria		Gramm-negative bacteria		Fungus
	B.subtilis	*S. aureus*	*E. coli*	*P.aeruginosa*	*C. albicans*
Essential oil Ph. isochila	15.04±0.10	10.08±0.12	6.08±0.12	6.12±0.13	6.04±0.10
Ampicillin (10 µg/disk)	27.04±0.10	26.08±0.12	NT	NT	NT

Ceftriaxone (30 µg/disk)	NT	NT	27.08±0.1	25.12±0.13	NT
Fluconazole (25 µg/disk)	NT	NT	NT	NT	30.04±0.10

*NT - not tested

Conclusion. Thus, the study of the chemical composition of volatile compounds of the aerial parts of the *Phlomoides isochila* plant showed that the main components of the essential oil are bicyclic monoterpene - *β*-Thujone, monoterpene ketone - Kamphora and monocyclic terpene-1,8-cineol. The essential oil exhibits varying degrees of antimicrobial activity against all strains of microorganisms tested, with the highest antibacterial activity occurring against Gramm-positive bacteria.

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