

# Distribution, morphology, phytochemical composition, and antioxidant activity of *Mediasia macrophylla* (Apiaceae) from the Western Tien Shan, Kazakhstan

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**Abstract.** Ubaev FA, Esimova AM, Aimenova ZE, Iskakova ZB, Kolpek A, Tekebayeva ZB, Bazarkhankyzy A, Askarova N. 2026. Distribution, morphology, phytochemical composition, and antioxidant activity of *Mediasia macrophylla* (Apiaceae) from the Western Tien Shan, Kazakhstan. *Biodiversitas* 27 (3): d270315. <https://doi.org/10.13057/biodiv/d270315>. *Mediasia macrophylla* (Apiaceae) is a perennial Central Asian species with reported medicinal relevance; however, integrated ecological, morphological, and phytochemical data for populations in Kazakhstan remain limited. The present study provides a preliminary, exploratory, site-based characterization of *M. macrophylla* from two natural populations within the Aksu-Zhabagly State Nature Reserve (Western Tien Shan, southern Kazakhstan). Habitat conditions, quantitative morphology, essential oil composition, and antioxidant activity of solvent extracts were assessed. Field surveys confirmed that the species occurs in restricted, localized populations associated with rocky limestone slopes and meadow-steppe habitats. Quantitative morphometric analysis of mature, flowering individuals (n = 20) showed moderate variation in vegetative traits and comparatively conservative reproductive and fruit morphology, consistent with stable diagnostic morphology at the local population level. Essential oil composition was determined by GC-MS using a single pooled sample, revealing 29 compounds accounting for 95.83% of the total oil. Antioxidant activity of pooled solvent extracts was evaluated using FRAP and DPPH assays with technical replication. Phytochemical and antioxidant data were obtained from pooled material and technical replicates only and are therefore interpreted descriptively. A clear solvent-dependent pattern was observed: the ethyl acetate extract showed the highest Ferric Reducing Capacity (FRAP absorbance 1.17 at 1.0 mg/mL), whereas the ethanol extract exhibited the strongest radical scavenging activity (80.62% DPPH inhibition at 1.0 mg/mL), approaching that of gallic acid (86.14%). The chloroform extract displayed consistently weak activity. Overall, the results provide descriptive, hypothesis-generating data on the ecological context, morphology, and phytochemical and antioxidant profiles of *M. macrophylla* within the Aksu-Zhabagly Reserve and highlight the need for replicated, multi-site, and multi-season studies to assess population-level variability and potential chemotypic patterns.

**Keywords:** Antioxidant activity, Central Asia, essential oil, *Mediasia macrophylla*, Western Tien Shan

## INTRODUCTION

The family Apiaceae (Umbelliferae) is one of the most ecologically and economically important families of flowering plants, comprising approximately 450 genera and nearly 4,000 species distributed in the Northern Hemisphere. Members of this family such as *Ferula*, *Heracleum*, and *Angelica*, are known for producing diverse metabolites, including essential oils, coumarins, flavonoids, and polyacetyles, which function in defense against herbivores and environmental stress (Bouchra et al. 2017). Within this family, the genus *Mediasia* represents a specialized lineage of the Irano-Turanian floristic region, characterized by distinct carpological features and a narrow ecological range (Bahora et al. 2026).

*Mediasia macrophylla* (Regel & Schmalh.) Pimenov is robust perennial herb distributed mainly in the mountainous

regions of Central Asia. The species has been used in ethnomedicine for the treatment of chronic rheumatism, nephritis, inflammatory conditions, and dermatological disorders (Chernenko and Glushenkova 2003). Earlier phytochemical investigations, particularly those involving populations from Uzbekistan, identified unsaturated fatty acids, essential oils, phenolic compounds, and uncommon polyacetylene glucosides (Baser et al. 1997; Kurimoto et al. 2010), highlighting the species' substantial chemical diversity.

The Western Tien Shan, spanning Kazakhstan, Uzbekistan, and Kyrgyzstan, is a recognized biodiversity hotspot characterized by pronounced altitudinal gradients, continental climatic conditions, and predominantly calcareous substrates. These environmental factors are known to influence secondary metabolite biosynthesis in Apiaceae species, often resulting in site-specific or population-level

variation in terpenoid and phenolic profiles (Salehi et al. 2018; Kozhantayeva et al. 2024, 2025).

*Mediasia macrophylla* typically inhabits rocky limestone slopes, steppe hillsides, and subalpine meadows at elevations of 900-2200 m, where ecological filtering may shape both morphological traits and chemical composition. Morphologically, the species is characterized by a dense rosette of deeply dissected basal leaves, pinnatisect cauline leaves, compound umbels with numerous small flowers, and schizocarpic fruits with well-developed vittae typical of Apiaceae (Chernenko et al. 2002). The species has undergone several taxonomic reassignments before being placed in the genus *Mediasia* based on fruit anatomy and umbel architecture (Chernenko and Glushenkova 2003).

The metabolic plasticity of Apiaceae species is intrinsically linked to their adaptive responses to abiotic stressors (Herden and Friesen 2019). In high-altitude environments, plants are subjected to intense solar radiation, fluctuating thermal regimes, and varying levels of moisture availability. These factors frequently act as elicitors for the phenylpropanoid and terpenoid pathways, leading to the accumulation of specific antioxidants that mitigate oxidative damage within plant tissues (Gulcin 2020). The synthesis of phenolic compounds and volatile oils is often enhanced as a protective response to UV stress and herbivory (Crestani et al. 2023). This implies that *M. macrophylla*'s phytochemical profile is a dynamic representation of its ecological niche rather than just a taxonomic characteristic (Zhumanova et al. 2021). Researchers can gain a better understanding of the functional significance of these metabolites in the plant's survival strategy, which determines the quality and potency of the plant material for pharmaceutical application, by looking at the relationship between environmental factors and chemical output (Suntar 2020).

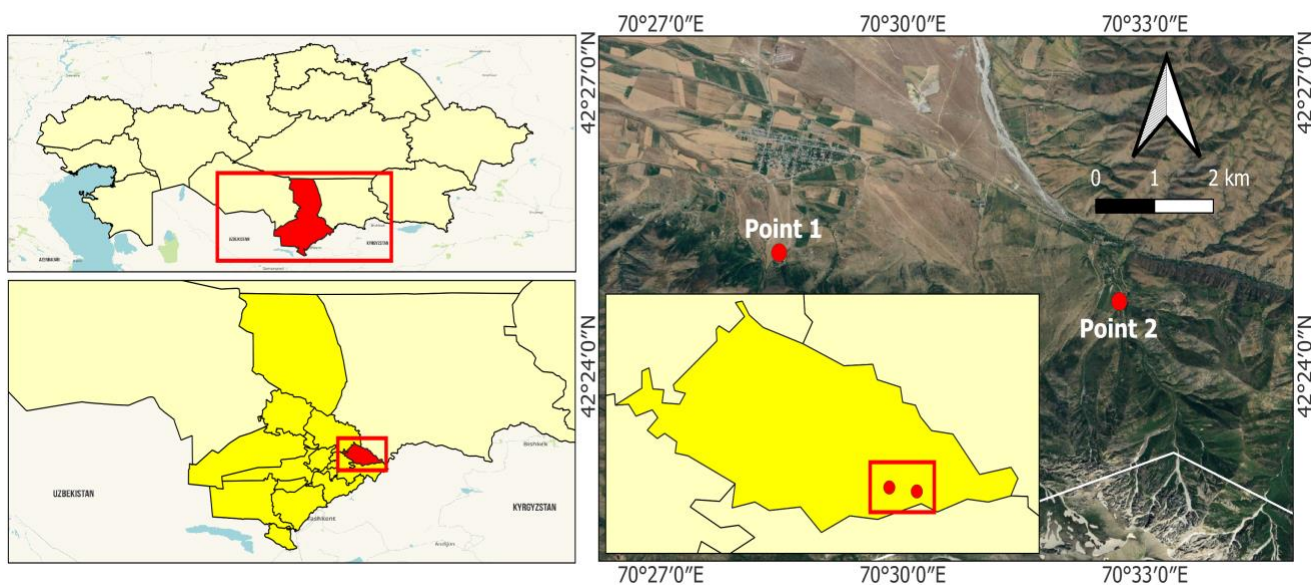
Beyond taxonomic and phytochemical interest, many Apiaceae taxa exhibit significant antioxidant activities (da Silva et al. 2021). *M. macrophylla* from other regions has been studied; however, the chemical profile and biological potency of populations from Central Asia, particularly Kazakhstan, remain unexplored. The unique environmental stressors of the Aksu-Zhabagly region—characterized by specific altitudinal gradients, UV exposure, and soil composition—likely trigger distinct adaptive metabolic pathways. Such "chemical polymorphism" can lead to significant variations in essential-oil yields and antioxidant capacity compared to populations from other regions. Hence, a comprehensive study integrating quantitative morphology and phytochemical profiling is essential to establish the specific chemotype and therapeutic potential of the Kazakhstan populations.

Accordingly, this study was designed as a site-based exploratory investigation to provide a preliminary integrative characterization of *M. macrophylla* populations in the Aksu-Zhabagly State Nature Reserve, southern Kazakhstan. Specifically, habitat conditions and quantitative morphology were assessed, essential oil composition was determined by GC-MS, and antioxidant and antiradical activities of the extracts were evaluated using FRAP and DPPH assays.

## MATERIALS AND METHODS

### Study area

The study was conducted in the Aksu-Zhabagly State Nature Reserve in the Western Tien Shan, southern Kazakhstan (42°30'N, 70°38'E) (Figure 1). The reserve spans 1100-4000 m asl, with a sharply continental climate and predominantly shallow, well-drained lithozem and meadow-steppe soils on limestone bedrock.



**Figure 1.** Map locations of the two sampling points (Point 1 and Point 2) situated inside the reserve at the Aksu-Zhabagly State Nature Reserve in the Western Tien Shan, Kazakhstan



**Figure 2.** A. *Mediasia macrophylla* in the natural habitat, B. Individual plant of *Mediasia macrophylla* growing on a rocky mountain slope, C. Mericarps of *Mediasia macrophylla*, D. Transverse section of the mericarp showing internal anatomical structure (light microscopy, objective 10×)

During the 2023 vegetation season, *M. macrophylla* (Figure 2) was recorded at two sites: A northeast-facing rocky limestone slope in open juniper woodland (~1850 m asl) and a steep talus slope with meadow-steppe vegetation (~2000 m asl). The species occurred in scattered or clustered patches within these microhabitats, often associated with *Ferula* spp., *Artemisia* spp., and *Poa bulbosa*. GPS coordinates and ecological parameters were recorded at both sites, and voucher specimens were deposited in the Herbarium of the Altai Botanical Garden (Ridder) and at the Institute of Botany of the Republic of Uzbekistan.

#### Sampling design and plant material

Sampling was conducted at two natural populations of *M. macrophylla* during the 2023 vegetation season. Individual plants (10 per site, 20 total) were analyzed separately for morphological and morphometric characterization. For phytochemical and antioxidant analyses, a single pooled composite sample was prepared from the aerial parts of all sampled individuals across both sites. This pooled sample was used for extraction using several solvents (ethanol, chloroform, ethyl acetate) and essential oil distillation. No independent biological replication was performed, and all results are intended solely for preliminary, descriptive characterization and do not represent site-level or population-level comparisons.

#### Morphological and morphometric analysis

Morphological and morphometric analyses were performed on the same set of 20 mature, flowering individuals (10 per site). The parameters for quantitative traits included basal leaf length and width, umbel diameter, number of primary rays per umbel, and mericarp length and width. All traits were measured on each individual. Leaf and umbel measurements were taken on fresh material, whereas mericarp traits and anatomical observations were conducted on air-dried material; differences between fresh and dried material are negligible for this study. Linear measurements were taken using a digital caliper (0.01 mm

accuracy), and mericarp anatomy was examined with a stereomicroscope (Leica EZ4) and light microscope (Olympus CX23). Minimum, maximum, mean, and standard deviation were calculated for each trait.

#### Plant material and extraction

Aerial parts of *M. macrophylla* were collected from both sites during a single vegetation season (May–August 2023). Plant material from all 20 sampled individuals across both sites was pooled into a single composite sample representative of the surveyed populations. This pooled material was air-dried, finely powdered, and used for all phytochemical and antioxidant analyses. No independent biological replication was performed; results are descriptive. The extraction solvents were ethanol, chloroform, and ethyl acetate. 5 g of dried material was extracted using a Soxhlet apparatus with 75 mL of solvent per extraction. Extracts were filtered, concentrated under reduced pressure, and stored at 4°C. Essential oil was obtained from fresh pooled aerial parts via hydrodistillation using a Clevenger-type apparatus for 3 h, dried over anhydrous sodium sulfate, filtered, and stored at 4°C. Extraction and essential oil yields are expressed as % (w/w and v/w, respectively).

#### GC-MS analysis

The chemical composition of *M. macrophylla* essential oil was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Analyses were performed on an Agilent 7890B gas chromatograph coupled to a 5977B mass-selective detector equipped with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. Before analysis, the essential oil was diluted to 1% (v/v) in n-hexane, and 0.2 μL of the diluted sample was injected in splitless mode. The injector temperature was set to 280°C. The oven temperature was held at 50°C for 2 min, then ramped to 280°C at 4°C/min and maintained at 280°C for 20 min in isothermal mode. Mass spectra were recorded in Electron Impact (EI) mode at 70 eV over a mass range of m/z 10–350. GC-MS analysis was performed

once on a single pooled essential oil sample, providing a qualitative-to-semi-quantitative characterization of the essential oil composition.

Only compounds with Linear Retention Indices (LRI) between 900 and 2200 were considered, encompassing the typical range of monoterpenes, sesquiterpenes, and their oxygenated derivatives commonly reported for Apiaceae essential oils analyzed on non-polar HP-5MS-type columns (Stashenko and Martinez 2017). Tentative compound identifications were based on comparison of mass spectra with the NIST 17 library and by matching LRIs with literature values. Co-elution was checked whenever possible. Relative quantification was performed by internal normalization of peak areas without the use of response (correction) factors. Accordingly, the reported percentages represent relative abundances of detected compounds rather than absolute concentrations.

#### FRAP assay

The Ferric Reducing Antioxidant Power (FRAP) of the extracts was determined according to the method of Benzie and Devaki (2018). An aliquot of 0.1 mL of each extract, prepared at concentrations of 0.25, 0.5, 0.75, and 1.0 mg/mL, was mixed with 0.25 mL of 0.2 M phosphate buffer (pH 6.6) and 0.25 mL of 1% potassium ferricyanide [ $K_3Fe(CN)_6$ ]. The reaction mixture was incubated at 50°C for 20 min, followed by the addition of 0.25 mL of 10% Trichloroacetic Acid (TCA). After centrifugation at 3000 rpm for 10 min, 0.5 mL of the supernatant was combined with 0.5 mL of distilled water and 0.1 mL of 0.1% ferric chloride ( $FeCl_3$ ).

Absorbance was measured at 700 nm using a UV-Vis Spectrophotometer (Shimadzu UV-1280, Shimadzu Corporation, Japan). Gallic acid was used as a reference antioxidant. A reagent blank containing all reaction components except the plant extract was used for baseline correction. All measurements were performed in triplicate as technical replicates; no independent biological replicates were included. Results are interpreted descriptively.

#### DPPH radical scavenging assay

The free radical scavenging activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay according to the method of Gulcin and Alwasel (2023). An aliquot of 0.1 mL of each extract (0.25-1.0 mg/mL) was mixed with 3.0 mL of freshly prepared  $6 \times 10^{-5}$  M DPPH solution in methanol. The mixture was vortexed briefly, protected from light, and incubated at room temperature for 30 min.

Absorbance was measured at 520 nm using the same UV-Vis spectrophotometer as described for the FRAP assay (Shimadzu UV-1280, Shimadzu Corporation, Japan). The control consisted of 0.1 mL of the corresponding extraction solvent mixed with 3.0 mL of DPPH solution, prepared and incubated under identical conditions. Radical Scavenging Activity (RSA) was calculated using the formula:

$$RSA (\%) = \frac{A_0 - A_t}{A_0} \times 100$$

Where :

$A_0$  : Absorbance of the control

$A_t$  : Absorbance of the test sample

All measurements were performed in triplicate as technical replicates; no independent biological replicates were included. Results are interpreted descriptively.

#### Statistical analysis

Antioxidant data (FRAP and DPPH) were obtained from technical replicates only, as described in the Methods, and are presented descriptively. Thus, statistical comparisons among extracts should be interpreted with caution, and values are best viewed as indicative trends. Morphometric data are reported as ranges and mean $\pm$ SD. Extraction and essential oil yields refer to single extraction/distillation batches. Data visualization was performed using GraphPad Prism 9.0.

## RESULTS AND DISCUSSION

#### Morphological characteristics and morphometric variation

Morphological examination of *M. macrophylla* from two populations/sites within the Aksu-Zhabagly State Nature Reserve (Western Tien Shan) revealed a consistent set of diagnostic vegetative and reproductive characters, accompanied by quantitative variation among individuals. Vegetative traits exhibited greater intraspecific variation than reproductive traits between the two sites. Basal leaf length ranged from 25 to 45 cm and width from 12 to 28 cm, forming a dense rosette typical of the species, whereas cauline leaves were consistently smaller (10-22 cm). Compound umbels measured 6.5-12.0 cm in diameter and comprised 15-30 primary rays, indicating a relatively narrow range of variation for reproductive traits compared to vegetative traits (Table 1).

**Table 1.** Pooled morphometric characteristics of *M. macrophylla* from two sampling sites within the Aksu-Zhabagly State Nature Reserve (Western Tien Shan)

Character	Range	Mean $\pm$ SD
Basal leaf length (cm)	25-45	34.2 $\pm$ 5.6
Basal leaf width (cm)	12-28	19.3 $\pm$ 4.1
Cauline leaf length (cm)	10-22	15.8 $\pm$ 3.2
Umbel diameter (cm)	6.5-12.0	9.1 $\pm$ 1.8
Number of primary rays per umbel (count)	15-30	22 $\pm$ 4
Mericaip length (mm)	4.2-6.1	5.1 $\pm$ 0.6
Mericaip width (mm)	2.0-3.2	2.6 $\pm$ 0.4

Note: Values are presented as ranges and mean $\pm$ Standard Deviation (SD) based on pooled data from two sites (Site 1 and Site 2, n = 10 per site, total n = 20). Due to the limited sample size per site, inter-site statistical comparisons were not conducted

Fruit morphology was comparatively consistent. Mericarps were ovoid to ellipsoid, with lengths of 4.2-6.1 mm and widths of 2.0-3.2 mm. For anatomical observations, three well-developed mericarps per individual were examined. Transverse sections consistently revealed well-developed vallecular and commissural vittae, confirming diagnostic fruit-anatomical features typical of Apiaceae. The relative stability of mericarp dimensions across individuals supports their taxonomic value in distinguishing *M. macrophylla* from morphologically related taxa.

Quantitative morphometric parameters are summarized in Table 1. Data from both sites were pooled and presented as ranges and mean±Standard Deviation (SD). Given the limited sample size per site, formal inter-site statistical comparisons were not performed; therefore, the morphometric data should be interpreted as a preliminary assessment of intraspecific variation rather than population-level differentiation.

### Distribution and habitat characteristics

Based on field surveys conducted during the 2023 growing season within the Aksu-Zhabagly State Nature Reserve, *M. macrophylla* was recorded at only a few localities associated with rocky limestone slopes and meadow-steppe habitats of the Western Tien Shan. Within the surveyed area, populations occurred as spatially discontinuous patches rather than continuous stands and occupied a relatively narrow altitudinal range.

Observations of population structure were based on general field reconnaissance rather than on systematic demographic sampling (e.g., transects or quadrat counts). At Site 1, plants were observed in small, spatially fragmented groups typically consisting of fewer than 20 individuals, whereas at Site 2, plants occurred in more aggregated clusters comprising several tens of individuals. These estimates reflect qualitative field impressions of local abundance and spatial arrangement and should therefore be regarded as approximate.

In both localities, populations were dominated by mature, flowering individuals, while juvenile plants were encountered only occasionally. Such site-specific observations suggest that the local occurrence of *M. macrophylla* within the reserve is associated with particular habitat conditions, including shallow calcareous soils, rocky substrates, and high solar exposure. However, due to the qualitative nature of the field data, no quantitative estimates of population density or spatial structure can be provided. The observed patchiness and limited distribution suggest that *M. macrophylla* may be vulnerable to habitat disturbance, highlighting the importance of monitoring and conservation efforts within the reserve. A formal demographic assessment employing standardized sampling methods would be required to accurately characterize population size, density, and age structure across the species' range.

### Extraction and isolation of compounds

Aerial parts of *M. macrophylla* were pooled from both sites and used to extract and distill essential oil as described in Materials and Methods. Extraction yields for ethanol, chloroform, and ethyl acetate were 2.50%, 3.00%, and

4.00% (w/w), respectively, while hydrodistillation of fresh aerial parts yielded 0.35% (v/w) essential oil (Table 2). These values correspond to single extraction/distillation batches and are presented descriptively.

**Table 2.** Extract codes, solvents, and yields of *M. macrophylla*

Code	Solvent	Plant material	Yield (%)
Mmacr-1	Ethanol	Pooled aerial parts from both sampling sites	2.50 (w/w)
Mmacr-2	Chloroform	Pooled aerial parts from both sampling sites	3.00 (w/w)
Mmacr-3	Ethyl acetate	Pooled aerial parts from both sampling sites	4.00 (w/w)
EO	Hydrodistillation	Fresh aerial parts	0.35 (v/w)

Note: Extraction yields are expressed as a percentage of dry extract mass relative to dry plant material (w/w), and essential oil yield as volume per 100 g of fresh plant material (v/w). Values are reported for a single extraction or distillation batch using pooled plant material

**Table 3.** Chemical composition of the essential oil of *M. macrophylla* obtained by hydrodistillation

RT (min)	Compound	Content (%)
7.686	5,5-dimethyl-5(H)-furan-2-one	0.08
8.856	B-myrcene	0.43
9.143	Unknown-1	1.16
9.258	A-phellandrene	0.40
9.941	Meta-cymene	20.28
10.087	3-carene	0.55
10.166	1,8-cineole	0.54
10.442	Cis-β-ocimene	0.80
11.612	Trans-furan linalool oxide	2.66
12.579	Linalool	11.93
13.140	B-thujone	0.67
13.300	Cis-para-menth-2en-1-ol	1.87
13.931	Menth-2en-1-ol	1.40
14.112	Camphor	0.51
14.985	Cis-pyran linalool oxide	0.40
15.265	Terpinen-4-ol	0.39
15.545	P-cymen-8-ol	15.25
15.734	A-terpineol	0.65
15.901	Methyl salicylate	0.63
16.326	Cis-piperitol	0.83
17.410	Cuminaldehyde	5.75
17.687	Carvotanacetone	0.98
19.147	Cuminol	1.62
19.513	Thymol	2.34
20.110	Hexyl senecioate	0.45
25.430	2-phenylethyl, 3-methylbutanoate	2.34
26.647	Kessone	3.23
26.958	Unknown-2	6.27
28.265	Caryophyllene oxide	12.76

Note: Identification of volatile components was achieved by comparison of mass spectra with the NIST 17 library and, where possible, by comparison of Linear Retention Indices (LRIs) with literature data. Compound names follow NIST library nomenclature. Relative content was calculated from normalized peak area without correction factors. Unknown components (Unknown 1 and Unknown 2) showed insufficient spectral similarity for unambiguous assignment

### Chemical composition of the essential oil

The essential oil profile of *M. macrophylla* was determined using Gas Chromatography-Mass Spectrometry (GC-MS). A total of 29 components were detected, representing 95.83% of the total essential oil composition (Table 3). Of these, 27 compounds were tentatively identified, while two components (7.42%) remained unidentified. The essential oil was dominated by monoterpene-derived constituents, with meta-cymene (20.28%), p-cymen-8-ol (15.25%), caryophyllene oxide (12.76%), and linalool (11.93%) as the major components. Based on structural classification, oxygenated monoterpenes constituted 38.06% of the oil, followed by monoterpene hydrocarbons (22.45%), sesquiterpene oxides (15.99%), and aromatic aldehydes and alcohols (7.37%). The Total Ion Chromatogram (TIC) is shown in Figure 3.

The predominance of cymene derivatives and oxygenated monoterpenes is broadly consistent with essential oils reported for several Apiaceae taxa rich in aromatic monoterpenoids, including species of *Cuminum*, *Bunium*, and related genera (An et al. 2021; Das et al. 2023; Kozhantayeva et al. 2024). The detection of cuminaldehyde and cuminol, compounds frequently reported in related genera such as *Cuminum* and *Bunium*, further supports this general phytochemical affinity. It should be emphasized that compound percentages represent relative abundances based on normalized GC-MS peak areas obtained from a single analysis of pooled essential oil material.

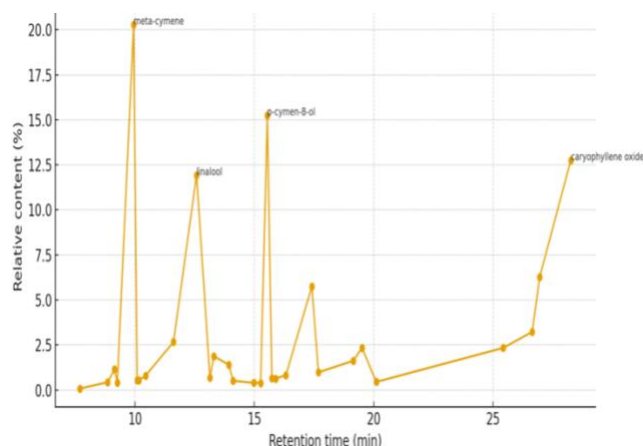
Based on structural classification, the detected components were grouped into eight chemical classes: oxygenated monoterpenes (38.06%), monoterpene hydrocarbons (22.45%), sesquiterpene oxides (15.99%), aromatic aldehydes and alcohols (7.37%), esters (3.42%), phenolic compounds (2.34%), unknown constituents (7.42%), and other compounds (0.08%) (Figure 4). Classification was based on dominant structural features following standard phytochemical conventions.

The two unidentified peaks (Unknown-1 and Unknown-2) could not be assigned to specific compounds due to insufficient spectral similarity and the absence of matching reference standards. Therefore, no definitive conclusions regarding their chemical identity or functional properties can be drawn from the present data.

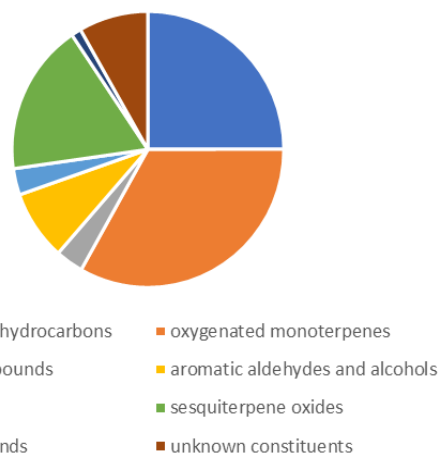
These compounds contribute to the plant's characteristic aroma and are commonly associated, in the literature, with various biological activities in the essential oils of Apiaceae species. The predominance of oxygenated monoterpenes (e.g., linalool, p-cymen-8-ol, thymol) and sesquiterpene oxides (e.g., caryophyllene oxide) indicates a chemically diverse essential oil profile. Similar qualitative compositional patterns have been reported for *M. macrophylla* populations from other regions of Central Asia, although variations in the relative proportions of individual constituents have been noted (Iskakova et al. 2022; Kozhantayeva et al. 2024).

Several major constituents identified in the essential oil, including linalool, p-cymen-8-ol, and caryophyllene oxide, have been reported in previous studies to exhibit antimicrobial, antioxidant, or anti-inflammatory activities when tested as individual compounds or as components of other plant essential oils (An et al. 2021; Das et al. 2023;

Pedrali et al. 2019; Elangovan and Mudgil 2023). Caryophyllene oxide, in particular, has been widely investigated for its pharmacological properties, including anti-inflammatory and antiproliferative effects, often discussed in the context of CB2 receptor modulation (Gyrdymova and Rubtsova 2022). However, it should be emphasized that the essential oil of *M. macrophylla* was not subjected to direct biological testing in the present study, and any discussion of potential bioactivity is based solely on previously published data and should therefore be regarded as hypothetical.



**Figure 3.** Total Ion Chromatogram (TIC) of the essential oil of *M. macrophylla* obtained by GC-MS. Major identified peaks include meta-cymene, linalool, p-cymen-8-ol, and caryophyllene oxide. Peak intensities represent relative abundances based on normalized peak areas from a single GC-MS run of pooled essential oil



**Figure 4.** Distribution of chemical groups identified in the essential oil of *M. macrophylla* based on GC-MS analysis. The proportions represent relative abundances calculated from normalized peak areas obtained in a single GC-MS run of a pooled essential oil sample. Classification into chemical groups was based on dominant structural features of the identified compounds

### Ferric Reducing Antioxidant Power (FRAP)

The ferric-reducing antioxidant power of the ethyl acetate, ethanol, and chloroform extracts of *M. macrophylla* increased in a concentration-dependent manner (Figure 5). Gallic acid showed the highest FRAP values across all tested concentrations. Among the plant extracts, the ethyl acetate extract (Mmacr-3) demonstrated the strongest reducing activity, followed by the ethanol extract (Mmacr-1). The chloroform extract (Mmacr-2) consistently had lower FRAP values throughout the tested concentration range. For example, at 1.0 mg/mL, the FRAP absorbance of the ethyl acetate extract reached 1.169, compared to 0.968 for the ethanol extract and 0.694 for the chloroform extract, demonstrating the concentration-dependent trend without consulting Table 4. Detailed numerical FRAP values are provided in Table 4.

### DPPH radical scavenging activity of the extracts

The antiradical activity of gallic acid and the three solvent extracts of *M. macrophylla* was evaluated using the DPPH assay (Figure 6). The ethanol extract (Mmacr-1) exhibited a pronounced concentration-dependent increase in radical scavenging activity, reaching 80.62% RSA at 1.0 mg/mL, closely approaching the activity of gallic acid (86.14% RSA at 1.0 mg/mL). In contrast, the chloroform extract (Mmacr-2) and the ethyl acetate extract (Mmacr-3) showed lower activity, with RSA of 19.63% and 14.73%, respectively, at the highest concentration tested.

A minor non-monotonic variation observed for the ethyl acetate extract at the highest concentration is considered negligible and likely reflects experimental variability inherent to technical replicates. Overall, these results indicate that the antiradical activity of *M. macrophylla* extracts is strongly solvent-dependent, with ethanol being the most effective extractant for radical-scavenging constituents under the conditions tested. Detailed percentage values for all concentrations are provided in Table 5.

### Discussion

The morphometric data obtained in this study indicate a relatively stable morphological pattern of *M. macrophylla* within the examined area of the Western Tien Shan. The observed ranges of vegetative and reproductive traits are consistent with earlier botanical descriptions from Central Asia. It supports the taxonomic stability of the species and suggests relatively limited morphological variation within the examined populations. The absence of pronounced differentiation between the two sampling sites, despite differences in substrate composition and slope exposure, may reflect ecological specialization to rocky calcareous habitats. Similar patterns of morphological conservatism across heterogeneous montane and steppe microhabitats have been reported for other Apiaceae species, including *Heracleum* (Bahadori et al. 2016; Changxing et al. 2019), *Cuminum* (Singh et al. 2017), and *Angelica* (Topal et al. 2021). In these taxa, strong environmental filtering favors conservative trait syndromes.

From a distributional perspective, differences in population structure between the two surveyed sites suggest that local habitat characteristics primarily influence population density and spatial arrangement rather than individual morphology. Although the present study was not designed as a comprehensive demographic assessment, these site-specific observations provide preliminary insight into the occurrence of *M. macrophylla* within protected areas of the Western Tien Shan. In this context, the results underscore the importance of limestone slopes and meadow-steppe habitats as key microenvironments supporting the persistence of this species, highlighting the conservation value of protected mountain ecosystems such as the Aksu-Zhabagly State Nature Reserve.

From a phytochemical perspective, the essential oil composition of *M. macrophylla* from the Western Tien Shan shows qualitative similarity, but differs in the relative proportions of meta-cymene and p-cymen-8-ol compared with previously reported profiles from other parts of Central Asia (Baser et al. 1997; Kozhantayeva et al. 2024). While earlier studies described linalool- and cymene-rich essential oils, the profile observed here is characterized by relatively higher levels of meta-cymene and p-cymen-8-ol, together with a substantial contribution of caryophyllene oxide. These observations are based on a single pooled essential oil sample, and any apparent differences remain preliminary and cannot be statistically validated.

**Table 4.** FRAP absorbance values of *M. macrophylla* extracts and gallic acid at different concentrations

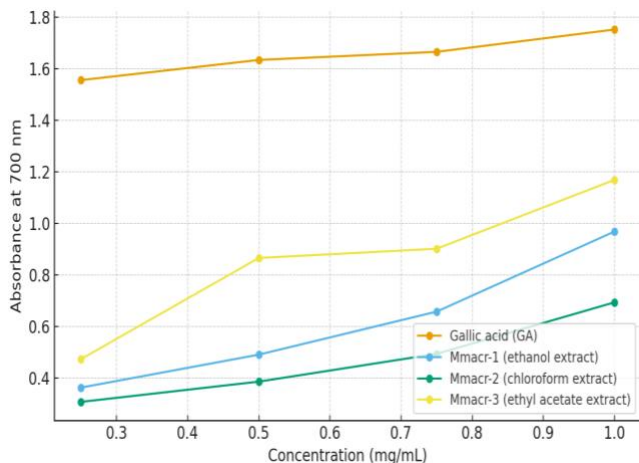
Sample	0.25 mg/mL	0.5 mg/mL	0.75 mg/mL	1.0 mg/mL
Gallic acid	1.5559	1.6342	1.6656	1.7522
Ethanol extract (Mmacr-1)	0.3631	0.4909	0.6578	0.9684
Chloroform extract (Mmacr-2)	0.3077	0.3862	0.4940	0.6937
Ethyl acetate extract (Mmacr-3)	0.4738	0.8660	0.9018	1.1688

Note: Values are expressed as mean absorbance values±SD of three technical replicates (n = 3). No inferential statistical analysis was performed due to the absence of independent biological replicates

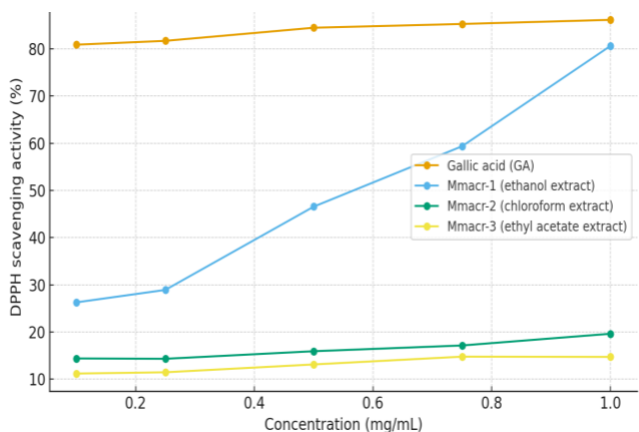
**Table 5.** DPPH radical scavenging activity (%) of *M. macrophylla* extracts and gallic acid at different concentrations

Sample	0.25 mg/mL	0.5 mg/mL	0.75 mg/mL	1.0 mg/mL
Gallic acid	81.69	84.47	85.27	86.14
Ethanol extract (Mmacr-1)	28.92	46.56	59.36	80.62
Chloroform extract (Mmacr-2)	14.33	15.93	17.15	19.63
Ethyl acetate extract (Mmacr-3)	11.47	13.13	14.78	14.73

Note: Values are expressed as mean±SD of technical replicates (n = 3). No inferential statistical analysis was performed due to the absence of independent biological replicates



**Figure 5.** Ferric Reducing Antioxidant Power (FRAP) activity of *M. macrophylla* extracts at different concentrations (0.25-1.0 mg/mL). GA: Gallic Acid (reference antioxidant), Mmacr-1: Ethanol extract, Mmacr-2: Chloroform extract, Mmacr-3: Ethyl acetate extract. Error bars represent mean $\pm$ SD of technical replicates (n = 3). Data are presented descriptively; no inferential statistical analysis was performed due to the absence of independent biological replicates



**Figure 6.** DPPH radical scavenging activity of *M. macrophylla* extracts at concentrations of 0.25-1.0 mg/mL. GA: Gallic Acid (reference standard), Mmacr-1: Ethanol extract, Mmacr-2: Chloroform extract, Mmacr-3: Ethyl acetate extract. Error bars represent mean $\pm$ SD of technical replicates (n = 3). Results are presented descriptively

The antioxidant behavior of *M. macrophylla* extracts reflects the polarity-driven selectivity of the extraction solvents and the underlying mechanisms captured by the applied assays. The contrasting patterns observed between FRAP and DPPH assays suggest that different classes of metabolites contribute to reducing power and radical-scavenging capacity through distinct mechanisms. Similar solvent-dependent antioxidant patterns have been reported in other Apiaceae genera, including *Heracleum* (Bahadori et al. 2016), *Cuminum* (Singh et al. 2017), and *Angelica* (Topal et al. 2021), where medium-polar extracts exhibited

higher FRAP activity, and more polar extracts showed enhanced DPPH radical scavenging.

These contrasting FRAP and DPPH responses likely reflect mechanistic differences between Single-Electron Transfer (SET) and Hydrogen Atom Transfer (HAT) assays. The ethyl acetate extract, enriched in medium-polar constituents such as oxygenated aromatic compounds and moderately polar phenolics, exhibited higher reducing capacity in the SET-based FRAP assay. By contrast, the ethanol extract, which contained a broader spectrum of hydrogen-donating metabolites including more polar phenolics and flavonoid glycosides, showed stronger radical-scavenging activity in the HAT-based DPPH assay (An et al. 2021; Das et al. 2023).

Taken together, the divergent antioxidant profiles of *M. macrophylla* extracts underscore the importance of solvent polarity and assay mechanism in interpreting antioxidant data. The results suggest that the antioxidant potential of this species is primarily associated with non-volatile, semi-polar to polar constituents rather than with the essential oil fraction. No direct antioxidant assays were performed on the essential oil itself; therefore, any statements regarding its potential antioxidant activity are based solely on literature and remain hypothetical, in line with general phytochemical patterns reported for Apiaceae species.

An intriguing hypothesis emerging from our observations and previous literature is that morphological stability may mask underlying phytochemical variation in *M. macrophylla*. Despite differences in local habitat conditions and population structure, vegetative and reproductive traits showed limited variation, indicating a conservative morphological strategy within the studied area. In contrast, the essential oil profile suggests qualitative and semi-quantitative differences relative to previously reported populations from other regions of Central Asia, pointing to potential chemical differentiation that is not mirrored by morphology. Future replicated, site-specific chemical analyses are required to validate this hypothesis and disentangle morphological conservatism from adaptive chemical plasticity.

Several methodological limitations should be acknowledged. Essential oil composition was determined from a single pooled sample, and antioxidant assays were performed using technical replicates only, without independent biological replication. Sampling was restricted to two sites within a single protected area and a single vegetation season, precluding assessment of spatial, seasonal, and inter-individual variation. Consequently, the GC-MS data should be regarded as qualitative to semi-quantitative, and antioxidant results as descriptive and exploratory.

These constraints limit the generalizability of the findings, highlighting the need for future studies with replicated, site-specific sampling, multi-seasonal collections, and independent biological replicates to fully assess intraspecific chemical variability, ecological adaptation, and the biological relevance of *M. macrophylla*. Future research should therefore prioritize replicated, population-level sampling across multiple sites and seasons, coupled with independent biological replicates. Targeted isolation

and structural characterization of active constituents, quantitative profiling of phenolic and related compound classes, and direct bioassays of essential oil fractions will be essential to resolve intraspecific chemical variability and to establish mechanistic links between phytochemistry, ecology, and biological activity. Such studies will further support biodiversity-focused research and the rational exploration of natural products derived from *M. macrophylla*.

In conclusion, this study provides an exploratory, integrative overview of the morphology, habitat characteristics, phytochemistry, and antioxidant behavior of *M. macrophylla* within a protected area of the Western Tien Shan, representing the first combined dataset of this type for the species in Kazakhstan. The results confirm morphological stability across the examined sites, the presence of a distinctive essential oil profile, and a pronounced solvent-dependent antioxidant response in non-volatile extracts, collectively highlighting the value of integrating ecological, morphological, and chemical approaches when investigating poorly studied Apiaceae taxa.

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