

Morphological and Chemical Diversity of *Artemisia Herba Alba* in Jabal Al-Akhdar of Libya

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Abstract:

This study investigated the morphological and phytochemical variability of *Artemisia herba-alba* across three ecologically distinct populations in Jabal Al-Akhdar, Libya. The results demonstrated marked population-level differences primarily driven by elevation, grazing intensity, and localized environmental conditions. Plants collected from Ras El-Hilal exhibited the strongest vegetative performance, recording the highest shoot perimeter (94.5 cm) and the greatest total, fresh, and dry biomass. Conversely, the El-Maslaqoun site, characterized by severe overgrazing, displayed a substantial reduction in growth parameters, with the shoot perimeter decreasing significantly to 16.3 cm and the total plant weight declining sharply from 143.7 g (Ras El-Hilal) to 35.1 g. Consistent trends were observed for root length, shoot length, and overall plant height, with the Ras El-Hilal and El-Maslaqoun populations consistently showing superior performance compared to El-Maslaqoun. The phytochemical composition also varied significantly across the populations. Total phenolic content ranged from 115.5 to 124.8 mg gallic acid equivalents per gram dry matter, while total flavonoid content spanned 78.1 to 94.8 mg catechin equivalents per gram. Notably, the highest levels of both phenolics and flavonoids were recorded in the El-Maslaqoun population, despite its diminished vegetative growth. This finding suggests that environmental stress may effectively stimulate the accumulation of these secondary metabolites. Furthermore, a moderate positive correlation ($R^2 = 0.4669$) was observed between total phenols and total flavonoids, indicating a coordinated biochemical response to ecological pressures. These findings highlight the substantial ecological plasticity of *A. herba-alba* and clearly demonstrate the influence of environmental gradients on both morphological traits and phytochemical richness. These insights are crucial for developing effective conservation strategies and optimizing the sustainable utilization of this medicinally important species.

Keywords: wormwood, morphological characters, conservation, medical plants.

التنوع الشكلي والكيميائي لنبات الشيح *Artemisia Herba Alba* في الجبل الأخضر بليبيا

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الملخص

حققت هذه الدراسة في التباين الشكلي والكيميائي النباتي لنبات *Artemisia herba-alba* عبر ثلاث مجموعات سكانية متميزة بيئياً في الجبل الأخضر بليبيا. أظهرت النتائج اختلافات واضحة على مستوى المجموعات السكانية، مدفوعة في المقام الأول بالارتفاع، وكثافة الرعي، والظروف البيئية المحلية.

أظهرت النباتات التي جُمعت من رأس الهلال أقوى أداء خضري، حيث سجلت أعلى محيط للساق (94.5 سم) وأكبر كتلة حيوية إجمالية، طازجة، وجافة. على العكس من ذلك، أظهر موقع المسلقون، الذي يتسم بالرعي الجائر الشديد، انخفاضاً كبيراً في معايير النمو، حيث انخفض محيط الساق بشكل ملحوظ إلى 16.3 سم وتراجع الوزن الإجمالي للنبات بشكل حاد من 143.7 جرام (رأس الهلال) إلى 35.1 جرام. لوحظت اتجاهات متسقة بالنسبة لطول الجذر، وطول الساق، والارتفاع الكلي للنبات، حيث أظهرت مجموعات رأس الهلال والمثيل أداءً متفوقاً باستمرار مقارنةً بالمسلقون. كما تباين التركيب الكيميائي النباتي بشكل كبير عبر المجموعات السكانية. تراوح إجمالي محتوى الفينولات من 115.5 إلى 124.8 ملجم مكافئ حمض الغاليك لكل جرام مادة جافة، بينما امتد إجمالي محتوى الفلافونويدات من 78.1 إلى 94.8 ملجم مكافئ كاتيشين لكل جرام. ومن الجدير بالذكر، أنه تم تسجيل أعلى مستويات لكل من الفينولات والفلافونويدات في مجموعة المسلقون السكانية، على الرغم من انخفاض نموها الخضري. تشير هذه النتيجة إلى أن الإجهاد البيئي قد يحفز بشكل فعال تراكم هذه المستقلبات الثانوية. علاوة على ذلك، لوحظ ارتباط إيجابي معتدل ($R^2 = 0.4669$) بين إجمالي الفينولات وإجمالي الفلافونويدات، مما يشير إلى استجابة كيميائية حيوية منسقة للضغوط البيئية. تسلط هذه النتائج الضوء على المرونة البيئية الكبيرة لنبات *A. herba-alba* وتوضح بوضوح تأثير التدرجات البيئية على كل من السمات الشكلية والثراء الكيميائي النباتي. تعتبر هذه الرؤى بالغة الأهمية لتطوير استراتيجيات حفظ فعالة ولتحسين الاستخدام لهذا النوع الهام طبيًا.

الكلمات المفتاحية: الشج، الصفات الشكلية، الحفظ، النباتات الطبية.

Introduction

Artemisia herba-alba (commonly known as white wormwood or desert wormwood) is a plant of considerable ethnopharmacological importance, traditionally utilized in folk medicine for treating numerous ailments with reportedly minimal side effects. Experimental studies corroborate its medicinal value, demonstrating that decoctions of *A. herba-alba* possess beneficial antioxidant properties. This biological activity is attributed to its rich phytochemical profile, which includes tannins, volatile oils, alkaloids, flavonoids, glycosides, saponins, and coumarins (Rizk, 1986; Al-Ghzawi et al., 2012). Beyond therapeutics, the plant is also employed in the fragrance and cosmetic industries (Hirasa & Takemasa, 1998).

Jabal Al-Akhdar in the northeastern region of Libya (32°-33° N and 20°-23° E) is characterized by distinct ecological heterogeneity. This area is a biodiversity hotspot, hosting approximately 1300 plant species, representing 70-80% of the entire Libyan flora, including *Artemisia herba-alba*. The region's topography consists of a series of escarpments that establish three climatically differing altitudinal zones: (1) a coastal plain (up to 200 m altitude) with a typical Mediterranean climate, (2) a middle zone (200 m to 600 m), and (3) a high plateau zone (600 m to 880 m) (Mahklouf & Etayeb, 2019). These climatic and topographical variations significantly influence the composition and characteristics of the local vegetation, resulting in substantial environmental diversity. This diversity is a critical determinant of the quality and quantity of active secondary metabolites in medicinal plants and may also drive genetic variation within plant populations. However, this fragile ecosystem is susceptible to changes induced by external pressures such as climate change, urbanization, destruction of natural vegetation, overgrazing, and accelerated land degradation and desertification (El-Barasi & Saaed, 2013).

Biodiversity encompasses the variety and variability of living organisms, the biological communities they inhabit, and the ecological and evolutionary processes that sustain them. It serves as a vital measure of ecosystem health, indicating the long-term viability of species assemblages (UNESCO, 1994). The worldwide loss of biodiversity represents a critical global issue. The current pace of habitat exploitation and species eradication exposes thousands of plant species to the threat of extinction, which inevitably leads to catastrophic impacts on fundamental ecosystem functions. The primary anthropogenic drivers of this loss include

habitat fragmentation, overexploitation of natural resources, land clearing, and pollution. Consequently, an accurate and systematic assessment of regional biodiversity is essential not only for addressing fundamental ecological questions but also for developing effective conservation and therapeutic strategies, such as those related to medicinal plants (Abdelmoumen et al., 2017; Salem et al., 2025).

The analysis of morphological parameters is a foundational and commonly implemented method for identifying intraspecific variation and assessing diversity levels within plant species. Vegetative traits are widely accepted as effective quantitative parameters for plant characterization (Elhoumaizi et al., 2002). Therefore, the objective of this study was to employ both morphological and phytochemical tools to investigate the extent of variation and the diversity level of *Artemisia herba-alba* across different altitudinally distinct populations within Jabal Al-Akhdar, Libya.

Material and Methods

2.1. Sample Collection and Study Sites

A total of 30 mature, whole plants of *Artemisia herba-alba* were collected. Sampling was conducted across three ecologically distinct populations, with ten replicate plants collected from each location. These sites were selected to represent areas with varying altitudinal and topographical levels in Jabal Al-Akhdar, Libya:

1. **Ras El-Hilal:** Representing the coastal region, located at an altitude of 30–40 meters above sea level (m.a.s.l.), at coordinates 32° 54' 05" N, 22° 10' 05" E.
2. **El-Maslaqoun:** Located south of Elfataeah at an altitude of 480 m.a.s.l., at coordinates 30' 16" N, 22° 33' 40" E.
3. **El-Mashel:** Situated south of Aslanta, near the city of El-Bayda, at an altitude of 675 m.a.s.l., at coordinates 32° 31' 02" N, 21° 43' 27" E. as shown in (Figure 1).

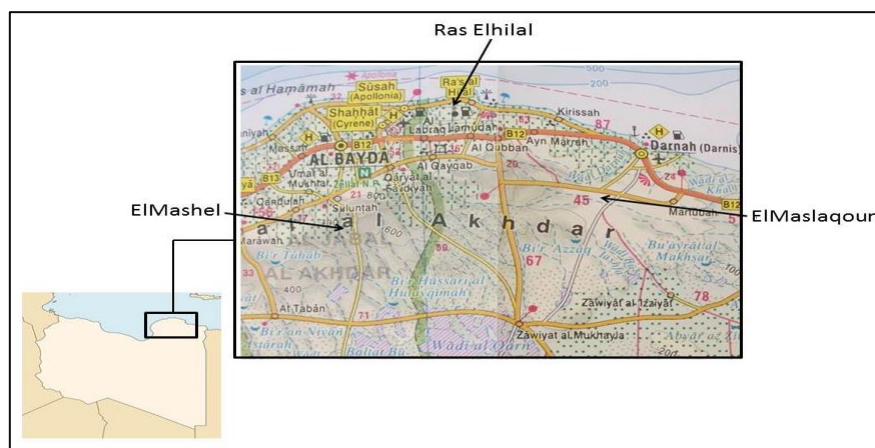


Figure 1. Map of Libya showing the location of samples.

2.2. Morphological Measurement and Statistical Analysis

The following morphological parameters were determined for each plant: shoot perimeter (cm), total plant weight (g), shoot fresh weight (g), shoot dry weight (g), root fresh weight (g), root dry weight (g), total plant length (cm), shoot length (cm), root length (cm), number of branches, shoot moisture content (%), and shoot dry matter (%).

Data analysis was performed using the ASSISTAT statistical program (Version 7.7 beta, 2016). The means of the data sets were compared using Tukey's test at a significance level of 5% ($p < 0.05$).

2.3. Extraction and Determination of Phenols and Flavonoids

Sample Preparation: Leaves and stems were washed and dried in an oven at 35°C. The samples were then subjected to vacuum drying (50 bars) for 2 to 3 days. Each sample was separately ground into a fine powder. The powdered samples were stored in sealed, dark jars to prevent degradation from light and moisture.

Extraction Procedure: A specific mass (3 g) of the powdered sample was accurately weighed and dissolved in 150 mL of 80% methanol. The mixture was placed on a shaker (vibrator) for 6 hours and subsequently filtered three times. The resulting methanol extract was concentrated using a Rotary Evaporator and then subjected to lyophilization (freeze-drying).

Phytochemical Analysis: Total phenols were determined using the Folin-Ciocalteu reagent method, with sodium carbonate reagent (Singleton et al., 1999). Total flavonoids were estimated using the aluminum chloride (AlCl₃) reagent method (Kosalec et al., 2004).

Results and Discussion

Morphological Variability Across Populations

The vegetative growth of *Artemisia herba-alba* was significantly influenced by the geographical location of the populations. The shoot perimeter exhibited the most pronounced variation, with the highest value (94.5 cm) recorded in the Ras El-Hilal area, while the lowest value (16.3 cm) was observed in the El-Maslaqoun location. The El-Mashel population showed an intermediate shoot perimeter (71 cm).

This significant disparity is strongly attributed to the localized environmental pressures, particularly the intense overgrazing noted in the El-Maslaqoun area. Overgrazing is known to induce severe physiological and structural changes in plant populations, affecting their dynamics and overall ecosystem stability, as observed in semi-arid shrublands (Navarro et al., 2006). Since *A. herba-alba* is considered a reliable and abundant source of forage for sheep and livestock in highland regions (Benmansour & Bendiab, 1998), its biomass is heavily reduced in areas subjected to uncontrolled grazing.

A similar trend was evident across all measured biomass characteristics, including total plant weight, shoot fresh weight, and shoot dry weight, with the highest values consistently recorded in the Ras El-Hilal population (Table 1). The total plant weight decreased drastically from a mean of 143.7 g in Ras El-Hilal to 35.1 g in El-Maslaqoun. This sharp decline in biomass can be further linked to potential drought conditions in the El-Maslaqoun area, which often correlates with lower rainfall compared to the coastal and elevated El-Mashel site. Water stress significantly impairs plant growth; for instance, severe water scarcity can dramatically increase the rate of leaf senescence and mortality (Guenauoui et al., 2009).

Vegetative Characteristics and Environmental Determinants

Beyond the shoot parameters, the Ras El-Hilal and El-Mashel populations also recorded higher values for root fresh weight, root dry weight, total plant length, shoot length, root length, and shoot dry matter when compared to the El-Maslaqoun area (Table 2).

The collective results of this study strongly indicate that the morphological performance of *A. herba-alba* in Jabal Al-Akhdar is primarily dictated by environmental heterogeneity, rather than inherent genetic factors. This finding is consistent with previous research demonstrating that the growth and phytochemical composition of *Artemisia* species are more profoundly influenced by environmental stressors than by underlying genetic variation (Thu et al., 2011), a pattern also observed in other plant species (Salem & Lakwani, 2024).

Furthermore, edaphic factors, such as soil type, play a crucial role in vegetative growth. For example, a significant increase in vegetative growth characteristics has been documented in plants cultivated in clay soils compared to those grown in sandy soils (Omer et al., 2013). Given the varied altitudinal and topographical levels of the study sites, differences in soil composition, nutrient availability, and water retention likely contribute to the morphological superiority

observed in the Ras El-Hilal and El-Mashel populations over the heavily stressed El-Maslaqoun population.

Table 1. Morphological characters of *Artemisia herba alba* in different locations of Libya.

NO.	character	RasElhilal	ElMaslaqoun	ElMashel
1	perimeter of Shoot / cm	94.5 ^a	16.3 ^c	71 ^b
2	Total weight/g	143.7 ^a	35.1 ^c	85 ^b
3	Shoot fresh weight/g	116.65 ^a	27.8 ^c	65 ^b
4	Shoot dry weight / g	76.75 ^a	13.92 ^c	42 ^b
5	Root fresh weight/g	27 ^a	7.3 ^b	20 ^a
6	Root dry weight / g	19.25 ^a	3.67 ^b	14.4 ^a
7	Total plant length/cm	61.3 ^a	37.3 ^b	60.9 ^a
8	Shoot length /cm	36.55 ^a	21 ^b	35.5 ^a
9	Root length / cm	24.75 ^a	16.3 ^b	25.4 ^a
10	Number of branches	6.8 ^a	4.8 ^{ab}	4 ^b
11	Shoot moisture content	34.07% ^b	47.77% ^a	37.15% ^b
12	Shoot dry matter	65.93% ^a	52.23% ^b	62.85% ^a

Separation of means by Tukey test at the 5% level and means with the same letter are not significantly different.

Phytochemical Variability and Environmental Stress

The results indicate significant variability in the total phenolic content of *Artemisia herba-alba* across the different collection sites (Figure 2). The highest phenolic quantity was 124.8 mg Gallic Acid Equivalents (GAE) per gram of dry matter (DM), recorded in the El-Maslaqoun location, followed by 122.5 mg GAE/g DM in El-Mashel. The lowest content (115.5 mg GAE/g DM) was noted in Ras El-Hilal. Despite recording the lowest value among the study sites, the phenolic content of the Ras El-Hilal population was notably high, approximately double the quantity reported for *Artemisia* growing in Saudi Arabia (Mobin et al., 2015). This wide variation in phenolic content is consistent with reports indicating that secondary metabolite levels are significantly affected by environmental factors, genetic variations, specific plant parts sampled, sampling time, and the extraction/analysis methods used (Bouayed et al., 2007; Shan et al., 2005).

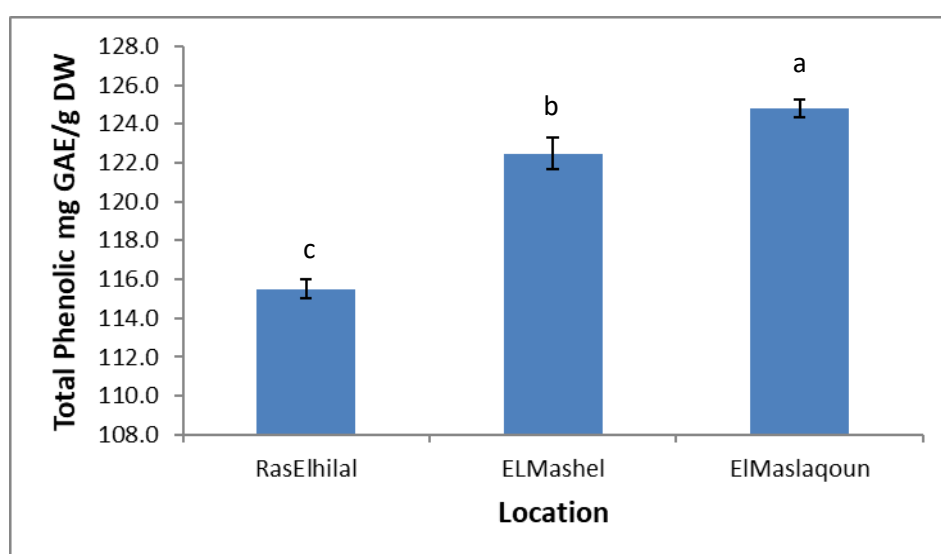


Figure 2. Phenolic compounds mg GAE/g equivalent for each gram of dry matter in three sites in Jabal al-Akhdar Libya.

These findings align with studies conducted on *Artemisia* collected from Tunisia, where total phenols and flavonoids contents were found to vary according to the geographical origin of the plants (Bourgou et al., 2016). Furthermore, the observation confirms that the content of phenolic compounds and flavonoids is dependent on the specific growth area (Moufid & Eddouks, 2012).

Similarly, the total flavonoid content followed the inverse morphological trend, with the highest value (94.8 mg Catechin Equivalents (CE) per gram of DM) recorded in El-Maslaqoun, followed by 81.3 mg CE/g DM in El-Mashel, and the lowest value (78.1 mg CE/g DM) in Ras El-Hilal (Figure 3).

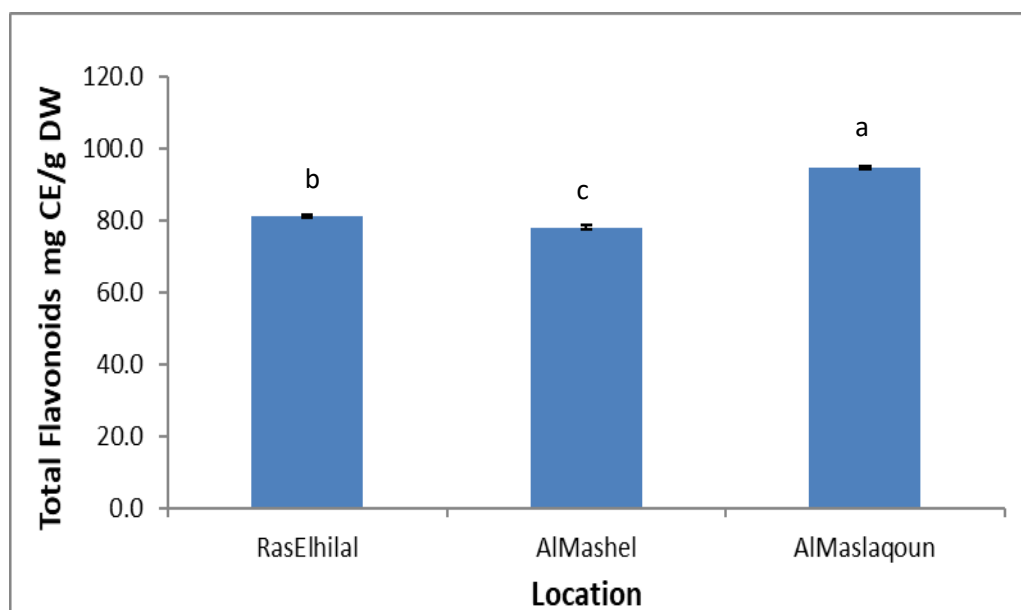


Figure 3. Flavonoid compounds mg CE/g equivalent for each gram of dry matter in three sites in Jabal al-Akhdar Libya

The finding that the El-Maslaqoun population—which exhibited the most significant reduction in vegetative growth due to severe grazing and potential drought stress—also accumulated the highest levels of phenolics and flavonoids is particularly important. This result supports the hypothesis that environmental stress serves as a crucial elicitor for the biosynthesis and accumulation of secondary metabolites in plants. Numerous studies have confirmed the pharmacological efficacy of medicinal plants, including *A. herba-alba*, growing in arid zones, confirming the abundance of phenolic compounds and antioxidants in the aerial parts of *A. herba-alba* (Dif et al., 2016). The total flavonoid content reported in this study is notably higher than the 18.3 mg CE/g DM reported by Mobin et al. (2015) and surpasses values obtained in other studies (Djeridane et al., 2006).

Correlation between Phenols and Flavonoids

This study established a **moderate positive correlation** between the total phenolic content and the total flavonoid content in *Artemisia herba-alba* growing in the Jabal Al-Akhdar region ($R^2 = 0.4669$) (Figure 4).

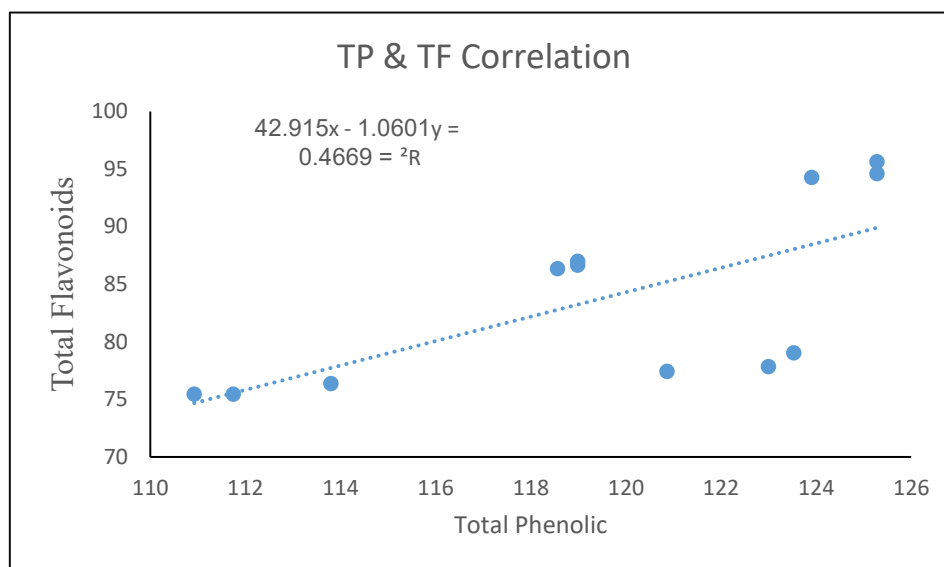


Figure 4. Correlation between total phenolic and total flavonoids content of *Artemisia herba alba* growing in Jabal al-Akhdar Libya.

This correlation suggests that the levels of total phenolics in *A. herba-alba* populations may serve as an indicator of the total flavonoid content, implying a coordinated metabolic pathway response to ecological conditions. This result is consistent with findings by Amoateng et al. (2011), who observed a high correlation between total antioxidant capacity and total phenol content in *Synedrella nodiflora*, a species also belonging to the Asteraceae family as *A. herba-alba*. Furthermore, Djeridane et al. (2006) previously reported a strong positive correlation between total phenolic content and antioxidant activity ($R^2 = 0.7802$).

Conclusion

This study provides fundamental data on the morphological and phytochemical diversity of *Artemisia herba-alba* grown across ecologically disparate locations in Jabal Al-Akhdar, Libya. The results demonstrate that geographical locations exert a significant influence on vegetative parameters, including shoot perimeter, total weight, root characteristics, and moisture content. The Ras El-Hilal area yielded the highest values for most studied morphological traits, likely due to its distinct, more favorable environmental and topographical setting. Conversely, the El-Maslaqoun site, despite exhibiting the lowest vegetative performance due to environmental stress, recorded the highest accumulation of total phenolic and flavonoid compounds. This inverse relationship highlights the substantial ecological plasticity of *A. herba-alba* and confirms the role of environmental stressors in driving the biosynthesis of high-value secondary metabolites. These insights are essential for developing appropriate conservation strategies and optimizing the targeted harvesting of this medicinally important species.

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Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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