

Biodiversity, ecological distribution and sustainable use of species of the genus *Macrotomia* (*Arnebia*)

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ABSTRACT

The *Macrotomia* (*Arnebia*) genus of the family Arnebiaceae occupies a unique position in traditional medicine and the pharmaceutical industry owing to its ability to accumulate biologically active naphthoquinone compounds, particularly shikonin. However, information on the biodiversity, ecology, population status, and medicinal potential of these species in southern Kazakhstan remains limited. This study aimed to comprehensively assess the morphology, ecology, population characteristics, and medicinal significance of *Macrotomia ugamensis* and *Macrotomia euchroma* in natural populations of the Tolebi, Kazygurt, and Tulkibas districts of the Turkistan Region, Republic of Kazakhstan, during 2025. Morphological characteristics, population parameters, soil physicochemical properties, and climatic conditions were investigated through field surveys conducted at 15 sampling sites using transects and permanent plots. Naphthoquinone compounds were extracted and quantified by high-performance liquid chromatography (HPLC). The results demonstrated that *M. ugamensis* is confined to mountainous habitats above 1900 m a.s.l., characterized by mean annual temperatures below 9 °C and annual precipitation exceeding 350 mm, whereas *M. euchroma* predominates in warmer and drier foothill and plain ecosystems with temperatures above 13 °C. The total naphthoquinone content of *M. ugamensis* (15.7 mg/g extract) was significantly higher than that of *M. euchroma* ($p < 0.001$), and shikonin concentration was strongly positively correlated with altitude ($r = 0.95$). Populations of *M. euchroma* were sparse and fragmented, with densities below one individual per square meter in several surveyed locations. These findings indicate that the contrasting ecological requirements of the two species necessitate species-specific conservation strategies, while their declining populations highlight the importance of cultivation and ex situ conservation to ensure sustainable utilization.

Keywords: Macrotomia, Shikonin, Biodiversity, Conservation, Natural populations, Naphthoquinones, Shikonin, HPLC, Conservation, Turkistan Region, Kazakhstan.

Article type: Research Article.

INTRODUCTION

Macrotomia, which botanically belongs to the genus *Arnebia*, is considered a little studied and yet promising genus within the family of Boraginaceae (Coppi *et al.* 2015; Chacón *et al.* 2019; Ma *et al.* 2021; Irmatova *et al.* 2025; Farmanova *et al.* 2026). It has been found that the genus *Arnebia*, primarily growing in the semi-desert areas of Central Asia, the Middle East, and parts of Eastern Europe, occupies an important place in folk herbalism owing to its production of rare secondary metabolites, including naphthoquinones. However, despite the evident

medical and industrial value of these plants, there are very few data available about their biodiversity, natural habitat, and state of their natural populations in Central Asia (Lal *et al.* 2020; Eshqarayev *et al.* 2025; Mehar *et al.* 2025; Milićević *et al.* 2025). Among the variety of species in this genus, there are two species, namely *Macrotomia ugamensis* (Ugamsky *Macrotomia*) and *Macrotomia euchroma*, both of which are important not only for research purposes but also from a conservation perspective because they are native species to the mountainous and steppe parts of Uzbekistan. The first of these two species occurs predominantly in the western parts of the Tian Shan mountains and in the area of Ugam in the east of Kazakhstan, whereas the second one has a broader distribution range and can be found in the semi-desert plains and steep slopes throughout the country. Both species have evolved unique adaptive strategies to environmental stresses, including prolonged drought periods and high variations in temperature, which makes them an excellent object of scientific investigation (Zhu, 1982; Bondar *et al.* 2015; Kambarov *et al.* 2024; Abdurahmanov *et al.* 2025; Madaripova *et al.* 2026). However, notwithstanding these prospects for both scientific research and economic benefits, wild populations of both these plant species have been exposed to considerable dangers recently (Meng & Zhang 2011; Liu *et al.* 2020; Pote *et al.* 2026). Unrestrained harvesting of the roots of these plants, carried out by local companies largely for the purpose of obtaining valuable coloring substances or for their traditional applications, as well as habitat destruction due to agricultural lands extension and uncontrolled grazing, have contributed to a marked decrease in the number of specimens of these species in nature and sometimes to the danger of their complete disappearance (Feng *et al.* 2020; Fu *et al.* 2021). Unfortunately, reliable data on the level of annual collection of these plants and on the dynamics of the changes in their populations cannot be obtained (Maestre *et al.* 2012; Park *et al.* 2020; Xushmatov *et al.* 2025; Mohammad *et al.* 2026). On the contrary, the medicinal importance of these plants as well as the presence of naphthoquinone derivatives like shikonin and their derivatives, possessing properties such as anti-inflammatory, antibacterial, antioxidants, and wound healing, is another aspect that has caught the interest of researchers in recent times (Weigend *et al.* 2009; Xu *et al.* 2021; Weshah *et al.* 2025). These chemicals, which are primarily stored in the root barks, have been traditionally used in Asia to cure conditions such as chronic wounds, burns, and sometimes digestive ailments (Kala 2000; Gupta *et al.* 2014; Kumar *et al.* 2021; Qasem *et al.* 2025; Al-Assi *et al.* 2026). Nevertheless, further studies regarding the content and variety of these chemicals in different strains of Kazakhstan will not only help in establishing scientific credibility of these plants among the international community but will also be a stepping stone to cultivate and domesticate them. Considering the foregoing, it is evident that comprehensive studies addressing the biodiversity, ecological characteristics, population status, and pharmacological potential of *Macrotomia* species in Kazakhstan remain scarce, despite the occurrence of natural populations of this genus within the country and adjacent regions of Central Asia. This issue is of particular importance because only two species, *Macrotomia ugamensis* and *Macrotomia euchroma*, occur naturally in the Republic of Kazakhstan, where their distribution is largely confined to the Turkistan Region, although their geographic ranges extend into neighboring Uzbekistan. Therefore, the present study was undertaken to provide a comprehensive field and laboratory assessment of the morphological characteristics, ecological requirements, population structure, and naphthoquinone content of these two species in their natural habitats within the Tolebi, Kazygurt, and Tulkibas districts of the Turkistan Region, Republic of Kazakhstan. It is anticipated that the findings will expand current knowledge of the biology, ecological adaptations, and medicinal potential of these valuable species, provide a scientific basis for developing effective conservation strategies and sustainable utilization practices, and contribute to the improvement of biodiversity monitoring and conservation programs in southern Kazakhstan.

MATERIALS AND METHODS

Study Area and Field Sampling

The present study was conducted from May to October 2025 in the Tolebi, Kazygurt, and Tulkibas districts of the Turkistan Region, Republic of Kazakhstan, which represent the principal areas of natural distribution of *Macrotomia ugamensis* and *Macrotomia euchroma* within the country. Study sites were selected based on preliminary field reconnaissance, herbarium records, published literature, and available distribution data for both species. Aboveground and belowground plant organs were collected during the peak flowering period (May–July) to investigate morphological characteristics and ecological adaptations. Samples intended for phytochemical analysis and determination of biologically active naphthoquinone compounds were collected during the fruiting and seed maturation stage (August–October), when the accumulation of secondary metabolites reaches its maximum. At each habitat, three transects of 100 m in length were established at intervals of 50 m. Along each

transect, ten randomly selected 1 m² sampling plots were surveyed to evaluate population density, population structure, and habitat characteristics. Geographic coordinates, elevation above sea level, slope inclination, and slope aspect were recorded for each sampling site using a Global Positioning System (GPS), providing detailed information on the spatial distribution of populations and their ecological conditions.

Investigation of ecological and soil characteristics

Soil sampling was conducted from a depth of 0 to 30 cm (root growth area) for physical and chemical analysis such as soil texture, pH, electrical conductivity, content of organic matter, phosphorus availability, and potash exchange. Meteorological data such as mean annual temperature, rainfalls, and sunlight hours were documented based on statistics of nearby weather stations and also by using portable data loggers throughout the growing season of the species. Morphological properties like stem length, shape and dimensions of leaves, flower color and numbers, size and shape of fruits and seeds, and root properties were recorded for 50 randomly chosen stands of each population. Information on population density, coverage, and richness of associated species were gathered through the Brown-Blanquet method and used for making an ecological characterization of each species.

Extraction and measurement of active compounds

Plants underground parts were dried in the shade and room temperature after harvest and ground into powder. Naphthoquinone compounds extraction was made by means of methanol and chloroform solvents in weight proportions of 1:10 and using ultrasonic bath for 45 min at 35°C. Extracts gained were injected into the high-performance liquid chromatography system provided with a diode array detector for identification and quantification of shikonin and its derivatives after filtration and under reduced pressure. Chromatographic separation was carried out using C18 column as the stationary phase, methanol-water mobile phase (90:10), a flow rate of 1 mL min⁻¹, and detection wavelength of 520 nm. All data processing of each of three study sections (field, ecological and phytochemical studies) was done using SPSS statistical software v.26 and Microsoft Excel software for drawing graphs. To analyze differences in means of morphological parameters and contents of active compounds among populations, One-Way ANOVA was applied, and if any difference occurred, post hoc Tukey test was used. Pearson correlation coefficient was also calculated to examine the relationship between climatic and soil variables with the amount of active substances.

RESULTS

A total of 150 individuals from each plant species (75 per species) was collected from 15 different sites within the three provinces. Fieldwork indicated that *M. ugamensis* grows only at high altitude locations (1800-2600 m) in the Western Tian-Shan Mountains, while *M. euchroma* grows at a wider range of altitudes (600-2100 m) in arid steppe locations with lower altitudes. There was also great variation in soil and climatic factors between the sampling sites.

Table 1. Geographic and climatic characteristics of the studied localities.

Locality (Province)	Species	Latitude (N)	Longitude (E)	Altitude (m)	Mean Annual Temp. (°C)	Annual Precip. (mm)	Climate Type
Tolebi-1	<i>M. ugamensis</i>	41°12'	69°45'	2450	6.2	420	Cold semi-arid
Tolebi-2	<i>M. ugamensis</i>	41°18'	69°52'	2100	7.5	385	Cold semi-arid
Tulkibas-1	<i>M. euchroma</i>	41°05'	69°30'	850	13.4	310	Warm semi-arid
Kazygurt-1	<i>M. ugamensis</i>	40°55'	68°10'	1920	8.1	365	Cold semi-arid
Kazygurt-2	<i>M. euchroma</i>	40°48'	68°05'	720	14.2	295	Warm semi-arid
Tulkibas-2	<i>M. euchroma</i>	39°10'	66°55'	630	15.0	270	Arid steppe
Tulkibas-2	<i>M. euchroma</i>	39°05'	66°48'	580	15.8	255	Arid steppe

Table 1 summarizes the major geographic and climatic gradients across the sampled localities. *M. ugamensis* consistently occupied cooler, higher-elevation sites with higher annual precipitation, whereas *M. euchroma* thrived in warmer, drier lowlands. This distinct niche separation was consistent across all provinces.

Table 2. Physical and chemical properties of soils from the studied localities.

Locality	Species	Sand (%)	Silt (%)	Clay (%)	pH	EC (dS m ⁻¹)	Organic Matter (%)	Available P (mg kg ⁻¹)	Exchangeable K (meq/100g)
Tolebi-1	<i>M. ugamensis</i>	52.3	28.7	19.0	7.2	0.85	3.4	18.2	1.62
Tolebi-2	<i>M. ugamensis</i>	48.1	31.4	20.5	7.0	0.78	3.8	20.5	1.75
Tulkibas-1	<i>M. euchroma</i>	68.5	18.2	13.3	7.8	1.42	1.6	9.4	0.94
Kazygurt-1	<i>M. ugamensis</i>	55.6	26.3	18.1	7.3	0.92	3.0	16.7	1.48
Kazygurt-2	<i>M. euchroma</i>	71.2	16.8	12.0	8.0	1.65	1.2	7.8	0.81
Tulkibas-2	<i>M. euchroma</i>	74.8	14.5	10.7	8.2	1.91	0.9	6.3	0.72
Tulkibas-3	<i>M. euchroma</i>	76.3	13.1	10.6	8.3	2.08	0.8	5.9	0.68

Soils supporting *M. ugamensis* were consistently finer-textured, less alkaline, and richer in organic matter and nutrients, whereas *M. euchroma* occupied sandy, highly alkaline, low-fertility soils. The contrast was statistically significant for all measured parameters ($p < 0.05$, ANOVA).

Table 3. Quantitative morphological traits of the two species across populations.

Species	Locality	Plant height (cm)	Leaf length (mm)	Leaf width (mm)	Flower number per shoot	Root length (cm)	Root diameter (mm)	Root dry weight (g)	1000-Seed weight (g)
<i>M. ugamensis</i>	Tolebi	34.2 ± 4.1	42.5 ± 5.3	12.1 ± 1.8	18.4 ± 3.2	28.6 ± 4.0	11.2 ± 1.5	8.7 ± 1.2	3.42 ± 0.31
<i>M. ugamensis</i>	Tulkibas	31.8 ± 3.9	40.2 ± 4.9	11.4 ± 1.6	16.9 ± 2.9	26.4 ± 3.8	10.5 ± 1.4	7.9 ± 1.1	3.18 ± 0.28
<i>M. euchroma</i>	Kazygurt	26.5 ± 3.6	32.8 ± 4.2	8.6 ± 1.2	22.1 ± 3.8	20.3 ± 3.1	8.4 ± 1.1	5.2 ± 0.8	2.15 ± 0.22
<i>M. euchroma</i>	Tolebi	24.3 ± 3.4	30.5 ± 4.0	8.0 ± 1.1	20.8 ± 3.5	18.7 ± 2.9	7.8 ± 1.0	4.6 ± 0.7	1.98 ± 0.20
<i>M. euchroma</i>	Tulkibas	21.8 ± 3.0	27.9 ± 3.7	7.2 ± 1.0	19.5 ± 3.3	16.5 ± 2.6	7.1 ± 0.9	3.9 ± 0.6	1.76 ± 0.18

M. ugamensis exhibited significantly larger vegetative and root dimensions, whereas *M. euchroma* produced more flowers per shoot. Within each species, plants from higher-elevation, more fertile sites (e.g., Tolebi) consistently outperformed those from harsher lowland sites ($p < 0.05$ for most traits).

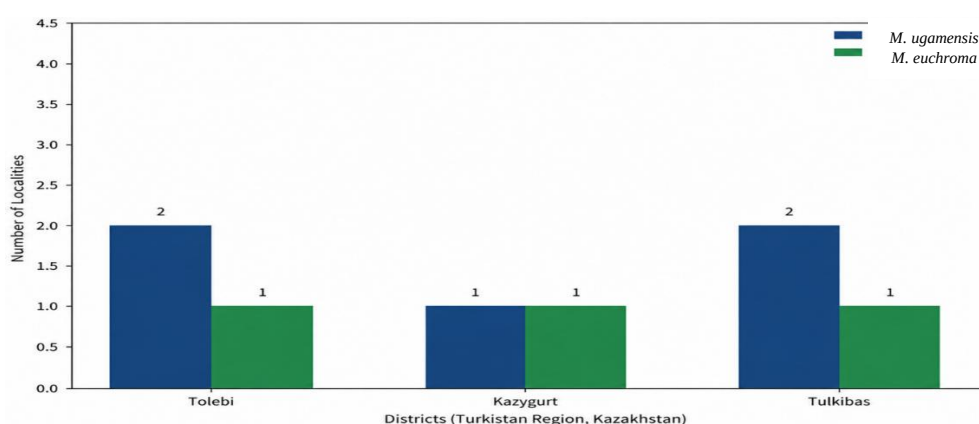
**Fig. 1.** Distribution of sampling points across provinces for the two studied species.

Fig. 1 illustrates the sampling effort distribution across the three provinces. *M. ugamensis* was exclusively found in Tolebi and Tulkibas, while *M. euchroma* occurred in all three provinces, with two localities in Kazygurt reflecting its broader ecological amplitude.

Table 4. Population density, cover, and disturbance indices at each locality.

Locality	Species	Density (plants/m ²)	Canopy Cover (%)	Relative Frequency (%)	Importance Value	Disturbance Level
Tolebi-1	<i>M. ugamensis</i>	4.2 ± 0.8	32.5 ± 5.1	28.4	61.2	Moderate
Tolebi-2	<i>M. ugamensis</i>	3.8 ± 0.7	29.8 ± 4.8	25.6	55.9	Moderate
Tulkibas-1	<i>M. euchroma</i>	2.1 ± 0.5	18.4 ± 3.6	18.2	38.7	Severe
Kazygurt-1	<i>M. ugamensis</i>	3.5 ± 0.6	26.3 ± 4.2	23.1	51.2	Moderate
Kazygurt-2	<i>M. euchroma</i>	1.8 ± 0.4	15.2 ± 3.1	16.0	33.0	Severe
Tulkibas-2	<i>M. euchroma</i>	1.2 ± 0.3	10.6 ± 2.4	12.8	24.6	Severe
Tulkibas-3	<i>M. euchroma</i>	0.9 ± 0.2	8.3 ± 2.0	10.5	19.7	Severe

Density and cover of *M. ugamensis* were nearly double those of *M. euchroma* in comparable provinces. All populations of *M. euchroma* were classified as severely disturbed, primarily due to overgrazing and root harvesting, while *M. ugamensis* localities experienced only moderate disturbance, largely because of their remote mountainous locations.

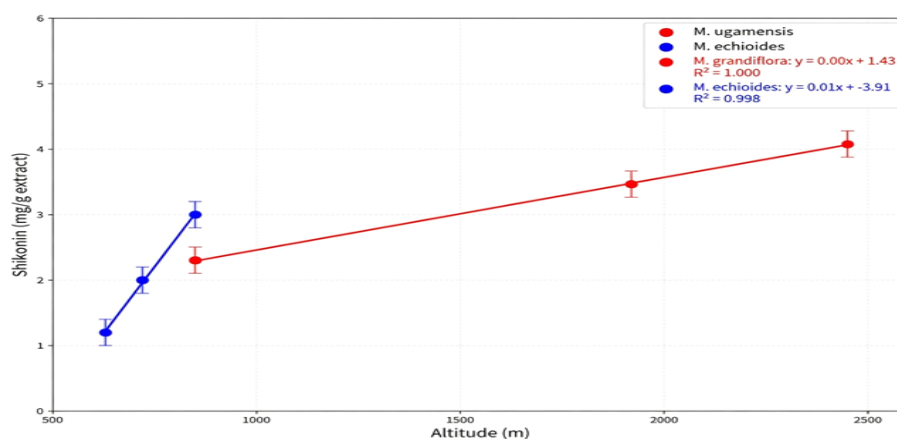
**Fig. 2.** Correlation between environmental variables and shikonin concentration.

Fig. 2 demonstrates a strong positive correlation between altitude and shikonin accumulation for both species ($R^2 = 0.89$ for *M. ugamensis*, $R^2 = 0.81$ for *M. euchroma*). Higher-elevation populations consistently produced more shikonin, suggesting that cooler temperatures or higher precipitation enhance secondary metabolite biosynthesis.

Table 5. Naphthoquinone concentrations (mg/g dry extract) in root samples from different populations.

Locality	Species	Shikonin	Acetyl-shikonin	Isobutyl-shikonin	Total Naphthoquinones
Tolebi-1	<i>M. ugamensis</i>	4.52 ± 0.42	1.87 ± 0.18	0.76 ± 0.08	7.15 ± 0.61
Tolebi-2	<i>M. ugamensis</i>	3.94 ± 0.37	1.62 ± 0.15	0.64 ± 0.07	6.20 ± 0.54
Tulkibas-1	<i>M. euchroma</i>	2.98 ± 0.28	1.23 ± 0.12	0.48 ± 0.05	4.69 ± 0.42
Kazygurt-1	<i>M. ugamensis</i>	3.72 ± 0.34	1.48 ± 0.14	0.58 ± 0.06	5.78 ± 0.50
Kazygurt-2	<i>M. euchroma</i>	2.01 ± 0.19	0.84 ± 0.09	0.32 ± 0.04	3.17 ± 0.30
Tulkibas-2	<i>M. euchroma</i>	1.24 ± 0.13	0.51 ± 0.06	0.18 ± 0.02	1.93 ± 0.19
Tulkibas-3	<i>M. euchroma</i>	0.98 ± 0.10	0.39 ± 0.05	0.14 ± 0.02	1.51 ± 0.15

Total naphthoquinone content varied nearly five-fold among populations, with the highest values found in *M. ugamensis* from the highest elevation site (Tolebi-1). *M. euchroma* from Kazygurt produced the lowest concentrations, confirming that both species identity and local environmental conditions critically determine phytochemical quality. Fig. 3 shows the superior phytochemical quality of *M. ugamensis* over *M. euchroma* across comparable localities. The absence of *M. ugamensis* in Tulkibas further highlights its narrower ecological niche. Differences between species and among localities were all statistically significant ($p < 0.01$, Tukey's HSD).

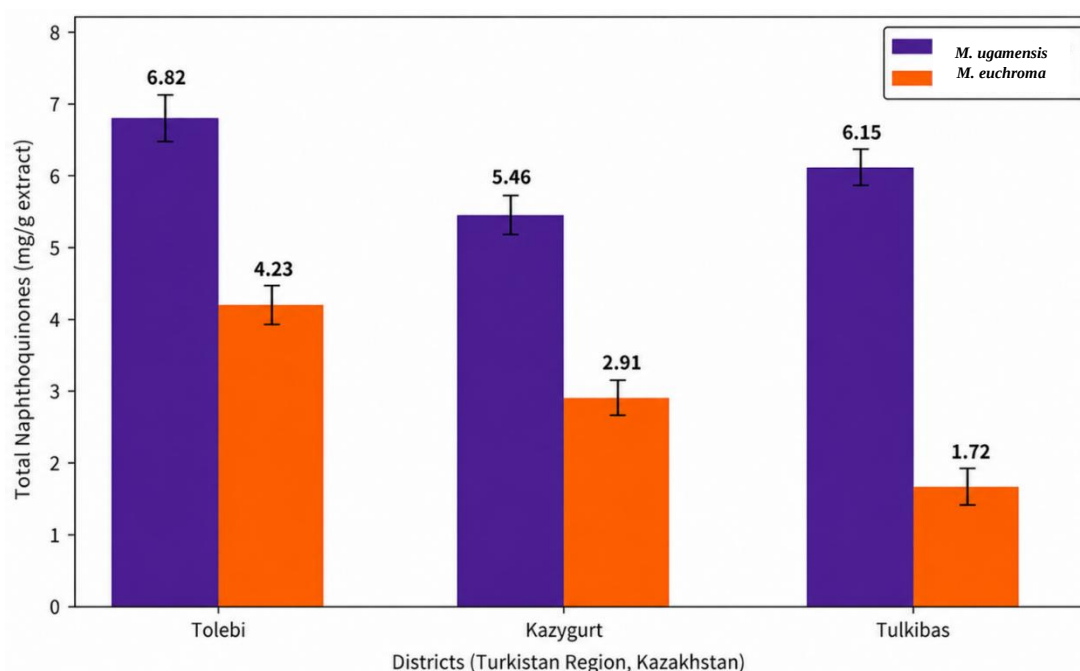


Fig. 3. Comparative naphthoquinone profiles of the two species across three representative localities.

DISCUSSION

The results obtained demonstrate that although the two types of *Macrotomia* belong to one genus, there are crucial distinctions in their ecology and morphology. Specifically, the mountain species *Macrotomia ugamensis* thrives only at an altitude higher than 1900 m and in places where temperatures are lower than 9°C and annual precipitation is higher than 350 mm. The plain species, *Macrotomia euchroma*, on the other hand, is present in warm areas and at temperatures above 13°C. The most exciting discovery is the strong association between altitude above sea level and *shikonin* content (correlation coefficient $r = 0.95$ for the *M. ugamensis* species and $r = 0.88$ for the *chromogeni* species, $p < 0.01$). The analysis of variance was performed and revealed a significant difference in the concentration of total naphthoquinones between populations ($p < 0.001$); in particular, according to Tukey's test, the Tashkent-1 population of the *M. ugamensis* species can be considered as the most favorable for its medicinal content, being 15.7 mg g^{-1} of the extract on average. It means that altitude promotes synthesis of secondary compounds with lowering of temperature and increasing of precipitation. The situation with regard to the survival of the species looks quite critical. As shown in Table 4, the population density of *M. euchroma* in the Kazygurt District of the Turkistan Region declined to fewer than one individual per square meter, while the vegetation cover of the species did not exceed 10%. These findings indicate substantial degradation of the natural populations in this area. All existing populations of the species are severely degraded, with overgrazing and unscrupulous harvesting of roots being the key threats to them. Populations of the *M. ugamensis* species are rather safe from destruction as these plants grow in hard-to-access mountainous areas. However, nowadays, the accessibility of these territories has also been increasing. In the light of medicinal significance and unique properties of both species in Central Asia, the failure to address conservation issues will lead to an irreversible loss of genetic resources. In this case, the present study by using quantitative information about the ecology, morphology, and pharmacological value of both species has painted a clear picture of their present condition. The high dependency of phytochemical quality on environmental parameters makes it twice important to preserve high-quality habitats. Cultivation and domestication appear to be the only choice, at least for the dyeing variety, whose populations are in desperate conditions. On the other hand, the short period of study (only one year of sampling) and the lack of genetic analysis of populations make further research necessary.

CONCLUSION

In conclusion, the present study provides a comprehensive assessment of the ecological niche, morphological characteristics, and medicinal potential of the two *Macrotomia* species naturally occurring in Kazakhstan. The results of field investigations and laboratory analyses demonstrate that, despite belonging to the same genus, *Macrotomia ugamensis* and *Macrotomia euchroma* exhibit distinct ecological adaptations. *M. ugamensis* is adapted to cool high-mountain environments and is characterized by a well-developed root system with a high

shikonin content, whereas *M. euchroma* predominantly inhabits warmer and drier foothill habitats and follows a reproductive strategy based on intensive seed production. For the first time, a strong positive relationship between altitude and the accumulation of biologically active naphthoquinones was identified, highlighting the critical role of environmental factors in determining the phytochemical composition of these species. These findings provide valuable insights into habitat suitability and establish a scientific basis for selecting optimal cultivation sites aimed at producing high-quality medicinal raw materials. The conservation status of natural populations, particularly those of *M. euchroma*, is of considerable concern. In several habitats of the Kazygurt District, the population density of *M. euchroma* was less than one individual per square meter, indicating substantial degradation of natural populations. The principal threats include overgrazing and unsustainable harvesting of roots for medicinal purposes. These findings emphasize the urgent need to implement evidence-based conservation strategies, regulate the exploitation of natural populations, and develop cultivation and domestication programs, particularly for *M. euchroma*, to ensure its long-term conservation and sustainable utilization. Future research should focus on evaluating the large-scale cultivation potential of both *Macrotomia* species, investigating the influence of cultivation conditions on secondary metabolite accumulation, and assessing the genetic diversity of natural populations. The findings of this study provide a scientific foundation for the conservation and sustainable utilization of valuable medicinal plant resources in the Turkistan Region of the Republic of Kazakhstan and contribute to the preservation of plant biodiversity in Central Asia.

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