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## Environmental influence on the phytochemical composition of *Calligonum leucocladum* populations in Kazakhstan

**Abstract.** *Calligonum leucocladum* (Polygonaceae) is a characteristic shrub of arid and semi-arid regions of Central Asia, where plant survival is limited by scarce water and extreme temperature fluctuations. This study investigated the phytochemical profiles of three spatially separated populations of *C. leucocladum* collected from natural habitats in Kazakhstan. The results demonstrated the presence of various phenolic compounds, organic acids, and amino acids. Among them, artemisinin C and caffeic acid phenethyl ester (CAPE) were detected in this species for the first time. Distinct differences in metabolite accumulation between populations were recorded. In particular, individuals from population P3, located in semi-arid mountainous terrain at an altitude of about 800 m above sea level, were characterised by increased concentrations of phenolic compounds, soluble carbohydrates and the osmoprotective amino acid proline. These population – specific metabolic features probably reflect biochemical adaptations to contrasting environmental influences. These results indicate that *C. leucocladum* is a valuable source of biologically active compounds, and variations in the composition of its metabolites are closely related to habitat conditions.

**Keywords:** *Calligonum leucocladum*, phenolic compounds, artemisinin C, proline, pharmacological potential, metabolic adaptation.

### Introduction

*Calligonum leucocladum* is a perennial shrub of the Polygonaceae family, naturally distributed in arid and semi-arid areas of southern Kazakhstan and adjacent regions of Central Asia. This species is adapted to an environment characterised by low rainfall and high heat stress, and is known to accumulate a number of biologically active compounds of ecological and potential medical interest [1].

Within its natural range, *C. leucocladum* contributes to the functioning of desert ecosystems in several ways. Its well-developed root system stabilises loose sandy substrates and reduces wind and water erosion, which is a major factor in land degradation in these regions. By influencing soil structure and moisture retention in the rhizosphere, the plant can locally mitigate microclimatic conditions, including extreme surface temperatures. In addition, *C. leucocladum* supports elements of local biodiversity by providing habitat and food resources for insects,

birds, and other fauna species [1]. Furthermore, it is important for the restoration of degraded landscapes, making it a key component of environmental rehabilitation programmes. The species’ resistance to high temperatures and low humidity demonstrates its adaptive capabilities in addressing climate change issues. Successful examples of its use in ecosystem rehabilitation can be seen in the sandy regions of southern Kazakhstan, where plantations have significantly reduced land degradation [2].

The uniqueness of the species *C. leucocladum* from a pharmacognostic perspective is determined not only by the standard set of polyphenols and organic acids but also by the presence of specific secondary metabolites. Study reports the successful identification of previously undescribed structures in this plant, particularly derivatives of phenylethyl acid and catechol [3]. Preliminary data indicate that the isolated compounds exhibit pronounced biological activity, opening avenues for their study in the context of developing antioxidant, anti-inflammatory

ry, and cytotoxic drugs. Other important compounds have also been found in the plant, including rutin, quercetin and chlorogenic acid, which are known for their medicinal properties [3]. In addition, the plant contains sugars and amino acids, which play an important role in metabolism. Quantitative studies have shown that the content of polymethoxyflavones in the leaves can be 20–30% higher than in the flowers. This chemical composition makes *C. leucocladum* a promising source of raw materials for the production of pharmaceuticals and functional foods [4]. It is also important to note that the chemical composition of the plant varies depending on the geographical location of the population, which highlights the need to study different populations [5].

Research on this plant opens unique perspectives for science and medicine. It is of great interest as a research object due to rich chemical composition, adaptation mechanisms, and broad spectrum of biological activity [6].



a)



b)

**Figure 1** – Morphological features of *C. leucocladum*  
 general habit of the plant in its natural habitat;  
 b) close-up view of branches and flowers

Future studies could focus on exploring the biosynthetic pathways of the plant's key compounds and investigating the interactions of its components with biological systems. Genetic analysis of various populations of this plant can shed light on intraspecific variability and mechanisms of adaptation to extreme environmental conditions [7]. The study of the pharmacological activity of *C. leucocladum* extracts on disease models will lay the foundation for the development of new drugs, which is particularly relevant for the medicine of the future [8-9].

Furthermore, attention should be given to developing sustainable cultivation methods for this plant, especially in degraded ecosystems. This will not only help preserve natural populations but also enable the use of this species for bioremediation, soil restoration, and maintaining ecological balance. Simultaneously, research into how climatic factors influence the plant's chemical composition is vital. Identifying these relationships will allow for the determination of optimal cultivation conditions, a prerequisite for its effective application in agriculture and pharmacology [10].

The plant we selected stands out among other species of the genus *Calligonum* due to its unique ability to survive under extreme conditions of low humidity and high temperatures. Its advantage lies in its high content of polyphenols and flavonoids, which give it pronounced antioxidant and anti-inflammatory properties. For example, compared to *Calligonum caput-medusae*, which also grows in Central Asia and is known for its ecological functions, *C. leucocladum* has greater therapeutic potential and a richer chemical composition [7]. *C. leucocladum* populations are threatened by intense human impact. The main issues include the destruction of natural habitats due to farming, construction, and landscape changes. An additional factor contributing to population decline is climate change, which leads to ecosystem degradation and species decline [11]. The decline in populations of this plant could lead to the loss of its ecological functions and pharmacological potential. Comprehensive measures are needed to preserve this species, including the creation of protected areas, population monitoring, and the implementation of restoration programs. In addition, the use of cultivation methods under controlled conditions can ensure stable production of biologically active substances without harvesting plants in the wild [12]. The decline in populations creates a risk of losing its key ecological functions, such as preventing soil erosion and maintaining biodiversity, as well as its pharmacological potential [13]. Comprehensive measures are needed to conserve and restore this species. One

step could be the creation of protected natural areas and regular monitoring of the population status. It is also important to develop population restoration programs, including the use of cultivation methods under controlled conditions. This approach will help to preserve natural populations and ensure stable production of biologically active compounds without harming ecosystems [13-15].

*C. leucocladum* possesses a rich and complex phytochemical profile, underpinning its considerable promise for the development of novel therapeutic agents [16]. Among its bioactive constituents, compounds with demonstrable anti-inflammatory effects show potential for managing conditions such as rheumatoid arthritis, inflammatory bowel disease, and asthma. Furthermore, its antioxidant properties are of significant interest for the prevention and therapeutic mitigation of neurodegenerative pathologies, including Alzheimer's and Parkinson's diseases [17]. The plant also has significant anticancer potential, as it inhibits the growth of cancer cells and reduces their proliferation. Its antibacterial and antifungal properties make it suitable for combating skin infections, respiratory infections, and infections of the genitourinary system [18]. In addition, it can regulate blood glucose levels, making it useful for the prevention and management of diabetes. Its wound-healing and antioxidant properties allow it to be used in dermatology to treat skin diseases and accelerate wound heal-

ing [19]. The plant's immunomodulatory properties open up possibilities for its use in immunodeficiency or autoimmune diseases. These properties may also be useful in cardiovascular diseases, including atherosclerosis, and as an adjunctive therapy in chronic inflammatory conditions [20].

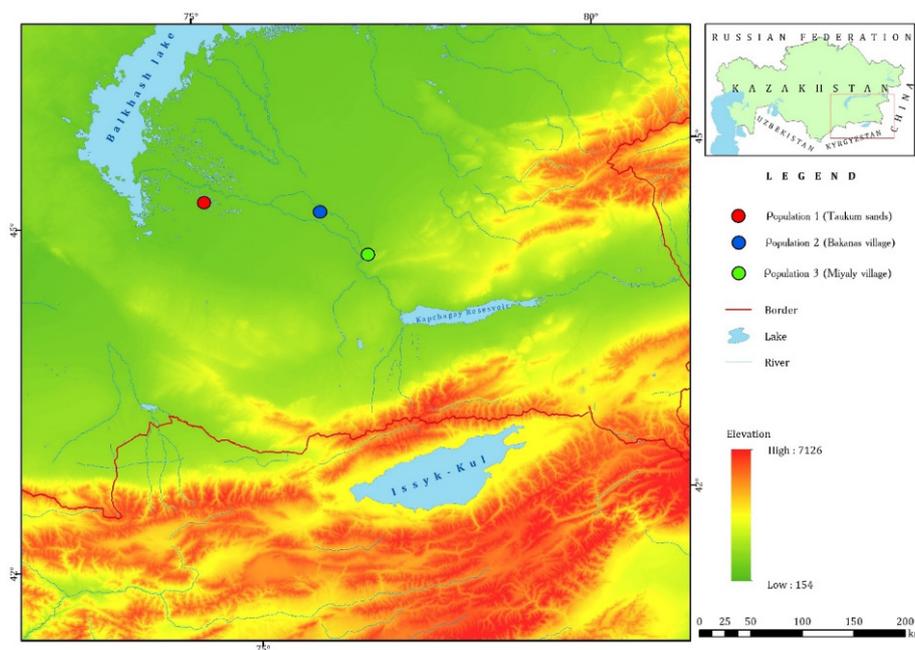
To fully unlock its potential, large-scale research, including clinical trials, is needed. This will not only confirm the plant's effectiveness in treating various diseases but also help determine the best ways to use it, safe dosages, and possible side effects. Only through a comprehensive approach can the full potential of this unique plant for science, medicine, and ecology be realised [21-23].

The aim of this study is to investigate chemical composition of three populations of *C. leucocladum* from different natural areas of Kazakhstan.

## Materials and methods

### *Collection and preparation of plant samples*

Plant samples of *C. leucocladum* were collected from three natural populations in July 2024. Population 1 was located in the Taukum sands, Balkhash district, Almaty region. Population 2 was sampled in the Saryesik-Atyrau sands, near Bakanas, in the same district and region. Population 3 was collected from the Miyaly area within the Saryesik-Atyrau sands, Balkhash district, Almaty region (Figure 2).



**Figure 2** – Map of the Balkhash district (Almaty region, Kazakhstan) indicating the sampling locations of the three *C. leucocladum* populations

For each location, geographical coordinates were recorded, as well as key environmental indicators such as altitude, ambient temperature and precipitation levels. Species identification was confirmed by a qualified taxonomist, and reference samples were transferred to the Herbarium of the Institute of Botany and Phytointroduction (Almaty, Kazakhstan; Index Herbariorum code: ALTB; specimen number 2200). The plant material was manually separated into morphological parts (flowers, leaves, and stems) and then dried naturally under stable conditions (25 °C, 40% relative humidity) in a shaded, well-ventilated environment for two weeks. The dried samples were ground into a fine powder using a grinder ZM200 (Retsch, Germany) and stored at -20 °C in sealed containers to preserve their integrity until analysis.

#### *Extraction of biologically active compounds*

A total of 1.5 g of powdered plant material was extracted using ultrasonic extraction (UAE) with an Transsonic T570 ultrasonic water bath (Elma, Germany) at a constant frequency of 40 kHz and a temperature of 40 °C. The extraction solvent consisted of 25 ml of 80% methanol (MeOH) acidified with 0.1% hydrochloric acid. The extraction process lasted 50 minutes, after which centrifugation was performed at 10,000 rpm for 10 minutes at 4 °C using a Beckman Coulter Allegra X-30R centrifuge (C0650 rotor, Beckman Coulter, USA). The supernatant was filtered through a 0.22 µm PTFE membrane filter (Millipore, USA) and stored at -80 °C for further analysis.

*Determination of total phenolic content.* Total phenolic content (TPC) was determined by colorimetric analysis using the Folin-Ciocalteu method in accordance with a standardised protocol [24]. In brief, 20 µl of extract was mixed with 100 µl of Folin-Ciocalteu reagent (Sigma-Aldrich, USA) and incubated at room temperature in the dark for 5 minutes. Then, 80 µl of 7.5% sodium carbonate was added and the reaction was allowed to proceed for 60 minutes. Absorbance was measured at 760 nm using a BioTek Epoch microplate reader (BioTek Instruments, VT, USA). A calibration curve was constructed using gallic acid (Sigma-Aldrich, USA) as a standard within a concentration range of 0.01–0.1 mg/mL. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g DW).

*Determination of total flavonoid content (TFC).* The total flavonoid content was quantified using an aluminium chloride-based colorimetric assay [25]. 100 µl of extract was mixed with 100 µl of 2% AlCl<sub>3</sub>

in methanol. After incubation at room temperature for 10 minutes, the absorbance was measured at 510 nm using a BioTek Epoch microplate reader (Agilent Technologies, USA). Quercetin (Sigma-Aldrich, USA) was used as the reference standard, and the results were expressed in mg of quercetin equivalent (QUE) per g of dry sample.

*Determination of total proanthocyanidin content.* Total proanthocyanidin content was determined using the vanillin-HCl method [26]. 100 µl of extract was mixed with 500 µl of 4% vanillin (Sigma-Aldrich, USA) in methanol and 250 µl of concentrated hydrochloric acid HCl 37%. Following a 15-minute incubation at room temperature, the absorbance of the reaction mixture was measured at 500 nm using a microplate reader (BioTek Epoch, Agilent Technologies, USA). The proanthocyanidin content was quantified by comparison with a catechin calibration curve (Sigma-Aldrich, USA), with results expressed as milligrams of catechin equivalents per gram of dry weight (mg CE/g DW).

*Determination of total carotenoid content.* Carotenoids were extracted using a hexane : acetone solvent system (7:3 by volume) [27]. 1 g of plant powder sample was homogenised and subjected to ultrasonic treatment (Elma, Germany) at 40 °C for 20 minutes. After centrifugation at 5000 rpm (C0650 rotor, Beckman Coulter, USA) for 5 minutes, the supernatant was collected and the absorbance was measured at 450 nm using a BioTek Epoch microplate reader (Agilent, USA). The total carotenoid content was expressed in mg of β-carotene per g of sample.

*Moisture content determination.* The moisture content of the dried plant samples was determined gravimetrically by drying 1 g of powdered material in an oven at 105 °C to constant weight. The moisture content was calculated as the percentage of weight loss relative to the initial sample mass.

*Determination of protein and ash content.* The total protein content was quantified using the Bradford method [28] with bovine serum albumin (BSA) as a standard. For protein extraction, plant tissues were first homogenized in phosphate buffer (0.1 M, pH 7.0) and centrifuged at 12,000 × g for 15 min at 4°C; the supernatant was used for protein content determination. The ash content was determined gravimetrically by incinerating 1 g of dry, powdered sample in a muffle furnace at 550°C for 4–6 hours until a constant weight was obtained.

#### *Antioxidant activity analysis*

*CUPRAC assay.* The cupric ion reducing antioxidant capacity (CUPRAC) assay was performed by mixing 10 µL of plant extract with 90 µL of CU-

PRAC reagent (neocuproine, copper(II) chloride, and ammonium acetate; Sigma-Aldrich, USA) [29]. After incubation in the dark (30 min), the absorbance was read at 450 nm using a BioTek Epoch microplate reader (Agilent Technologies, USA). Antioxidant activity was expressed as mg of Trolox equivalents (TE) per g of dry sample.

**DPPH assay.** The free radical scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method adapted from [30]. A 0.1 mM DPPH (Sigma-Aldrich, USA) solution in methanol was prepared. Then, 100  $\mu$ l of the methanolic extract was mixed with 100  $\mu$ l of the DPPH solution and incubated for 30 minutes in the dark at room temperature. The absorbance was recorded at 510 nm using a BioTek Epoch microplate reader (Agilent, USA), and the results were expressed in milligrams of Trolox equivalents (TE) per gram of dry sample.

#### *LC-MS/MS analysis of polyphenols*

**Extraction of polyphenols.** Polyphenols were extracted by homogenising 100 mg of plant material in 1 ml of methanol : water : formic acid (80:19:1 by volume). The mixture was subjected to ultrasonic treatment Transsonic T570 ultrasonic bath (Elma, Germany) and centrifuged Beckman Coulter Allegra X-30R centrifuge (Beckman Coulter, USA) at 13,500 rpm for 5 minutes at 4 °C. The supernatant liquids were collected for LC-MS/MS analysis.

**LC-MS/MS conditions.** Polyphenols were identified and quantified using a Thermo TSQ Quantis LC-MS/MS system with an electrospray ionisation (ESI) source (Thermo Fisher Scientific, USA). Analyses were conducted on a Phenomenex C18 column (150  $\times$  2.1 mm, 2.6  $\mu$ m; 40 °C). The mobile phase, a mixture of 0.1% formic acid in water (A) and acetonitrile (B), was eluted under a gradient program (5–95% B in 20 min) at 0.3 mL/min. The injection volume was set to 5  $\mu$ L. Compound quantification relied on external calibration curves generated from high-purity ( $\geq$ 95%) reference standards (Sigma-Aldrich, USA).

**Extraction and LC-MS/MS analysis of free amino acids.** For the extraction of free amino acids, a methanol : water : formic acid mixture (80:20:1, v/v/v) was employed following an adapted protocol [31]. In brief, 100 mg of powdered plant material was homogenized with 1 mL of this solvent. The homogenate was subsequently centrifuged at 14,000 rpm for 30 minutes at 4 °C in a Beckman Coulter Allegra X-30R centrifuge (Beckman Coulter, USA). The resulting supernatant was carefully collected, passed through a 0.22  $\mu$ m PTFE membrane filter, and prepared for chromatographic analysis.

The quantitative profiling of amino acids was conducted using the same Thermo Scientific TSQ Quantis LC-MS/MS instrument applied for polyphenols, albeit with a modified chromatographic method optimized for amino acid separation. Definitive identification and quantification were accomplished by matching the retention times and specific mass-to-charge (m/z) transitions of the analytes to those of pure amino acid standards (Sigma-Aldrich, USA). A series of external calibration curves, spanning a concentration range from 0.1 to 100  $\mu$ g/mL, were established for each target compound to enable precise quantification.

**Determination of soluble sugars.** The concentrations of glucose, fructose, and sucrose were quantified using high-performance liquid chromatography with refractive index detection (HPLC-RID) [32]. Briefly, 100 mg of dry plant powder was extracted with 5 ml of 80% (v/v) aqueous ethanol at 80°C for 30 minutes. The extract was centrifuged at 10,000 rpm for 10 minutes, filtered through a 0.22  $\mu$ m PTFE syringe filter, and analyzed. Chromatographic separation was performed on a Rezex™ ROA-Organic Acid H+ (8%) column (300  $\times$  7.8 mm; Phenomenex, USA) maintained at 60 °C. An isocratic mobile phase of 5 mM sulfuric acid was used at a flow rate of 0.6 mL/min. Detection was carried out using a Refractive Index Detector (RID) (model, e.g., Waters 2414 or equivalent). Quantification was achieved by comparison with external calibration curves constructed from authentic standards of glucose, fructose, and sucrose (Sigma-Aldrich, USA).

**Statistical analysis.** All measurements were conducted in three independent replicates (n = 3). The data are expressed as the mean  $\pm$  standard deviation (SD). To assess the significance of differences between the studied groups, a one-way analysis of variance (ANOVA) was applied, followed by Tukey's honest significant difference (HSD) post hoc test for pairwise comparisons. A p-value of less than 0.05 was considered statistically significant. All statistical computations were performed using GraphPad Prism software (version 9).

## **Results and discussion**

### *Biochemical composition and population variability*

The high content of polyphenols in various populations of *C. leucocladum* is associated with the plant's adaptation mechanisms to changing climatic conditions. The P3 population, collected in a semi-arid high-altitude region (about 800 m above sea level),

showed the greatest accumulation of polyphenols, probably due to the combined effects of environmental factors such as increased ultraviolet radiation, lower temperatures and moderate drought. In such conditions, polyphenols act as antioxidants, protecting cells from oxidative damage. The P1 population located in an arid lowland area (about 300 m above sea level) demonstrated moderate levels of polyphenols, which may reflect adaptation to chronic heat and aridity. The lowest accumulation of polyphenols was observed in the P2 population, which taken in a more humid environment at an average altitude ( $\approx 500$  m above sea level), which indicates a decrease in the synthesis of secondary metabolites with a decrease in abiotic stress. Thus, the metabolic profile of *C. leucocladum* is formed as a phenotypic reaction to environmental conditions, while populations from more stressful habitats show distinct phytochemical signs.

This pattern is further confirmed by the analysis of floral tissues, in which the concentrations of the main biologically active compounds varied significantly depending on the population (Table 1). Phenolic compounds, which play a key role in plant protection and can be potentially beneficial to human health, reached the highest levels in flowers of P3 ( $2.13 \pm 0.14$  mg GAE/g), which indicates a strong antioxidant potential. Flowers P1 and P2 contained lower levels of phenol ( $1.31 \pm 0.03$  and  $1.34 \pm 0.03$  mg GE/g, respectively), although these concentrations can still provide biological activity. Flavonoids, which also contribute

to antioxidant protection, are most abundant in P3 ( $40.24 \pm 1.23$  mg QE/g), which indicates an increased ability of this population to neutralize free radicals. The flavonoid content in P1 and P2 was about half that in P3. Analyses of antioxidant activity (CUPRAC and DPPH) confirmed these data, while P3 extracts demonstrated the greatest ability to remove radicals. Proanthocyanidins, known for their antioxidant and anti-inflammatory properties, prevailed in P3 ( $24.32 \pm 0.65$  mg CE/g), which was twice the levels in other samples. Carotenoids, important for plant photoprotection and used in the cosmetics and food industries, reached their maximum concentration in P2 ( $3909.11 \pm 298.50$  micrograms of  $\beta$ -carotene/g), while P1 and P3 contained significantly lower amounts.

The interpopulation differences were also evident in protein accumulation: in P1 ( $0.71 \pm 0.06\%$ ), the protein content was significantly higher than in P2 ( $0.46 \pm 0.02\%$ ). This variability may reflect differences in nitrogen metabolism or biosynthetic activity. On the contrary, the ash content in different populations varied minimally, stabilizing at about 3.6%, which indicates the constant assimilation and precipitation of minerals regardless of local growing conditions.

Each studied population has different biochemical characteristics: P3 has a high content of antioxidants, P2 has a high content of carotenoids, and P1 has a high protein content. These characteristics point to various potential applications, from pharmacology to the food and cosmetics industry.

**Table 1** – Biochemical composition of *C. leucocladum* flowers from three populations grown under different environmental conditions

Populations	Total Phenols, mg GAE/g	Total Flavonoids, mg QUE/g	CUPRAC, mg TE/g	DDPPH, mg TE/g	Proanthocyanidin, mg CE/g	Total Carotenoids, mg $\beta$ -carotene/g	Total Protein, %	Ash, %
P1 flower	$1.31 \pm 0.03$	$22.47 \pm 2.34$	$4.03 \pm 0.14$	$7.49 \pm 0.34$	$12.69 \pm 0.36$	$2.71 \pm 0.11$	$0.71 \pm 0.06$	$3.59 \pm 0.27$
P2 flower	$1.34 \pm 0.03$	$23.96 \pm 1.10$	$3.66 \pm 0.06$	$6.42 \pm 0.55$	$10.61 \pm 0.35$	$3.91 \pm 0.30$	$0.46 \pm 0.02$	$3.69 \pm 0.35$
P3 flower	$2.13 \pm 0.14$	$40.24 \pm 1.23$	$5.70 \pm 0.36$	$7.02 \pm 0.43$	$24.32 \pm 0.65$	$2.07 \pm 0.11$	$0.64 \pm 0.01$	$3.59 \pm 0.36$

Note: P1: arid region, slightly alkaline soils; P2: moderately humid region, neutral soils; P3: semi-arid highland region, slightly acidic soils. Values are presented as mean  $\pm$  standard deviation (n = 3) on a dry weight basis

Analysis of leaf tissues revealed significant differences in the content of protective phytochemicals, which may also be beneficial to human health (Table 2). Phenolic compounds known for their antioxidant properties were present in the highest concentration in the leaves of Population

P3 ( $3.60 \pm 0.14$  mg GAE/g), which indicates a high protective ability. The levels of P2 ( $3.01 \pm 0.20$  mg GAE/g) and P1 ( $2.77 \pm 0.10$  mg GAE/g) were moderately lower, but remained significant. Flavonoids, which support plant resistance to stress and exhibit bioactive potential,

showed a similar trend: the largest amount accumulated in P3 ( $59.48 \pm 3.32$  mg units/g), followed by P2 ( $44.63 \pm 3.89$  mg units/g) and P1 ( $38.77 \pm 0.95$  mg units/g).

Analyses of antioxidant activity confirmed these observations. CUPRAC values were highest in leaves of P3 ( $10.49 \pm 0.26$  mg TE/g), exceeding the values of P2 ( $8.76 \pm 0.33$  mg TE/g) and P1 ( $7.86 \pm 0.29$  mg TE/g). Similarly, DPPH radical scavenging activity was most pronounced in P3 ( $12.54 \pm 1.03$  mg TE/g), while P1 ( $10.42 \pm 0.90$  mg TE/g) and P2 ( $9.73 \pm 0.46$  mg TE/g) showed lower but significant activity. Proanthocyanidins, which are valued for their antioxidant and anti-inflammatory properties, were also most abundant in P3 ( $48.53 \pm 1.07$  mg CE/g), while P2 ( $35.95 \pm 2.37$  mg CE/g) and P1 ( $27.44 \pm 0.45$  mg CE/g) were found in lower amounts.

On the contrary, carotenoids, the key compounds for photoprotection, reached their maximum concentration in the leaves of P1 ( $22\,178.24 \pm 963.96$  micrograms of  $\beta$ -carotene/g). The beta-carotene content in leaves P2 and P3 was relatively low ( $18,269.13 \pm 298.50$  and  $16,753.35 \pm 390.83$  micrograms/g, respectively), although it still remained significant. The protein content, indicating metabolic activity, was highest in the P1 group ( $1.43 \pm 0.11\%$ ), followed by P3 ( $0.95 \pm 0.07\%$ ) and P2 ( $0.71 \pm 0.07\%$ ). Conversely, the ash content reflecting the mineral fraction remained relatively the same in different populations, ranging from  $10.24 \pm 0.72\%$  (P3) to  $10.92 \pm 0.96\%$  (P2), indicating stable absorption of minerals regardless of differences in habitat.

Collectively, the metabolic profiles of the leaves indicate different functional capabilities: P3 is characterized by pronounced antioxidant and anti-inflammatory properties, P1 is characterized by an in-

creased content of carotenoids and protein, and P2 is characterized by intermediate but noticeable levels of several classes of biologically active substances. These differences in composition highlight the variety of applications of *C. leucocladum* leaf extracts in pharmaceutical, nutraceutical, and cosmetic products.

A comparative analysis of metabolites in the tissues of flowers and leaves revealed pronounced organ-specific accumulation patterns. In all populations, the leaves consistently contained significantly higher concentrations of the main antioxidant compounds. For example, in a population with high stress levels of P3, the total phenol content in leaves was about 70% higher ( $3.60$  mg GAE/g) compared to flowers ( $2.13$  mg GAE/g). Similar differences were observed in the total amount of flavonoids and proanthocyanidins, which confirms the role of leaves as the main site of biosynthesis of these protective secondary metabolites. Accordingly, the *in vitro* antioxidant capacity, measured by CUPRAC and DPPH assays, was significantly higher in extracts obtained from the leaves.

In contrast, the tissues of the flowers show a distinct metabolic effect, expressed in selective enrichment with certain specialized flavonoids. For example, the concentration of hyperoside in P3 flowers exceeds the concentration in leaves by more than ten times. This differential separation implies a potential ecological function – such as attracting pollinators or protecting gametophytes – in reproductive structures. Taking together, these results highlight the crucial influence of tissue type on phytochemical composition, which directly influences the targeted collection of plant raw materials in various fields of application, from pharmacology to the development of functional ingredients.

**Table 2** – Biochemical analysis of *C. leucocladum* leaves from three populations grown under different environmental conditions

Populations	Total Phenols, mg GAE/g	Total Flavonoids, mg QUE/g	CUPRAC, mg TE/g	DDPPH, mg TE/g	Proanthocyanidin, mg CE/g	Total Carotenoids, mg $\beta$ -carotene/g	Total Protein, %	Ash, %
P1 leaf	$2.77 \pm 0.10$	$38.77 \pm 0.95$	$7.86 \pm 0.29$	$10.42 \pm 0.90$	$27.44 \pm 0.45$	$22.17 \pm 0.96$	$1.43 \pm 0.11$	$10.41 \pm 0.38$
P2 leaf	$3.01 \pm 0.20$	$44.63 \pm 3.89$	$8.76 \pm 0.33$	$9.73 \pm 0.46$	$35.95 \pm 2.37$	$18.27 \pm 0.30$	$0.71 \pm 0.07$	$10.92 \pm 0.96$
P3 leaf	$3.60 \pm 0.14$	$59.48 \pm 3.32$	$10.49 \pm 0.26$	$12.54 \pm 1.03$	$48.53 \pm 1.07$	$16.75 \pm 0.39$	$0.95 \pm 0.07$	$10.24 \pm 0.72$

Note: P1: arid region, slightly alkaline soils; P2: moderately humid region, neutral soils; P3: semi-arid highland region, slightly acidic soils. Values are presented as mean  $\pm$  standard deviation (n = 3) on a dry weight basis

### Identification of phenolic compounds

A total of 23 phenolic compounds representing a wide range of biologically active components with documented pharmacological activity were identified in the analyzed samples of *C. leuocladum*. These include antioxidants such as epicatechin [33], apigenin [34], and quercetin [35], as well as compounds with pronounced anti-inflammatory (for example, gallic acid [36], hyperoside [37]), cardioprotective (chlorogenic acid [38]), and potential antitumor properties (chrysin [39]). Of particular importance was the discovery of artepillin C, a rare compound with anti-inflammatory and antimicrobial effects [40].

Significant qualitative and quantitative differences in the content of polyphenols were observed both in plant organs and in populations (Table 3). A constant trend was a noticeably higher accumulation of most phenolic compounds in leaf tissues compared to parts of the flower, which makes the foliage the main site of biosynthesis of these pharmacologically significant metabolites.

As a result of the analysis, clear signs characteristic of a particular population were identified. The P3 population demonstrated the highest concentrations of epicatechin, especially in flowers ( $2322.67 \pm$

$170.68$  mg/kg) and leaves ( $1874.59 \pm 137.75$  mg/kg), indicating its potential as a rich source of this powerful antioxidant. On the contrary, the leaves of P2 were characterized by an increased content of chlorogenic acid ( $5.56 \pm 0.33$  mg/kg), which indicates differences in the metabolism of phenolic acids. The P1 population was characterized by a pronounced accumulation of anti-inflammatory compounds with particularly high concentrations of gallic acid ( $39.19 \pm 1.41$  mg/kg) and chrysin ( $82.20 \pm 5.57$  mg/kg). Of particular phytochemical interest was the detection of hesperidin exclusively in P1 leaves ( $12.88 \pm 0.40$  mg/kg) and trace amounts of artepillin C exclusively in P2 leaves ( $0.04 \pm 0.0004$  mg/kg), indicating unique biosynthetic capabilities or strictly regulated metabolic pathways characteristic of these populations.

The results confirm that each *C. leuocladum* population has special phenolic properties: P3 is characterized by high antioxidant activity, P1 by anti-inflammatory action, and P2 by a more balanced distribution of key metabolites. These chemical characteristics specific to a particular population highlight the significant potential of this species as a source of naturally occurring bioactive substances for pharmaceutical and biotechnological applications.

**Table 3** – Phenolic Compounds in flowers and leaves of *C. leuocladum* from three populations (mg/kg, on dry base)

№	Phenolic compound	Formula	Population 1		Population 2		Population 3	
			flower	leaf	flower	leaf	flower	leaf
1	(-)-Epicatechin	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	1099.72 ± 80.81	1697.06 ± 124.71	861.36 ± 63.30	2021.26 ± 148.53	2322.67 ± 170.68	1874.59 ± 137.75
2	Apigenin	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	3.44 ± 0.18	4.19 ± 0.22	3.23 ± 0.17	3.17 ± 0.17	3.85 ± 0.20	3.58 ± 0.19
3	Artepillin C	C <sub>19</sub> H <sub>24</sub> O <sub>3</sub>	nd	0.07 ± 0.001	0.04 ± 0.001	nd	0.04 ± 0.001	nd
4	Caffeic acid phenethyl ester	C <sub>17</sub> H <sub>16</sub> O <sub>4</sub>	0.25 ± 0.002	0.31 ± 0.003	0.25 ± 0.002	0.26 ± 0.002	0.25 ± 0.002	0.27 ± 0.002
5	Catechol	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	0.06 ± 0.001	0.02 ± 0.002	0.13 ± 0.001	0.09 ± 0.001	0.10 ± 0.001	0.12 ± 0.001
6	Chlorogenic acid	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	1.98 ± 0.12	2.01 ± 0.12	2.46 ± 0.14	5.56 ± 0.33	1.48 ± 0.09	1.36 ± 0.08
7	Chrysin	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	67.65 ± 4.58	82.20 ± 5.57	62.25 ± 4.22	68.57 ± 4.65	69.10 ± 4.68	63.63 ± 4.31
8	Galangin	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	57.90 ± 2.65	71.56 ± 3.27	57.75 ± 2.64	65.29 ± 2.99	58.35 ± 2.67	59.64 ± 2.73
9	Gallic acid	C <sub>7</sub> H <sub>6</sub> O <sub>5</sub>	16.41 ± 0.59	39.19 ± 1.41	21.03 ± 0.76	12.84 ± 0.46	23.32 ± 0.84	14.99 ± 0.54
10	Hesperidin	C <sub>28</sub> H <sub>34</sub> O <sub>15</sub>	12.88 ± 0.40	nd	nd	nd	nd	nd
11	Hyperosid	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	883.26 ± 56.97	125.64 ± 8.10	562.24 ± 36.27	98.68 ± 6.37	1055.85 ± 68.11	90.54 ± 5.84
12	Kaempferol	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	6.89 ± 0.36	13.62 ± 0.71	7.82 ± 0.41	12.39 ± 0.65	5.33 ± 0.28	8.80 ± 0.46
13	Luteolin	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	1.05 ± 0.02	3.91 ± 0.07	1.45 ± 0.02	2.11 ± 0.04	0.98 ± 0.02	1.32 ± 0.02
14	Myricetin	C <sub>15</sub> H <sub>10</sub> O <sub>8</sub>	0.41 ± 0.01	1.18 ± 0.03	0.34 ± 0.01	0.73 ± 0.02	0.71 ± 0.02	0.82 ± 0.02
15	Naringenin	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	2.98 ± 0.21	3.50 ± 0.24	3.04 ± 0.21	3.15 ± 0.22	3.55 ± 0.25	3.18 ± 0.22

Continuation of the table

№	Phenolic compound	Formula	Population 1		Population 2		Population 3	
			flower	leaf	flower	leaf	flower	leaf
16	Neochlorogenic acid	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	2.10 ± 0.17	1.84 ± 0.15	2.33 ± 0.18	5.15 ± 0.41	1.65 ± 0.13	1.22 ± 0.10
17	Protocatechuic acid	C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>	4.78 ± 0.22	4.43 ± 0.20	3.60 ± 0.16	3.68 ± 0.17	4.54 ± 0.21	3.78 ± 0.17
18	Pinocembrin	C <sub>15</sub> H <sub>12</sub> O <sub>4</sub>	47.50 ± 2.60	54.74 ± 2.99	51.57 ± 2.82	47.73 ± 2.61	48.14 ± 2.63	52.80 ± 2.89
19	Quercitrin	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	51.06 ± 1.13	1.66 ± 0.04	0.10 ± 0.002	0.09 ± 0.002	0.10 ± 0.002	0.14 ± 0.003
20	Rutin	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	14.93 ± 0.23	2.70 ± 0.04	5.31 ± 0.08	0.72 ± 0.01	5.04 ± 0.08	0.87 ± 0.01
21	t-Cinnamic acid	C <sub>9</sub> H <sub>8</sub> O <sub>2</sub>	1.23 ± 0.06	2.58 ± 0.12	1.04 ± 0.05	2.08 ± 0.10	3.68 ± 0.18	1.83 ± 0.09
22	t-Caffeic acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	nd	nd	43.46 ± 2.56	nd	nd	nd
23	t-Ferulic acid	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	2.40 ± 0.09	12.58 ± 0.48	2.08 ± 0.08	5.68 ± 0.22	3.18 ± 0.12	10.18 ± 0.39

Note: nd – not detected.

P1: arid region, slightly alkaline soils; P2: moderately humid region, neutral soils; P3: semi-arid highland region, slightly acidic soils. Values are presented as mean ± standard deviation (n = 3) on a dry weight basis

The novelty of the study results is highlighted by the identification of several specialized metabolites that had not previously been reported in the genera *Calligonum*. The key discovery was the discovery of artepillin C, a compound not previously described in *C. leucocladum* or its relatives. Artepillin C, the main biologically active component of Brazilian propolis, is known for its powerful anti-inflammatory, antitumor, and antioxidant properties [41]. His presence in S. The results, confirmed by mass spectrometry and chromatographic analysis, indicate the existence of unexplored biosynthetic pathways in this species, which opens up new opportunities for studying the chemical adaptation of plants. This discovery positions *C. leucocladum* as a potential source of artepillin C for the development of pharmaceutical drugs, especially in oncology and for anti-inflammatory therapy [42].

Equally notable was the discovery of caffeic acid phenethyl ether (CAPE), a compound commonly associated with bee products (such as propolis) and rarely found in higher plants. CAPE exhibits strong antioxidant and anti-inflammatory activity, mitigating oxidative stress at the cellular level [43]. Its presence in *C. leucocladum* means that this species can serve as an alternative source of this valuable plant-based metabolite, which contributes to research into its biosynthesis and sustainable production for therapeutic purposes.

In addition, catechol was first discovered in *C. leucocladum*. This phenolic compound plays

a crucial role in the biosynthesis of lignin and tannin and helps protect plants from abiotic stressors such as drought and ultraviolet radiation, as well as from pathogens [44]. Its accumulation may reflect an adaptive response to extreme environmental conditions of the species. Catechol is promising as an object of fundamental research in the field of plant stress physiology, as well as a leading compound for the development of antioxidant agents and biochemical markers.

In addition to these new findings, the exceptionally high concentrations of hyperoside and rutin observed, in particular, in the P3 population, deserve special attention. Both flavonoids are well-established antioxidants with proven cardioprotective, neuroprotective, and anti-inflammatory activities [45]. Their significant accumulation once again highlights the pharmacological potential of *C. leucocladum*, especially for use in vascular integrity disorders and chronic inflammatory diseases.

Another notable discovery was the identification of transferulic acid, a compound not previously described in the scientific literature for this species. It has powerful antioxidant activity, contributing to the protection of plant cells from oxidative stress. This biological activity indicates its potential use in dermatological and pharmaceutical preparations designed to counteract photoaging and mitigate skin damage caused by ultraviolet radiation. The study of its biosynthetic pathway, metabolic function, and therapeutic potential is justified, since transferulic acid can serve as a valuable component in new anti-

oxidant and anti-inflammatory drugs [46].

#### *Amino acid profile and adaptive metabolic responses*

The analysis extended to the profile of free amino acids, determining the number of 36 compounds in the tissues of flowers and leaves of three populations (Table 4). Amino acids play an important role not only as fundamental building blocks for proteins necessary for human health – they play an important role in collagen synthesis (proline, hydroxyproline), neurotransmission (tryptophan as a precursor to serotonin), and tissue repair [47] -but also play a central role in plant physiology and stress adaptation.

A clear pattern specific to the organ was revealed: leaves constantly have a higher concentration of most amino acids compared to flowers. This is consistent with the role of leaves as the main site of photosynthesis and the associated intensive metabolism. It is noteworthy that proline, a key osmoprotector, accumulates in exceptionally high concentrations in P3 leaves ( $21,128.47 \pm 1,116.16 \mu\text{mol/g DW}$ ), which underlines the importance of the osmotic regulation mechanism in response to drought or salinity stress.

The P3 population demonstrated the most pronounced accumulation of amino acids in general, which indicates increased biosynthetic activity as an adaptive strategy to its more extreme semi-arid high-altitude habitat conditions. This is evidenced by the maximum levels of serine (involved in the synthesis of proteins and metabolites) and taurine (a

membrane stabilizer and antioxidant). A significant accumulation of conditionally essential stress-sensitive amino acids was especially noticeable: gamma-aminobutyric acid (GABA) in P3 leaves ( $4358.80 \pm 181.51 \mu\text{mol/g DW}$ ) emphasizes its role in ionic homeostasis and signaling, while the increased content of beta-alanine and taurine indicates enhanced antioxidant protection.

Essential amino acids (leucine, isoleucine, threonine, valine) also reached peak concentrations in P3 leaves, supporting the need for stress-related protein synthesis. In addition, a noticeable accumulation of less common metabolites, such as beta-aminobutyric acid ( $2399.01 \pm 154.74 \mu\text{mol/g DW}$  in P3 leaves), indicates special protective functions. In contrast, amino acids that play a key metabolic role, such as glutamic acid and methionine, maintained stable concentrations in all populations, reflecting their main functions in the household.

Populations P1 and P2 exhibit a more moderate amino acid profile, which corresponds to growth under conditions of less severe environmental stress. The pronounced amino acid composition of P3, especially in leaves, demonstrates coordinated metabolic reprogramming towards the biosynthesis of protective compounds. These data not only reveal the biochemical basis of *C. leucocladum* adaptation, but also indicate the potential of P3 as a source of osmoprotectors and stress-related metabolites for the development of biostimulants that increase crop resistance.

**Table 4** – Free amino acid composition in flowers and leaves of three *C. leucocladum* populations with functional classification ( $\mu\text{mol/g}$ , on dry base)

№	Compound	Molecular weight	Population 1		Population 2		Population 3	
			flower	leaf	flower	leaf	flower	leaf
1	1-Methylhistidine	169.18	$43.75 \pm 1.18$	$53.66 \pm 1.45$	$46.69 \pm 1.26$	$60.27 \pm 1.62$	$33.62 \pm 0.91$	$76.07 \pm 2.05$
2	3-Methylhistidine	169.18	nd	$3.27 \pm 0.03$	$3.61 \pm 0.03$	$1.66 \pm 0.01$	nd	$0.73 \pm 0.01$
3	alpha-Aminoadipic acid	161.16	$114.5 \pm 6.0$	$193.2 \pm 10.1$	$80.78 \pm 4.22$	$293.7 \pm 154$	$96.97 \pm 5.07$	$379.9 \pm 19.9$
4	alpha-Aminobutyric acid	103.12	nd	$73.11 \pm 3.04$	nd	$42.96 \pm 1.79$	$53.70 \pm 2.24$	nd
5	Anserine	240.26	$0.59 \pm 0.01$	$1.25 \pm 0.01$	$0.52 \pm 0.01$	$0.38 \pm 0.01$	nd	$0.51 \pm 0.01$
6	Arginine	174.20	nd	$3.99 \pm 0.07$	$0.87 \pm 0.02$	$3.25 \pm 0.06$	$0.73 \pm 0.01$	$3.73 \pm 0.07$
7	Asparagine	132.12	$4,400 \pm 313$	$2,178 \pm 155$	$3,700 \pm 263$	$2,811 \pm 200$	$922.8 \pm 65.6$	$2,623 \pm 186$
8	Aspartic Acid	133.10	$1,209 \pm 42$	$872.4 \pm 30.6$	$1,403 \pm 49$	$999.3 \pm 35.1$	$1069.4 \pm 37.6$	$1067.9 \pm 37.5$
9	beta-Alanine	89.09	$232.9 \pm 16.4$	$217.2 \pm 15.3$	$119.0 \pm 8.4$	$614.0 \pm 43.1$	$96.06 \pm 6.74$	$744.3 \pm 52.3$

Continuation of the table

№	Compound	Molecular weight	Population 1		Population 2		Population 3	
			flower	leaf	flower	leaf	flower	leaf
10	beta-Aminoisobutyric acid	103.12	827.4 ± 53.4	18,787 ± 121	383.8 ± 24.8	2,285 ± 147	545.6 ± 35.2	2,399 ± 155
11	Carnosine	226.23	1.04 ± 0.01	1.09 ± 0.01	0.79 ± 0.01	3.10 ± 0.04	0.61 ± 0.01	1.64 ± 0.02
12	Cystathionine	222.26	0.55 ± 0.00	0.36 ± 0.00	0.06 ± 0.00	0.12 ± 0.00	0.08 ± 0.00	0.56 ± 0.00
13	Cystine	240.30	nd	nd	nd	8.27 ± 0.22	8.78 ± 0.23	nd
14	Citrulline	175.19	10.70 ± 0.40	36.59 ± 1.37	4.49 ± 0.17	84.86 ± 3.19	10.51 ± 0.39	45.58 ± 1.71
15	Ethanolamine	61.08	1,043 ± 78	1,441 ± 108	1,044 ± 78	2,033 ± 153	698.3 ± 52.5	2,340 ± 176
16	gamma-Aminobutyric acid	103.12	1,590 ± 66	3,460 ± 144	721.0 ± 30.0	4,109 ± 171	1,052 ± 44	4,359 ± 182
17	Glutamic Acid	147.13	782.3 ± 48.3	1,791 ± 111	1,525 ± 94	1,561 ± 96	1,068 ± 66	1,625 ± 100
18	Homocystine	268.40	37.17 ± 0.82	137.81 ± 3.04	23.40 ± 0.52	99.00 ± 2.18	41.39 ± 0.91	112.41 ± 2.48
19	Hydroxylysine	162.19	1,972 ± 136	1,972 ± 136	nd	1,971 ± 136	1,971 ± 136	nd
20	Hydroxyproline	131.13	128.4 ± 7.7	239.1 ± 14.4	135.2 ± 8.1	459.5 ± 27.7	198.3 ± 12.0	542.8 ± 32.7
21	Histidine	155.15	nd	11.07 ± 0.16	5.36 ± 0.08	10.35 ± 0.15	9.33 ± 0.14	10.11 ± 0.15
22	Isoleucine	131.17	173.8 ± 8.7	345.3 ± 17.2	192.2 ± 9.6	587.5 ± 29.3	280.1 ± 14.0	740.0 ± 36.9
23	Leucine	131.17	128.5 ± 9.2	232.7 ± 16.7	137.0 ± 9.8	437.6 ± 31.4	185.2 ± 13.3	546.0 ± 39.2
24	Lysine	146.19	1,668 ± 78	6,186 ± 288	907.2 ± 42.2	6,862 ± 319	1,400 ± 65	8,184 ± 381
25	Methionine	149.21	1,542 ± 50	1,737 ± 56	1,548 ± 50	1,677 ± 54	1,537 ± 50	1,774 ± 57
26	Ornithine	132.16	1,557 ± 107	1,564 ± 107	1,556 ± 107	1,562 ± 107	1,563 ± 107	1,563 ± 107
27	Phenylalanine	165.19	1,860 ± 27	2,415 ± 36	1,822 ± 27	2,544 ± 37	1,747 ± 26	2,005 ± 29
28	Phosphoethanolamine	141.06	14.00 ± 0.18	4.10 ± 0.05	3.06 ± 0.04	4.31 ± 0.06	13.38 ± 0.17	3.83 ± 0.05
29	Phosphoserine	185.07	2.33 ± 0.02	5.42 ± 0.05	5.45 ± 0.06	2.76 ± 0.03	nd	8.74 ± 0.09
30	Proline	115.13	1,348 ± 71	13,136 ± 693	1,126 ± 59	12,819 ± 677	1,146 ± 61	21,128 ± 116
31	Serine	105.09	1,335 ± 81	6,591 ± 402	1,557 ± 95	8,331 ± 508	1,170 ± 71	8,634 ± 526
32	Taurine	125.15	4,635 ± 370	8,127 ± 649	5,247 ± 419	7,042 ± 562	2,532 ± 202	10,683 ± 853
33	Threonine	119.12	949.0 ± 66.8	2,208 ± 155	1,375 ± 97	2,776 ± 195	855.2 ± 60.2	2809 ± 197
34	Tryptophan	204.22	530.1 ± 16.2	2,431 ± 74	552.9 ± 16.9	1,773 ± 54	230.5 ± 7.1	1,715 ± 53
35	Tyrosine	181.19	661.1 ± 15.3	1,396 ± 32	487.8 ± 11.3	1,444 ± 33	929.6 ± 21.6	1,329 ± 31
36	Valine	117.15	634.1 ± 27.5	1412.6 ± 61.4	501.1 ± 21.8	2349.5 ± 102.1	515.5 ± 22.4	3,012 ± 131

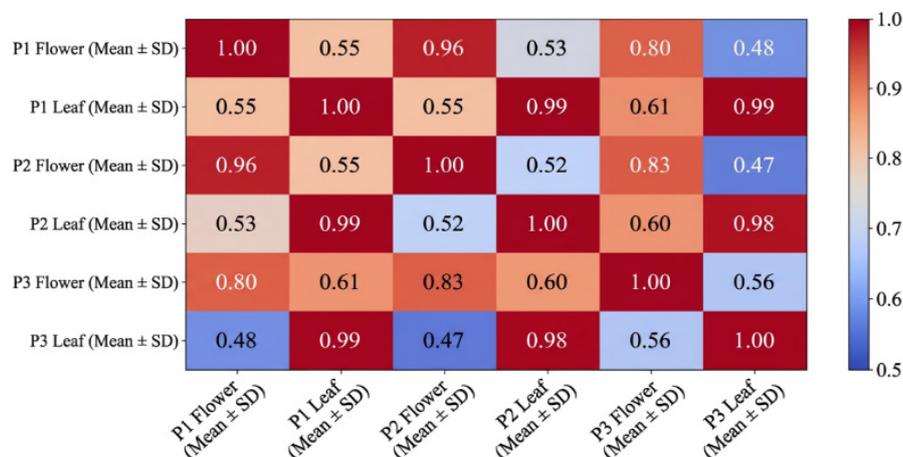
Note: nd – not detected.  
P1: arid region, slightly alkaline soils; P2: moderately humid region, neutral soils; P3: semi-arid highland region, slightly acidic soils.  
Values are presented as mean ± standard deviation (n = 3) on a dry weight basis

The correlation matrix demonstrates a strong positive relationship between amino acid concentrations in flowers and leaves within each population, indicating that the distribution of amino acids in different parts of the plant depends on general metabolic processes (Figure 3). For example, in population P1, the correla-

tion between flowers and leaves is 0.55, in population P2 it is 0.52, and in population P3 it is 0.56. Leaves from different populations show a high correlation, indicating similar amino acid synthesis processes in this part of the plant. For example, the correlation between leaves from populations P1 and P2 is 0.99, and

between P2 and P3 it is 0.98. Similarly, flowers from different populations also show high correlation: between flowers P1 and P2 it is 0.96, and between P2 and

P3 it is 0.83. This may reflect the conservative nature of the amino acid composition of flowers, which perform important reproductive functions.



**Figure 3** – Correlation matrix of mean amino acid concentrations across *C. leucocladum* populations (P1, P2, P3) and plant organs (flowers and leaves). Pearson correlation coefficients ( $r$ ): higher positive correlation values (closer to +1) are displayed in brighter colors (e.g., yellow), indicating strong similarity in amino acid composition; lower or negative values (closer to -1 or 0) are shown in darker colors (e.g., blue), indicating weak or inverse relationships. P1: arid region, slightly alkaline soils; P2: moderately humid region, neutral soils; P3: semi-arid highland region, slightly acidic soils.

The correlation between amino acid concentrations in the leaves of one population and the flowers of another is relatively lower. For example, the correlation between the leaves of population P1 and the flowers of population P3 is 0.48, and between the leaves of P2 and the flowers of P3, it is 0.47. This is probably due to differences in the functions of leaves and flowers, as well as specific metabolic processes in these organs. Population P3 shows a moderate correlation between leaves and flowers (0.56), which may be related to its adaptation to specific environmental conditions. At the same time, the leaves of this population have a high correlation with the leaves of other populations, which may indicate similarities in habitat conditions. The results emphasise the conservative nature of the amino acid composition of flowers and leaves, as well as their connection with general metabolic and ecological characteristics. The lower correlation between plant parts from different populations may be the result of adaptation to different habitat conditions. These results are important for further studies of the ecological and metabolic characteristics of *C. leucocladum* and for the development of biotechnological and pharmacological applications.

Population P3 is distinguished by high biosynthetic activity. Its leaves have the highest concentrations of amino acids such as proline, lysine and gamma-aminobutyric acid (GABA). This indicates the presence of specific adaptation mechanisms in this population that stimulate increased metabolism and the accumulation of key metabolites. Population P1 demonstrates a more balanced distribution of amino acids between leaves and flowers. At the same time, the leaves of this population show elevated levels of amino acids such as threonine and phenylalanine, indicating their importance for supporting physiological processes such as protein synthesis and photosynthesis. However, for some amino acids, such as 1-methylhistidine and carnosine, concentrations in flowers exceed those in leaves. This may indicate a special role for these compounds in protective and reproductive processes. Some amino acids, such as glutamic and aspartic acids, exhibit similar concentration levels in different populations and plant parts. This indicates their conservative role in key metabolic processes, such as protein synthesis, nitrogen metabolism, and energy metabolism.

The study of the biological functions of amino acids presented in this work allows us to draw sev-

eral important conclusions about plant metabolism and their adaptive abilities. Amino acids play an important role in numerous metabolic processes such as building blocks of proteins: they participate in the synthesis of secondary metabolites, regulate stress responses, promote nitrogen transport and accumulation, and support energy metabolism. 1-methylhistidine and 3-methylhistidine are involved in nitrogen metabolism and can serve as markers of stress and protein degradation. Their role in plants remains poorly understood, but their detection in this study highlights their potential as indicators of plant physiological status. Alpha-amino adipic acid is an intermediate product of lysine catabolism, which highlights its importance in energy metabolism. Alpha-aminobutyric acid is associated with osmoregulation and stress tolerance, for example, to drought, making it important for studying plant adaptation mechanisms. Anserine and carnosine are powerful antioxidants that protect cells from oxidative stress [48]. These compounds also act as pH buffers, helping to maintain metabolic stability under extreme conditions. Arginine and ornithine play a key role in nitrogen metabolism, serving as precursors to nitric oxide and polyamines, which regulate cell growth and stress protection. These amino acids are also involved in nitrogen recycling pathways in plants. Asparagine and aspartic acid are involved in nitrogen transport and storage. They are precursors of other amino acids and nucleotides, making them central components of plant metabolism. Ethanolamine and phosphoethanolamine are involved in the formation of phospholipids, key components of cell membranes. They play an important role in maintaining membrane integrity and allow plants to adapt to external stresses such as drought and high soil salinity. High concentrations of these compounds highlight their importance in plant responses to adverse environmental conditions. Gamma-aminobutyric acid (GABA) is found in significant amounts, especially in leaves. This compound performs important regulatory functions, including ion transport and stress adaptation. High levels of GABA in leaves may be related to its role in protecting plants from abiotic factors such as drought and soil salinity. Glutamic acid is one of the central molecules in nitrogen metabolism [49]. It serves as a precursor for the synthesis of other amino acids and is actively involved in nitrogen-related metabolic processes. Homocysteine and cystathionine play an important role in sulphur metabolism by supporting the synthesis of key compounds such as methionine and

cysteine. These amino acids are crucial for protein synthesis and the formation of antioxidant defence systems. Hydroxylysine and hydroxyproline are amino acids necessary for strengthening plant cell walls. They provide mechanical strength to tissues and help plants resist physical stress and infections. Histidine plays an important role in metal binding and antioxidant protection of cells. Its function is especially important for plants deficient in micronutrients such as iron and zinc [50].

#### *Organic acids in C. leucocladum*

The study of *C. leucocladum* composition reveals that organic acids play a significant role in the plant's central metabolism and adaptation to environmental stress. Malic acid, a key intermediate in the TCA cycle and stomatal regulation, was the dominant organic acid across all samples, with notably high concentrations in P1 tissues, indicating intense metabolic activity. Citric acid, another crucial TCA cycle component involved in metal chelation and stress response, reached its peak concentration in the leaves of population P3, suggesting its active role in adaptive mechanisms under semi-arid highland conditions. Fumaric and tartaric acids also showed elevated levels in P1 leaves, reinforcing the picture of active energy metabolism in this population from arid lowlands. In contrast, acetic, butyric, and propionic acids—often associated with secondary fermentation pathways—were present in lower and more variable concentrations, with localized accumulations (e.g., acetic acid in P2 leaves) that may reflect specific metabolic adjustments. The presence of trace amounts of 3-methylhistidine, particularly in P1 leaves, merits further investigation for its potential role in stress adaptation. Overall, the distinct profiles of organic acids (Table 5) reflect population-specific metabolic strategies, with P1 exhibiting a signature of high primary metabolic flux, while P3 accumulates specific acids like citrate likely involved in stress tolerance.

The analysis results show that malic acid is the dominant component in all plant samples (leaves and flowers). Its concentration is particularly high in sample P1, which may be linked to the plant's high metabolic activity. Malic acid plays a critical role in respiratory processes and may indicate favorable growing conditions or an active growth phase of the plants. Citric acid, the second most abundant acid, is especially prominent in the leaves of sample P3. This could be related to its role in metal detoxification or adaptation to environmental stress factors.

**Table 5** – Concentration of organic acids in flowers and leaves of three *C. leucocladum* populations ( $\mu\text{g/g}$ , on dry base)

№	Compound	Population 1		Population 2		Population 3	
		flower	leaf	flower	leaf	flower	leaf
1	Acetic acid	5.85 $\pm$ 0.98	6.54 $\pm$ 0.32	7.68 $\pm$ 0.24	18.08 $\pm$ 0.19	6.0 $\pm$ 1.05	2.78 $\pm$ 0.43
2	3-Methylhistidine	1.18 $\pm$ 0.14	19.64 $\pm$ 0.67	3.61 $\pm$ 0.40	5.70 $\pm$ 0.60	3.92 $\pm$ 0.18	2.60 $\pm$ 0.03
3	Propionic acid	1.59 $\pm$ 0.26	13.63 $\pm$ 1.36	4.18 $\pm$ 0.04	1.50 $\pm$ 0.17	2.74 $\pm$ 0.16	1.60 $\pm$ 0.01
4	Butyric acid	18.24 $\pm$ 0.40	55.99 $\pm$ 2.97	7.32 $\pm$ 0.28	44.7 $\pm$ 0.52	16.34 $\pm$ 0.14	31.76 $\pm$ 0.33
5	Lactic acid	12.53 $\pm$ 0.22	6.05 $\pm$ 0.34	1.38 $\pm$ 0.27	3.64 $\pm$ 0.01	2.51 $\pm$ 0.41	7.48 $\pm$ 0.71
6	Fumaric acid	9.90 $\pm$ 1.40	63.64 $\pm$ 6.79	7.71 $\pm$ 1.21	11.12 $\pm$ 0.97	7.40 $\pm$ 1.06	18.18 $\pm$ 1.05
7	Malic acid	356.64 $\pm$ 3.74	27.87 $\pm$ 0.96	54.39 $\pm$ 1.09	98.96 $\pm$ 0.41	72.64 $\pm$ 1.92	181.98 $\pm$ 4.45
8	Tartaric acid	76.07 $\pm$ 4.02	78.92 $\pm$ 5.65	12.84 $\pm$ 0.92	30.88 $\pm$ 3.58	26.62 $\pm$ 2.03	33.12 $\pm$ 1.84
9	Citric acid	170.25 $\pm$ 0.50	106.88 $\pm$ 14.36	38.7 $\pm$ 0.23	95.23 $\pm$ 1.20	118.48 $\pm$ 3.03	225.64 $\pm$ 4.35

Note: nd – not detected.  
P1: arid region, slightly alkaline soils; P2: moderately humid region, neutral soils; P3: semi-arid highland region, slightly acidic soils.  
Values are presented as mean  $\pm$  standard deviation (n = 3) on a dry weight basis.  
\*3-Methylhistidine is an amino acid derivative included here due to its analysis within the organic acid profile.

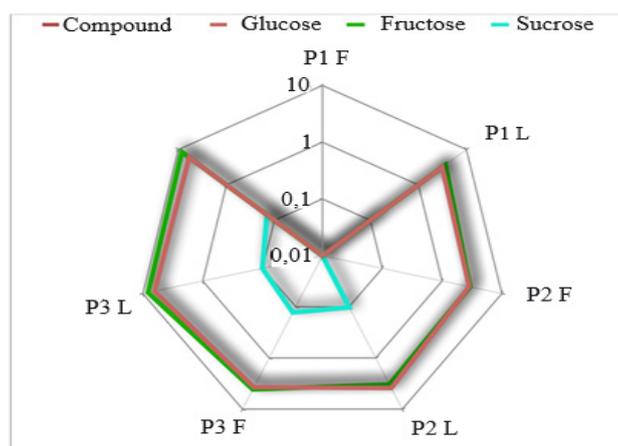
A comparison of leaves and flowers revealed that leaves contain higher concentrations of acids, which is expected given their primary function in photosynthesis and metabolism. In contrast, flowers show lower acid concentrations, as their role is more focused on reproductive processes and attracting pollinators.

Plants of population P1 stand out with the highest acid concentration, likely due to favorable environmental conditions or the physiological state of the plant. Samples P2 and P3 exhibit more uniform acid distribution, which may result from adaptations to various stresses or genetic traits. These differences in acid concentrations between samples could be attributed to environmental conditions, genetic characteristics of the plants, or growth stages. This data is valuable for further studies on plant adaptation mechanisms and their metabolic activity.

Analysis of soluble carbohydrate composition revealed distinct accumulation patterns between plant organs and among the studied populations (Figure 5). Glucose and fructose were the dominant sugars, showing nearly parallel concentration profiles and significantly higher levels in leaf tissues compared to flowers across all populations. This trend is con-

sistent with the role of leaves as the primary site of photosynthesis and carbon storage. The maximum concentrations of both hexoses were recorded in the leaves of population P3, indicating particularly active carbohydrate metabolism or storage under its semi-arid highland growth conditions. Conversely, floral tissues exhibited the lowest sugar levels, with minimal glucose and fructose content found in P1 and P2 flowers, reflecting the high metabolic demand of reproductive development.

Sucrose distribution contrasted sharply with that of the hexoses, displaying substantially lower concentrations overall. Its highest levels were detected in the leaves of populations P2 and P3, though they remained an order of magnitude lower than glucose or fructose. The notably low sucrose-to-hexose ratio, especially in flowers, suggests that sucrose may be rapidly hydrolyzed in these tissues or efficiently allocated for phloem transport rather than stored. These results underscore a fundamental metabolic division: leaves accumulate hexoses as a stable carbon reserve linked to environmental adaptation, while flowers prioritize sugar utilization for growth and development, with sucrose serving a more transient, transport-related role.



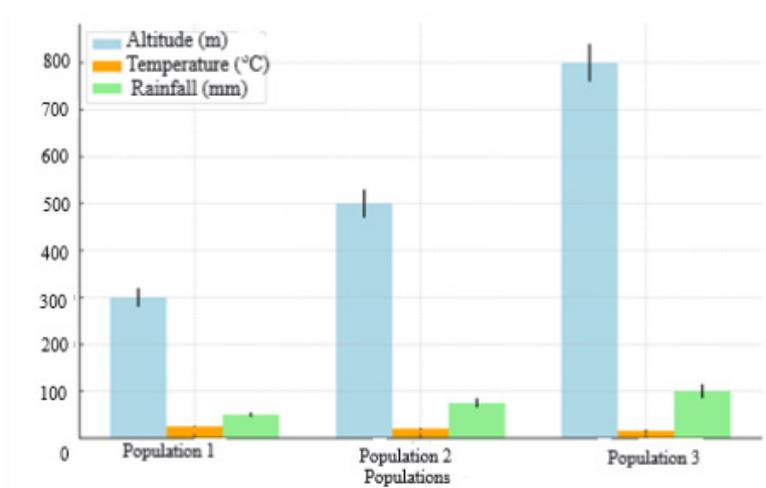
**Figure 5** – Comparative radar chart of soluble sugar concentrations (glucose, fructose, sucrose) in flowers (“F”) and leaves (“L”) of three *C. leuocladum* populations (P1: arid region, slightly alkaline soils; P2: moderately humid region, neutral soils; P3: semi-arid highland region, slightly acidic soils). Concentrations are presented on a logarithmic scale (ppm). The chart visualizes the pronounced accumulation of hexoses in leaves versus flowers and the overall low abundance of sucrose.

#### *Environmental stress and bioactive compound accumulation*

Quantitative phytochemical analysis revealed a clear gradient in the accumulation of bioactive compounds among the three *C. leuocladum* populations, which corresponds directly to the measured environmental parameters of their habitats (Figure 6).

Population 3, collected from a high-altitude semi-arid site ( $800 \pm 40$  m a.s.l.,  $15 \pm 2$  °C,  $100 \pm 15$  mm rainfall) with increased UV exposure, exhibited the most pronounced metabolic re-

sponse. It showed the highest concentrations of total phenolics ( $3.60 \pm 0.14$  mg GAE  $g^{-1}$ ), flavonoids ( $59.48 \pm 3.32$  mg QUE  $g^{-1}$ ), and proanthocyanidins, along with the strongest in vitro antioxidant activity (CUPRAC, DPPH). In contrast, population 1, originating from a warmer arid lowland ( $300 \pm 20$  m a.s.l.,  $25 \pm 1$  °C,  $50 \pm 5$  mm rainfall), displayed the lowest values for these parameters. Population 2, from a moderately humid mid-altitude region ( $500 \pm 30$  m a.s.l.,  $20 \pm 1.5$  °C,  $75 \pm 10$  mm rainfall), presented an intermediate profile.



**Figure 6** – Environmental factors by of three *C. leuocladum* populations (P1: arid region, slightly alkaline soils; P2: moderately humid region, neutral soils; P3: semi-arid highland region, slightly acidic soils)

This gradient demonstrates that the phytochemical composition of *C. leucocladum* is tightly coupled to abiotic stress intensity. The harsher conditions of the P3 habitat characterized by lower temperatures, higher solar radiation, and specific water availability act as a key driver, redirecting carbon flux toward the biosynthesis of protective secondary metabolites such as phenolics and flavonoids [51]. This stress-induced investment in chemical defense appears reduced under the more favorable growth conditions of the P1 site, where resources are likely prioritized for primary metabolism and biomass production.

Rainfall represents an additional key environmental factor influencing secondary metabolite accumulation by regulating water availability for biosynthetic processes. In the present study, P3 was collected from a site with comparatively higher annual precipitation ( $100 \pm 15$  mm), which likely ensured sufficient hydration to sustain active metabolite synthesis under concurrent abiotic stress (e.g., lower temperature, higher UV). This combination of adequate water supply and environmental stress appears critical for maximizing phytochemical production. In contrast, the limited rainfall at the P1 site ( $50 \pm 5$  mm) may constrain overall metabolic activity, contributing to its lower phenolic and flavonoid content (Tables 1, 2).

Soil properties and nutrient availability, while not directly assessed here, may further modulate the observed metabolic profiles. Future studies integrating soil chemistry data would provide a more complete understanding of the edaphic influences on *C. leucocladum* phytochemistry. Beyond immediate environmental effects, long-term genetic adaptation may also contribute to population-specific metabolic differences. Recurrent exposure to harsh conditions could select for genotypes with enhanced secondary metabolite biosynthesis, potentially explaining the robust metabolic phenotype of P3. Confirming this requires complementary genetic and transcriptomic analyses [7].

The strong environment–metabolism association has direct practical relevance. Our findings suggest that moderate, controlled abiotic stress simulating conditions of higher altitudes (cooler temperatures, increased radiation) or regulated water deficit could be employed in cultivation to enhance the yield of valuable antioxidants (phenolics, flavonoids) in *C. leucocladum*. Concurrently, these results underscore the importance of conserving natural populations and

their habitats, as environmental degradation could diminish the species' inherent biochemical potential.

The diverse biochemical profile of *C. leucocladum*, particularly in population P3, reflects an integrated adaptive strategy for arid ecosystems. Elevated levels of polyphenols, flavonoids, and proanthocyanidins mitigate oxidative stress, while specific organic acids (e.g., malic, citric acid) support energy metabolism and detoxification. The discovery of rare compounds (artepillin C, CAPE, catechol) indicates a previously unrecognized biosynthetic capacity that is likely activated under stress [52]. Furthermore, the extreme accumulation of proline and elevated levels of GABA provide direct biochemical evidence of osmotic adjustment and stress signaling, solidifying the classification of P3 as a stress-adapted phenotype [53].

## Conclusions

In summary, this study establishes that *C. leucocladum* harbors a rich and diverse phytochemical profile, with marked variation observed across different populations and plant organs. Notably, the P3 population, originating from a semi-arid highland habitat ( $\approx 800$  m a.s.l.), exhibited the most pronounced accumulation of bioactive compounds. This finding supports the hypothesis that abiotic stress acts as a key driver, stimulating the biosynthesis of antioxidant and protective metabolites as an adaptive response. The identification of rare specialized metabolites, including artemillin C, caffeic acid phenethyl ester (CAPE), and catechol, underscores the significant and underexplored pharmacological potential of this species and broadens the known chemodiversity of the genus *Calligonum*. Collectively, these results advance our understanding of biochemical adaptation in plants to environmental stress. Furthermore, they provide a robust scientific foundation for leveraging *C. leucocladum* extracts in applied sectors such as medicine, cosmetology, and the functional food industry. To translate this potential into tangible applications, future research should focus on: (1) elucidating the genetic and regulatory architecture underlying key biosynthetic pathways; (2) rigorously validating the bioactivity and safety profiles through targeted pharmacological models; and (3) developing sustainable, scalable agronomic practices for the commercial cultivation and production of these valuable phytochemicals.

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## Conflict of interest

The authors declare no competing interests.

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