

Morphology and phytochemical potential of *Vaccinium varingiifolium* in Mount Lawu, Karanganyar District, Central Java, Indonesia

EFRI ROZIATY^{1,2,✉}, SANTHYAMI^{1,2,✉✉}, PUTRI AGUSTINA¹, IMA ARYANI¹, MUHAMMAD ASYAM FATHIN¹, SALSABILLA ANIDA RAHMANIA¹, MUHAMMAD YAHYA NUR SHOLIHIN¹, DAWUD FA'I SALSABILLA¹

¹Department of Biology Education, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta. Jl. A. Yani, Sukoharjo 57162, Central Java, Indonesia. Tel./fax.: +62-271-717417 ext 2197, ✉email: er375@ums.ac.id, ✉✉email: san915@ums.ac.id

²Center of Environmental Study, Universitas Muhammadiyah Surakarta. Jl. A. Yani, Sukoharjo 57162, Central Java, Indonesia

Manuscript received: 17 September 2024. Revision accepted: 22 June 2025.

Abstract. Roziaty E, Santhyami, Agustina P, Aryani I, Fathin MA, Rahmania SA, Sholihin MYN, Salsabilla DF. 2025. Morphology and phytochemical potential of *Vaccinium varingiifolium* in Mount Lawu, Karanganyar District, Central Java, Indonesia. *Biodiversitas* 26: 3209-3217. *Vaccinium varingiifolium*, commonly known as Cantigi, is a subalpine shrub endemic to high-altitude ecosystems above 2,000 meters. Mount Lawu, located in Central Java, Indonesia, represents one of its key habitats. Despite its ecological relevance, the species remains underexplored, particularly in terms of its morphological and phytochemical traits. This study aimed to characterize the morphological features and phytochemical composition of *V. varingiifolium* to understand its adaptive strategies and ecological role better. A combination of qualitative and quantitative exploratory approaches was employed, using purposive sampling. Morphological assessments, including microscopic examinations, were conducted on stems, leaves, flowers, and fruits. Phytochemical screening using spectrophotometry targeted secondary metabolites, while ecological observations assessed environmental factors influencing adaptation. Morphological characterization of *V. varingiifolium* in Lawu Mountains revealed that young leaves are purplish-red, turning green with increased tissue density upon maturation. Stems are brown, globular, and lignified. Flowers are purplish-red, bell-shaped (campanulate), and axillary in position. Fruits are oval (5-6 mm in diameter), transitioning from green to black as they ripen. Phytochemical analyses revealed the presence of flavonoids and tannins, compounds known for their antioxidant, antibacterial, anti-inflammatory, and wound-healing properties. The plant thrives at elevations of 2,900-3,100 meters, where higher temperatures facilitate rapid regeneration. These findings provide important insights into the morphological adaptations and chemical defenses of *V. varingiifolium*, supporting conservation initiatives and enriching our understanding of plant survival mechanisms in subalpine environments, particularly in the context of climate change.

Keywords: Biopharmaceuticals, morphology, shrub, subalpine, *Vaccinium varingiifolium*

INTRODUCTION

Mt. Lawu (3,265 masl) is an extinct volcano located (111°15'E 7°30'S) between the provinces of Central Java and East Java, Indonesia. Administratively, it is split among the districts of Karanganyar, Magetan, Ngawi, and Sragen, Central Java, Indonesia. The topography of the northern part is conical, with the peak of Argo (Hargo) Dumilah as high as 3,265 meters above sea level (masl) (Yu et al. 2017). As the third highest mountain on Java Island, the mountain is home to various ecosystems ranging from the hill forest of the foothills to the subalpine and alpine (Cavieres et al. 2016). The subalpine and the alpine ecosystems of Lawu are of special interest as they form ecological islands within the already insular ecosystem of Java (Nakhutsrishvili et al. 2022). Among the endemic lifeforms found within the subalpine ecosystem of Lawu is *Vaccinium varingiifolium* (Blume) Miq, locally known as Cantigi (Argent 2019). *V. varingiifolium* is also known as "*manis rejo*" because of the sweet and slightly sour taste of its berries, which mountaineers often consume as a source of energy to supplement limited supplies (Tong et al. 2021). *V. varingiifolium* belongs to the Ericaceae family (Huang et al. 2022). This plant is native to the subalpine areas of Peninsular Malaysia and Indonesia,

including mountain slopes in Java Island, such as Lawu Mountain. It often thrives in high-altitude habitats where circumstances are colder and harsher, demonstrating its adaptation to subalpine settings (Tong et al. 2020). *V. varingiifolium* is a shrub native to the subalpine ecosystem of several volcanoes on Java (Yulyana et al. 2016) at altitudes more than 2,500 masl (Yu et al. 2017). The type of *V. varingiifolium* found on Mount Lawu is taxonomically classified as being of the division Magnoliophyta, subdivision Spermatophyta, class Magnoliopsida, order Ericales, and family Ericaceae (Blume 1825; Zehnder et al. 2020). This species is related to other berry-bearing species, such as bilberries, huckleberries, blueberries, and cranberries that have been widely researched. *Vaccinium* is a shrub that is widespread throughout the world, while *V. varingiifolium* is a woody shrub or small tree that can reach several meters in height. Its growth pattern is characteristic of plants acclimated to high elevations, with strong and sturdy structures (Cavieres et al. 2016).

The leaves of *V. varingiifolium* are green in color, have an elliptic to lanceolate shape, and a rough surface with a waxy covering aiding in water retention and enhancing resistance to cold temperatures. The plant produces petite, tubular, bell-shaped flowers, a distinguishing feature of the

Vaccinium genus. The flowers have most likely evolved for pollination by certain insects in their natural environment. The fruits are berries, similar to other *Vaccinium* species, and may have an ecological function in seed dissemination through birds and animals. *Vaccinium* is found in various types (Huang et al. 2022). It is a flowering plant characterized by short racemose-shaped flowers, very short flower stems, or 4-5 mm long, and a wide, bell-shaped crown of leaves that are yellow-green or red-colored.

Previous studies of metabolites of *V. varingiifolium* have been done in various mountainous regions, such as Mt. Tangkuban Perahu (Yulyana et al. 2023), Mt. Gede Pangrango, Mt. Papandayan, and even alpine tundra (Zhou et al. 2022). Also, the *V. varingiifolium*'s biodiversity and the types of *V. varingiifolium* growing in the research habitat have been explored (Cronin et al. 2014). However, information about the detailed morphological characters of *V. varingiifolium* in Lawu Mountain is still limited. Therefore, it is necessary to study the various morphological differences between those found on Lawu and compare them to those found on other mountains. It is noted that phenotypic variations may correspond to differences in the concentrations of bioactive compounds, depending on the source area. A previous study (Jurikova et al. 2019) has also indicated cytostatic and antioxidant potential in this species (Barcelo 2015). There are limited records regarding *Vaccinium* in

the mountains of Indonesia regarding its morphology and biopharmaceutical potential (Zhang et al. 2019). This study aimed to explore the morphological characters of *V. varingiifolium* typically grown in Mount Lawu and reveal its bioactive compounds.

MATERIALS AND METHODS

The study area (sampling sites)

After obtaining all necessary permits from the relevant authorities, a mountaineering expedition was conducted in June 2023 to collect samples for this study. The expedition followed the established Cemoro Kandang hiking trail to Mt. Lawu. Three stations in each plot were defined at 2,700-3,000 masl, 3,000-3,100 masl, and >3,100 masl, respectively, which are *Vaccinium* habitats in subalpine. Plot 1: 7°36'24"S 111°11'25"E, Plot 2: 7°36'54"S 111°11'51"E, and Plot 3: 7°37'14"S 111°11'45"E (Figure 1).

Upon the research team's arrival in the research area, sampling plots 5 × 5 m (Figure 2) were arranged. Sample specimens were then acquired according to purposive random sampling. All further analysis was conducted at the Biological Education Laboratory of Universitas Muhammadiyah Surakarta, Central Java, Indonesia.

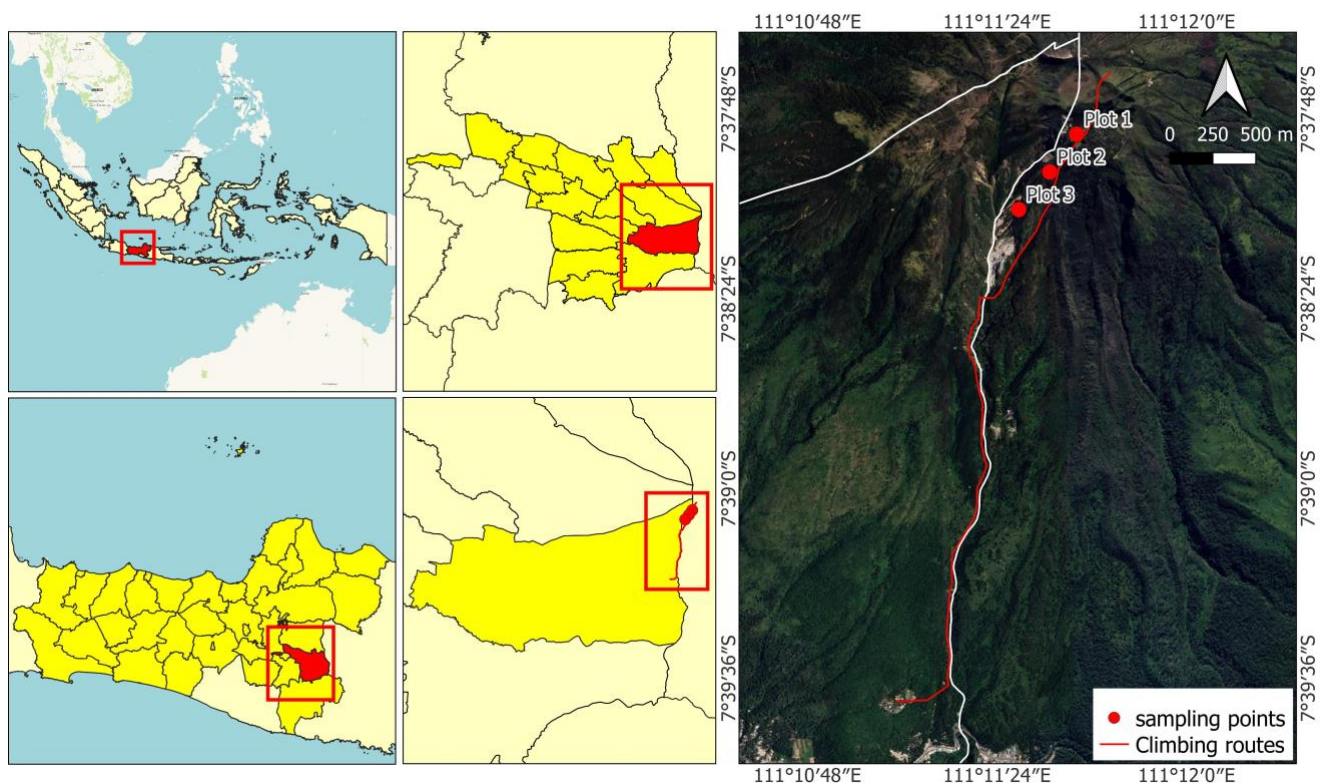


Figure 1. Site of plots in the Cemoro Kandang hiking trail to Mt Lawu, Karanganyar, Central Java, Indonesia. Note: See text for coordinate of Plot 1, 2, and 3

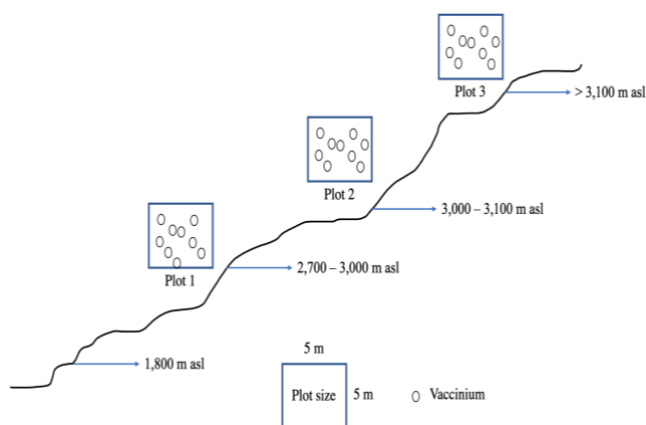


Figure 2. Sketch of a field research site in Cemoro Kandang hiking trail to Mt. Lawu, Karanganyar, Central Java, Indonesia

Research procedures

Morphological analyses of Vaccinium varingiifolium and ecological data

Further analyses comprised both morphological and chemical evaluations of the samples. Morphological assessment followed Rifnas and Vidanapthirana (2023) and consisted of detailed macroscopic examination of leaves, stems, flowers, and fruits. For each organ, precise measurements of size (length, width, thickness), shape, surface texture, and consistency were taken with a digital caliper and documented photographically. All observations qualitative descriptions and quantitative data were recorded in a standardized character matrix. Finally, voucher specimens were pressed, dried, and deposited in the dry herbarium of Biological Education Laboratory, Universitas Muhammadiyah Surakarta. Ecological data, presented in the results, were derived from field observations and habitat assessments, revealing key environmental factors influencing its abiotic factors and distribution. The collected abiotic data were temperature, altitude, and humidity.

Phytochemical analysis

Qualitative screening of flavonoids and tannins in *V. varingiifolium* leaf extracts was carried out using established colorimetric assays; the Bate-Smith method first detected flavonoids. Metcalf test: the extract was treated with dilute HCl and subjected to indirect heating at 100°C for 15 min; a reddish-brown coloration indicated a positive reaction (Jia et al. 2019). A separate aliquot of extract was mixed with 10% NaOH solution, and the appearance of a distinct yellow color confirmed the presence of flavonoids. Finally, an ammonia-sulfuric acid test ($\text{NH}_3 + \text{H}_2\text{SO}_4$) was performed by heating 1 mL of extract, filtering, then adding 0.5 mL of NH_3 , followed by titration with H_2SO_4 ; development of yellow coloration was taken as a positive response (Jia et al. 2019).

Three complementary methods qualitatively assessed tannins. In the ferric chloride test, treatment of the extract with FeCl_3 yielded a dark-blue precipitate. In the gelatin-salt test, 1% gelatin solution containing NaCl was added to the extract, and visible sedimentation indicated tannin-

protein complex formation. Finally, the Stiasny test was performed by adding 15 mL of Stiasny reagent (30% formaldehyde in concentrated HCl, 2:1 v/v) to 10 mL of extract and heating indirectly; light-red sediment confirmed a positive reaction for condensed tannins (Jia et al. 2019).

Quantitative determination of flavonoid and tannin contents was achieved by spectrophotometric analysis. For flavonoids, 5 g of dried leaf powder was extracted with 96% ethanol in a 100 mL volumetric flask and filtered; 1 mL of the filtrate was mixed with 2 mL of 5% AlCl_3 solution and brought to volume with 7 mL of ethanol. After homogenization, absorbance was measured at 415 nm against a reagent blank, and concentrations were calculated from a quercetin standard curve (Jia et al. 2019; Zhang et al. 2019).

For tannins, 5 g of the sample was pulverized, suspended in 100 mL of distilled water, stirred until homogeneous, and filtered. To 1 mL of filtrate, 0.5 mL of Folin-Ciocalteu reagent (1:1) and 1 mL of 10% Na_2CO_3 solution were added; the volume was then adjusted to 10 mL with distilled water. The mixture was stirred and allowed to react, after which the absorbance was read at 730 nm. Tannin content was quantified using a standard curve constructed from gallic acid equivalents (Jia et al. 2019).

Data analysis

Qualitative morphological data were analyzed using graphs and diagrams created in Excel applications. The phytochemical content of flavonoids and tannins was quantified based on the calibration curves derived from standard solutions using a spectrophotometer. The correlations between individual-found *V. varingiifolium* and the height of the research plot were analyzed using an Excel function.

RESULTS AND DISCUSSION

Vaccinium varingiifolium morphology

Habitus of *V. varingiifolium* are shrubs that can grow up to 10 m in height before branching out and forming a canopy. It is observed to have hard ligneous wood with a monopodial branch pattern where the main trunk (usually circular in cross-section) is distinguishably more substantial than the branches (Huang et al. 2022) (Figures 3.A and 3.B). Branches were observed to grow skywards with rough (ramosus) bark (Figure 3.C). Roots tend to be long, growing straight down with many branches (Huang et al. 2022).

Cantigi was observed in all three plots, with individuals observed beginning near plot 1 at 2,700 masl. The individuals observed were shrubs with red petioles. The young leaves were reddish-violet, and older leaves turned green. The leaves were measured between 2–6 cm in length and 1–2.5 cm in width. Leaves are generally oval with a cuneate base forming an inverted triangle, an entire leaf margin, and an acute tip. The leaves exhibit a pinnate venation. Leaf surfaces are leathery and waxy in texture and are generally dark green on the top side of leaves and a lighter green on the bottom side of leaves. Leaves are arranged in an alternate spiral along the stem. Leaf stalks

are glandular with modules containing a podium and a lamina. Cantigi shrubs produce small, bell-shaped flowers that are usually white or pale pink (Barcelo 2015). These flowers are arranged in clusters and have a delicate fragrance (Hussain et al. 2023). The flowers attract various pollinators, such as bees and butterflies. The phenotypical characteristics of leaves as plant organs tend to be constant within the species: however, flowers are more susceptible to changes in morphology in response to environmental triggers (Forney et al. 2012; Wang et al. 2019). It is also observed that leaf thickness decreases at higher altitudes (>1,480 masl) (Torres-Guevara et al. 2023).

Vaccinium varingiifolium is a shrub plant that can grow up to 10 m before finally branching a lot and forming a well-developed crown. The wood is very hard (lignosus), with a monopodial trunk where the main trunk is visible and larger (Argent and Wilkie 2020) (Figures 3.A and 3.B). *V. varingiifolium* has a cross-sectional shape of a rounded stem and a rough bark structure (Figure 3.C). The branches grow upward, and the stem is brown. The root is a branched taproot (ramosus), long, cone-shaped, growing straight down with numerous branches (Hussain et al. 2023). The identification results show that *V. varingiifolium* plants are shrubs that have red petioles, reddish-purple young leaves, and green older leaves (Figure 4). The leaves of *V. varingiifolium* measure 2-6 cm in length and 1-2.5 cm in width. The leaves have an oblong (ovate) shape, with a flat, entire leaf margin, pointed (acute) tips, and a cuneate base that forms a breech triangle (wedge-like) shape. The leaf venation is pinnate. The upper surface of the leaves is smooth. The color of the upper part of the leaf is dark green, and the underside is light green. The leaves are arranged alternately in a spiral pattern along the stem, with one leaf attached to each node, arranged around the twigs like a spiral. The petioles are glandular, with both an epipodium and lamina (Petruřová and Bačkorová 2024).

The fruit of *V. varingiifolium* is a small, round berry, typically measuring around 5-8 mm in diameter. When unripe, the berries are green, gradually turning reddish and eventually deep purple to black as they mature. The texture is smooth, with a slightly waxy surface. The fruit is juicy and contains multiple tiny seeds, similar to other species in the *Vaccinium* genus. Ecologically, the berries play an essential role in the subalpine ecosystem, serving as a food source for birds and other wildlife, which aid in seed dispersal. While not widely known or cultivated, the fruit is edible. It has a mildly sweet to slightly tart taste, potentially containing beneficial phytochemicals such as flavonoids and anthocyanins, similar to other *Vaccinium* species like blueberries.

Vaccinium varingiifolium has slightly thick leaves, a characteristic influenced by altitude, which plays a crucial role in the plant's adaptation to environmental conditions. The leaf tissue thickness shows a positive correlation with increasing altitude between 950-1,480 masl, likely as a response to greater exposure to sunlight, lower temperatures, and reduced moisture availability. However, at 1,760 masl, leaf thickness decreases, possibly due to physiological limitations, changes in nutrient availability, or adaptation to

different microclimatic conditions. These variations suggest that the species adjusts its morphological traits to optimize survival across different elevations within its natural habitat (Svanberg and Ståhlberg 2021).

A qualitative and quantitative test of a bioactive compound of *Vaccinium varingiifolium* leaves

Qualitative testing for the presence of flavonoid compounds in the leaves of *V. varingiifolium* was carried out using the Bate-Smithe, NaOH, and $\text{NH}_3 + \text{H}_2\text{SO}_4$ tests. The formation of a red color indicates a positive reaction in the Bate-Smithe test, while the formation of an orange color indicates a positive reaction in the NaOH test. The qualitative results were then confirmed using quantitative results (Table 1).

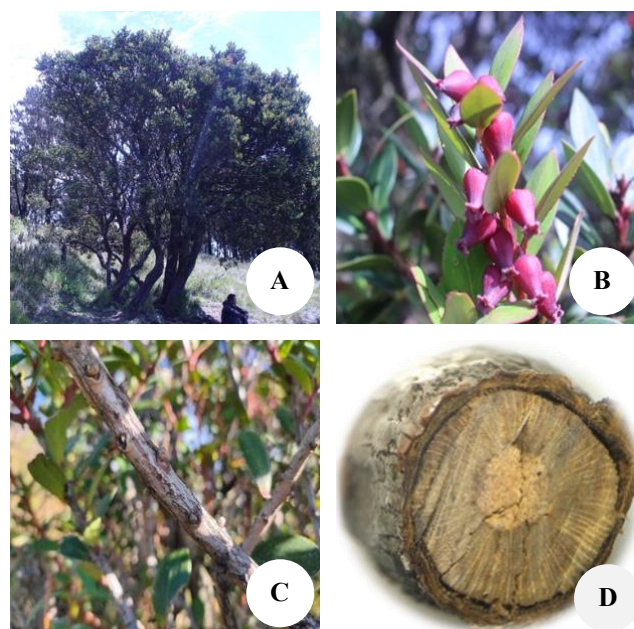


Figure 3. Morphological characters of *Vaccinium varingiifolium*. A. Individual *V. varingiifolium* shrubs that can reach 10 m in height, B. Fruits, C. Branch, D. Wood of *V. varingiifolium*



Figure 4. Leaf shapes of the *Vaccinium varingiifolium*

Table 1. Result of qualitative and quantitative tests of bioactive agent categories

Bioactive agent	Code	Method	Qualitative test			Code	Quantitative test
			Result	Color indicator	Sedimentation		Content (%)
Flavonoid	K1	Bate-Smite	+	Red	(-)	K1	1.0935
			+			K2	1.2333
	K1	NaOH	+	Crimson	(-)	K3	1.3211
			+			K4	1.4311
	K1	NH ₃ +H ₂ SO ₄	+	Reddish brown	(+)		
			+				
Tannin	K1	FeCl ₃	+	Dark blue	(-)	K1	1.2635
			+			K2	1.2333
	K1	Garam gelatin	+	Yellowish brown	(+)	K3	1.5643
			+			K4	2.2635
	K1	Stiasny	+	Yellowish brown	(+)		
			+				
Saponin	K1	Saponification test	+	Stable foam			
		Saponification test and HCl test	+	Stable foam			
	K2	Saponification test	+	Light foam			
		Saponification test and HCl test	+	Stable foam			
Alkaloid	K1	Dragendorf test	+	No color change	(-)		
		Mayer test	+	Dark brown	(+)		
	K2	Dragendorf test	+	No color change	(-)		
		Mayer test	+	Dark brown	(+)		

Note: (+): Positive, (-): Negative, K1: Sample taken from 1,800 masl, K2: Sample taken from 2,700-3,000 masl, K3: Sample taken from 3,000-3,100 masl, K4: Sample taken from >3,100 masl

A color change and a precipitate indicate a positive reaction in the NH₃ + H₂SO₄ test. The red color in the flavonoid test is due to the formation of flavylum salts (Zhang et al. 2019). All three tests confirmed the presence of flavonoids (see Table 1). Flavonoids play a role in plants in regulating growth, photosynthesis, and antimicrobial and anti-viral action. All groups of flavonoids play a significant role in human health. For example, isoflavonoids have a role in breast and prostate cancer therapy (Geng et al. 2016).

Qualitative testing for the presence of tannins was undertaken using the FeCl₃, gelatin, and Stiasny salts techniques. In the FeCl₃ test, a blackish-blue color was formed. In the gelatin salt test, a white precipitate was formed, in line with previous research findings. In the Stiasny test, a white precipitate is formed, which means it is positive for containing tannin compounds (Kaškonienė et al. 2020).

The results of qualitative tests using three methods are uniform, meaning that the leaves of the *V. varingiifolium* plant contain tannin compounds. Tannins protect plants from predation and regulate plant growth (Raudone et al. 2019). They have been used since the 1960s as adhesives in the wood industry and the production of corrosive chemicals. Moreover, tannins act as antioxidants and anti-carcinogens and play an important role as anti-inflammatory, cicatrizing, and anti-HIV agents (Tong et al. 2021).

Following the establishment of the qualitative presence of both flavonoids and tannins, similar to those found in hibiscus leaves and green apple peel, quantitative measurement of the total flavonoids and tannins were measured by spectrophotometer, following the preparation of test solutions for flavonoids and tannins according to established procedures. For flavonoids, absorbance was read with a wavelength of 415 nm, yielding results of 0.060, 0.115, 0.170, 0.218, and 0.270. The regression

equation obtained for flavonoid levels was $Y = 3.579x + 0.0046$, $Y = 3.579x + 0.0046$, $Y = 3.579x + 0.0046$, with an R² value of 0.9988. Based on the regression equation, the total flavonoid content was calculated using spectrophotometry as 1.0935% in sample K1 and 1.233% in sample K2 (Figure 5).

Following the preparation of the sample solution, the absorbance was read with a wavelength of 730 nm, yielding results of 0.108, 0.214, 0.292, 0.367, and 0.461. The total tannin content was determined using the regression equation of $Y = 7.7851x + 0.035$, with an R² value of 0.9976. Based on the regression equation, the total tannin content calculated using a spectrophotometer was 2.2635% for sample K1 and 2.8532% for sample K2.

All samples of *V. varingiifolium* collected during the expedition contained secondary metabolite compounds, namely flavonoids and tannins (Elferjane et al. 2024). These compounds are generally efficacious and are used as medicinal plants. These compounds are known as secondary metabolites produced by plants for self-defense. Several plants with biopharmaceutical potential have one or more secondary metabolite compounds, including alkaloids, flavonoids, saponins, tannins, triterpenoids, and steroids (Zoratti et al. 2015).

Flavonoids are a kind of polyphenolic chemical that exhibits antioxidant, anti-inflammatory, and antibacterial activities (Petruřová and Bačkorová 2024). Flavonoids can help alleviate oxidative stress in *V. varingiifolium* by scavenging reactive oxygen species, which are formed in response to environmental challenges such as UV radiation, dehydration, and infections (Hu et al. 2016). Flavonoids operate as natural sunscreens, absorbing UV radiation and protecting plant tissues from UV-induced damage (Vilkickyte and Raudone 2021). These chemicals may help plants

withstand microbial infections (Arivo et al. 2023) by suppressing pathogen development (Liu et al. 2014). *V. varingiiifolium* may help boost the plant's possible health benefits when ingested. It can help prevent oxidative stress-induced cell damage and reduce inflammation, both of which can help manage chronic illnesses. Flavonoids, notably anthocyanins (a kind of flavonoid), are responsible for berry coloring (Ștefănescu et al. 2020). These colors attract pollinators and help seed distribution by making fruits attractive to animals (Casolo et al. 2020).

Abiotic factors

Environmental factors, such as data analysis, indicate that temperature (°C) and air humidity (%) significantly affect the distribution and population of *V. varingiiifolium*. Higher temperatures accelerate its life cycle, while humidity variations influence leaf structure and regeneration capacity across different altitudes. The results from measuring abiotic factors obtained at different altitudes, characteristics of places, namely at station 1 sampling area with an altitude of 2,900 masl to station 3 sampling area with an altitude of 3,100 masl, can be explained (see Table 3).

The measurement of abiotic factors, including temperature and air humidity, on the number of individual populations of *V. varingiiifolium* plants at different altitudes yielded the following results. At station 1, located at 2,900 masl, the air temperature ranged from 25 to 29°C, with air humidity from 31 to 33%, and the average number of individuals was 1. At station 2, at 3,000 masl, the air temperature ranged from 21 to 25°C, with air humidity from 31 to 43%, and the average number of individuals was 13. Meanwhile, at station 3, with an altitude from 3,100 to 3,265 masl (top of Mt. Lawu, Central Java, Indonesia), the temperature ranged from 15 to 16°C. Air humidity ranged from 41 to 50%, and the average number of individuals was 17. The results showed that an increase in altitude of 200 masl causes a decrease in temperature of 4.5°C. The highest temperature of 29°C is at station 1 with an altitude of 2,700 masl, and the lowest air temperature is at station 3 with a temperature of 15°C (Table 2).

The increase in air temperature has a profound impact on the life cycle of individual plants. High temperatures accelerate their life cycle, leading to a rapid regeneration cycle. The degree of temperature significantly influences the rate of metabolism, photosynthesis, respiration, and plant transparency. The results of air humidity measurements showed a general increasing trend with altitude, ranging from 31-33% at 2,700 m, to 31-43% at 3,000 m, and 41-50% at 3,100 m. Therefore, as air humidity increases, the rate of transpiration slows down, while lower humidity accelerates the rate of transpiration (Stanisci et al. 2020). The effects of these abiotic parameters follow environmental circumstances, where the number of individual plants increases as altitude, air temperature, and humidity rise. Station 3 had the most individual plant populations, with 51 plants, while Station 1 had the fewest, only 3 individuals (Figure 6). The *Vaccinium* on Mt. Lawu can tolerate these environmental differences, demonstrating that this plant

has a wide range of tolerance to environmental stresses (Yu et al. 2017).

The ecological data have unveiled a significant correlation between the altitude of the research site and the number of individual shrubs at each station, as evidenced by the significance value of 0.05. This finding underscores the importance of altitude in understanding the population dynamics of *V. varingiiifolium* (Figure 7.A). However, the correlation test between air temperature and the number of individual shrubs did not yield a significant relationship, with the significance value of 0.06 exceeding the threshold of 0.05. This unexpected result, while not supporting our initial hypothesis, is of significant interest and prompts further investigation. As a result, the hypothesis was not accepted, indicating no correlation between the number of individual *V. varingiiifolium* and the air temperature (Figure 7.B).

Table 2. Abiotic factors at three research stations in Mt. Lawu, Central Java, Indonesia

Parameter	Altitude (masl)		
	2,700	3,000	3,100
Temperatures (°C)	25.1-29	21-25.7	15-16
Air humidity (%)	31-33	31-43	41-50
Several individuals encountered	3	39	51

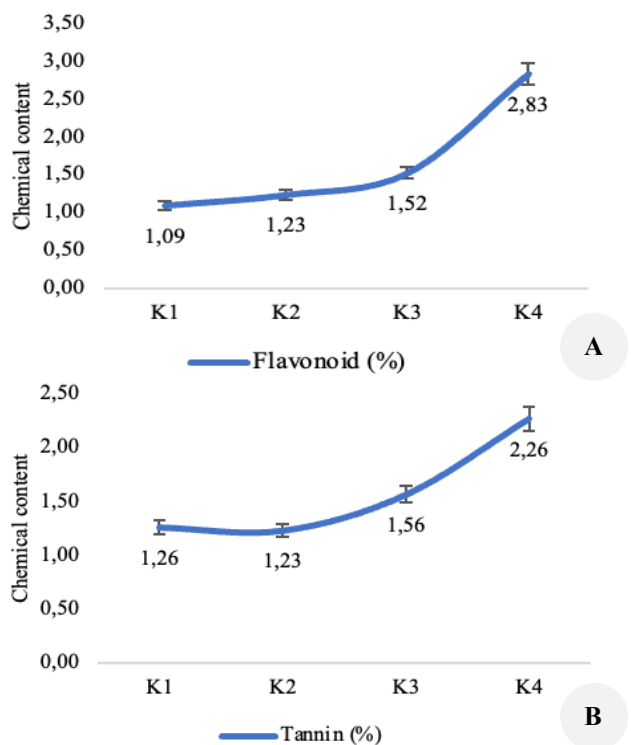


Figure 5. Visualization of the quantity values of bioactive agents: A. Flavonoids (%), and B. Tannins (%) in sampled *Vaccinium varingiiifolium* leaves. K1: Sample from 1,800 masl, K2: Sample from 2,700-3,000 masl, K3: Sample from 3,000-3,100 masl, and K4: Sample from >3,100 masl

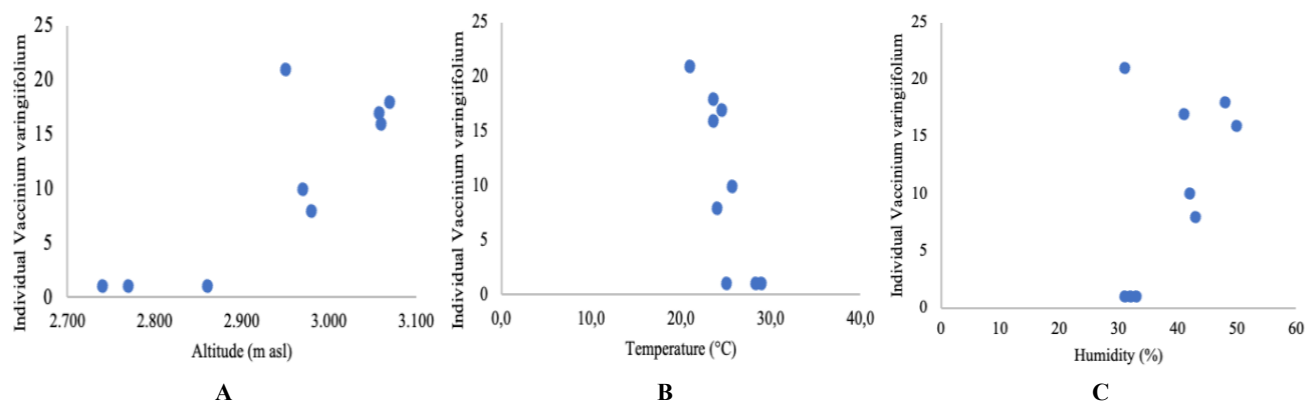


Figure 7. Correlation between the number of *Vaccinium varingiifolium* individuals found in the research station (Station 1 at 2,700 m asl; Station 2 at 3,000 m asl; and Station 3 at 3,100 m asl) to abiotic factors such as: A. Altitude, B. Temperature, and C. Humidity

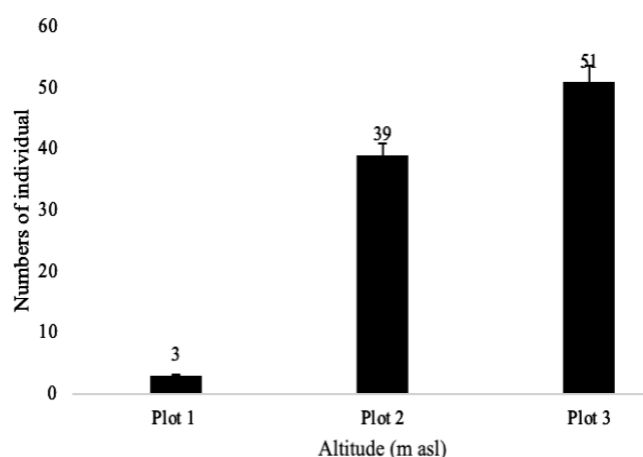


Figure 6. *Vaccinium varingiifolium* individuals were found in the research plot at 3 different heights, Plot 1 at 2,700 masl; Plot 2 at an altitude of 3,000 masl, and Plot 3 at an altitude of 3,100 masl

The correlation analysis between humidity and the number of individual *V. varingiifolium* plants yielded a significance value of 0.159 (Figure 7.C). Since this value is greater than 0.05, it indicates that humidity does not have a significant effect on the population of *V. varingiifolium* (Roziaty and Nurrahman Al Farisi 2022). In area 1, at an altitude of 2,700 masl, the air temperature ranged from 16 to 18°C with air humidity of 41 to 48%. Meanwhile, in area 2, at an altitude of 3,100 masl, the air temperature reaches 12 to 14°C, with air humidity reaching 54 to 56%. The *V. varingiifolium* plants on Mount Lawu demonstrate remarkable adaptability to the environment, exhibiting a broad tolerance to varying environmental factors. The differences in total compound content are depicted in Figure 7.

Figure 5 shows that flavonoid and tannin compounds vary at different altitudes in an area. Temperature, pH, and humidity are key abiotic variables influencing the process. A variety of internal and environmental variables regulate secondary metabolite molecules in plants, including flavonoids and tannins. External influencing factors include light, temperature, humidity, pH, soil nutrient concentration,

and altitude. The findings indicate that flavonoid and tannin compounds exhibit significant variation across different altitudes within a given area. Environmental conditions likely influence this variation, as temperature, soil pH, and humidity serve as key abiotic factors regulating the biosynthesis and accumulation of these secondary metabolites. Higher or lower altitudes may create distinct microclimates that impact plant metabolism, leading to differences in phytochemical concentrations. Understanding these patterns can provide valuable insights into how *V. varingiifolium* and other subalpine plants adapt to changing environmental conditions (Frazier and Brewington 2020).

The content of secondary metabolite compounds, like flavonoids and tannins, is influenced by environmental factors, including climate conditions, rainfall, altitude, air temperature, and soil physicochemical conditions, such as soil moisture. Meanwhile, variations that can increase the content of secondary metabolite compounds are influenced by biotic or abiotic factors (Casolo et al. 2020). As stated by Djamal et al. (2024) the flavonoid content in *Phyllanthus niruri* is affected by drought stress. Plants exhibit a remarkable ability to produce a diverse range of secondary metabolites, such as flavonoids and tannins, which play crucial roles in their survival and adaptation to the environment. These compounds are not directly involved in plants' primary growth and development, but they contribute to various functions, including defense mechanisms, signaling, and interactions with other organisms.

The content and accumulation of secondary metabolites in plants are strongly influenced by environmental factors (Devi et al. 2023; Reshi et al. 2023). Under stress conditions, such as exposure to high light, UV radiation, nutrient deficiency, or pathogen attack, plants often increase the production of these compounds as protective and adaptive responses. The underlying mechanism is that environmental stressors can alter the expression of genes involved in the biosynthetic pathways of secondary metabolites, leading to their enhanced accumulation (Mahajan et al. 2020). For example, the formation and accumulation of phenylpropanoids, anthocyanins, and polyamines have been reported as a response to various environmental stresses (Cetinkaya et al. 2017). These metabolites can act as antioxidants, UV

protectants, and signaling molecules, helping plants cope with adverse conditions. Furthermore, the balance between primary and secondary metabolism in plants is dynamic and can shift in response to environmental cues (Zehnder et al. 2020).

In conclusion, *V. varingiifolium* provides fascinating insights into its morphology and pharmacological potential. The plant's morphology reflects the adaptation to the typical subalpine settings at altitudes ranging from 2,819 to 3,265 masl. The plant flourishes most effectively between 3,025-3,192 masl, where maximum population density was observed. *V. varingiifolium* has adapted to the extreme environment of the subalpine zone, including distinctive leaf structures that may aid in water retention and UV radiation protection. Studies have emphasized the plant's antioxidant qualities, particularly in its leaves. These features are connected to bioactive molecules that may help prevent oxidative stress-related illnesses. A comparative study reveals commonalities with other species in the *Vaccinium* genus that are commercially and medicinally valuable, such as blueberries and cranberries. While the plant is native to the Indonesian mountains, its diversity on Mt. Lawu is limited, with only one dominant species discovered in this area. It is crucial that local populations, including environmental scientists, conservationists, and researchers, are educated about the conservation of this rare species. These initiatives are vital, as they seek to secure their long-term utilization while also preventing habitat deterioration on Mt. Lawu. This combination of ecological value and biopharmaceutical promise emphasizes the necessity of protecting *V. varingiifolium* while researching its potential for health and economic use. Its ability to thrive in extreme environmental conditions also makes it an important species for studying plant adaptations to subalpine climates, particularly in response to temperature fluctuations and soil conditions.

ACKNOWLEDGEMENTS

Acknowledgements are expressed to Universitas Muhammadiyah Surakarta, Indonesia, for funding this research through the Integrasi Tri Dharma (HIT) project grant, with the contract number 355/A.3-III/FKIP/IV/2022.

REFERENCES

- Argent G, Wilkie P. 2020. Six new species of *Vaccinium* (Ericaceae) from New Guinea. *Edinburgh J Bot* 77 (3): 439-453. DOI: 10.1017/S0960428620000104.
- Argent G. 2019. *Rigirolepis* and *Vaccinium* (Ericaceae) in Borneo. *Edinburgh J Bot* 76 (1): 55-172. DOI: 10.1017/S0960428618000276.
- Arivo D, Mubarik NR, Rusmana I, Batubara I. 2023. Antioxidant activity of various solvent extracts from endophytic bacteria isolated from girang (*Leea indica*) leaves. *Biodiversitas* 24 (1): 415-422. DOI: 10.13057/biodiv/d240148.
- Barcelo R. 2015. Phytochemical screening and antioxidant activity of edible wild fruits in Benguet, Cordillera Administrative Region, Philippines. *Electron J Biol* 11 (3): 80-89.
- Blume CL. 1825. *Bijdragen tot de flora van Nederlandsch Indië* (Vol. 1). Ter Lands Drukkerij, Batavia. [Dutch]
- Casolo V, Braidot E, Petrusa E, Zancani M, Vianello A, Boscutti F. 2020. Relationships between population traits, nonstructural carbohydrates, and elevation in alpine stands of *Vaccinium myrtillus*. *Am J Bot* 107 (4): 639-649. DOI: 10.1002/ajb2.1458.
- Cavieres LA, Hernández-Fuentes C, Sierra-Almeida A, Kikvidze Z. 2016. Facilitation among plants as an insurance policy for diversity in Alpine communities. *Funct Ecol* 30 (1): 52-59. DOI: 10.1111/1365-2435.12545.
- Cetinkaya H, Kulak M, Karaman M, Karaman HS, Kocer F. 2017. Flavonoid accumulation behavior in response to the abiotic stress: Can a uniform mechanism be illustrated for all plants? In: Justino GC (eds). *Flavonoids-From Biosynthesis to Human Health*. IntechOpen, London. DOI: 10.5772/68093.
- Cronin DT, Libalah MB, Bergl RA, Hearn GW. 2014. Biodiversity and conservation of tropical montane ecosystems in the Gulf of Guinea, West Africa. *Arct Antarct Alp Res* 46 (4): 891-904. DOI: 10.1657/1938-4246-46.4.891.
- Devi AM, Devi KK, Devi PP, Devi ML, Das S. 2023. Metabolic engineering of plant secondary metabolites: Prospects and its technological challenges. *Front Plant Sci* 14: 1171154. DOI: 10.3389/fpls.2023.1171154.
- Djamas N, Pancoro A, Devy L. 2024. The effect of drought stress on phyllanthin and quercetin contents of green meniran plant (*Phyllanthus niruri* L.). *Jurnal Hortikultura Indonesia* 15 (2): 112-118. DOI: 10.29244/jhi.15.2.112-118.
- Elferjane MR, Milutinović V, Krivokuća MJ, Taherzadeh MJ, Pietrzak W, Marinković A, Jovanović AA. 2024. *Vaccinium myrtillus* L. leaf waste as a source of biologically potent compounds: Optimization of polyphenol extractions, chemical profile, and biological properties of the extracts. *Pharmaceutics* 16 (6): 740. DOI: 10.3390/pharmaceutics16060740.
- Forney CF, Javorek SK, Jordan MA, Kloet SPV. 2012. Floral volatile composition of four species of *Vaccinium*. *Botany* 90 (5): 365-371. DOI: 10.1139/B2012-008.
- Frazier AG, Brewington L. 2020. Current changes in Alpine ecosystems of Pacific Islands. In: Goldstein MI, DellaSala DA (eds). *Encyclopedia of the World's Biomes*. Elsevier Inc, Amsterdam, Netherlands. DOI: 10.1016/b978-0-12-409548-9.11881-0.
- Geng Y, Zhang Y, Ranjitkar S, Huai H, Wang Y. 2016. Traditional knowledge and its transmission of wild edibles used by the Naxi in Baidi Village, Northwest Yunnan province. *J Ethnobiol Ethnomed* 12: 10. DOI: 10.1186/s13002-016-0082-2.
- Hu J, Wang J, Li S, Yang B, Gong M, Li X, Zhang L, Tian J. 2016. Phytochemical compositions, antioxidant and antimicrobial activities analysis of extracts from *Vaccinium bracteatum* Thunb. leaves. *J Appl Bot Food Qual* 89: 150-155. DOI: 10.5073/jabfq.2016.089.018.
- Huang YS, Nong SY, Li XK, Xie G, Tong YH. 2022. *Vaccinium bangliangense*, a new species of Ericaceae from limestone areas in Guangxi, China. *PhytoKeys* 194: 23-31. DOI: 10.3897/phytokeys.194.81018.
- Hussain S, Sharma S, Bhatti RC, Singh AN. 2023. Floral diversity expedition in Ladakh: An insight into the exploration, distribution pattern, ethnobotanical, phytochemical studies and conservation strategies. *Ethnobot Res Appl* 26: 1-32. DOI: 10.32859/era.26.43.1-32.
- Jia G, Zhao H, Hou D, Sun T, Lin W. 2019. Quantitative determination of total flavonoids from *Polygonatum sibiricum* by spectrophotometry. *IOP Conf Ser: Mater Sci Eng* 677: 022126. DOI: 10.1088/1757-899X/677/2/022126.
- Jurikova T, Skrovankova S, Mlcek J, Balla S, Snopek L. 2019. Bioactive compounds, antioxidant activity, and biological effects of European cranberry (*Vaccinium oxycoccos*). *Molecules* 24 (1): 24. DOI: 10.3390/molecules24010024.
- Kaşkonienė V, Bimbraitė-Survilienė K, Kaškonas P, Tiso N, Česonienė L, Daubaras R, Maruška AS. 2020. Changes in the biochemical compounds of *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, and forest litter collected from various forest types. *Turk J Agric For* 44: 557-566. DOI: 10.3906/tar-1912-41.
- Liu P, Lindstedt A, Markkinen N, Sinkkonen J, Suomela J-P, Yang B. 2014. Characterization of metabolite profiles of leaves of bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.). *J Agric Food Chem* 62 (49): 12015-12026. DOI: 10.1021/jf503521m.
- Mahajan M, Kuiry R, Pal PK. 2020. Understanding the consequence of environmental stress for accumulation of secondary metabolites in medicinal and aromatic plants. *J Appl Res Med Aromat Plants* 18: 100255. DOI: 10.1016/j.jarmap.2020.100255.

- Nakhtrishvili G, Batsatsashvili K, Bussmann RW, Ur Rahman I, Hart RE, Haq SM. 2022. The subalpine and alpine vegetation of the Georgian Caucasus—a first ethnobotanical and phytosociological synopsis. *Ethnobot Res Appl* 23: 1-60. DOI: 10.32859/era.23.12.1-60.
- Petrušová V, Bačkorová M. 2024. Determination of phytochemical quality of leaves *Vaccinium vitis-idaea* L. and *Vaccinium myrtillus* L. from the polluted and non-polluted areas. *Environ Monit Assess* 196 (11): 1135. DOI: 10.1007/s10661-024-13157-1.
- Raudone L, Vilkičkyte G, Pitkauskaitė L, Raudonis R, Vainoriene R, Motiekaitė V. 2019. Antioxidant activities of *Vaccinium vitis-idaea* L. leaves within cultivars and their phenolic compounds. *Molecules* 24 (5): 844. DOI: 10.3390/molecules24050844.
- Reshi ZA, Ahmad W, Lukatkin AS, Bin Javed S. 2023. From nature to lab: A review of secondary metabolite biosynthetic pathways, environmental influences, and in vitro approaches. *Metabolites* 13 (8): 895. DOI: 10.3390/metabo13080895.
- Rifnas LM, Vidanaphirana N. 2023. Plant Morphology. University of Colombo Institute for Agro-Technology and Rural Sciences, Sri Lanka.
- Roziaty E, Nurrahman Al Farisi A. 2022. Cantigi (*Vaccinium varingifolium* (Blume) Miq.) di jalur pendakian Gunung Lawu Kabupaten Karanganyar Jawa Tengah. *Bioeksperimen* 8 (1): 20-30. DOI: 10.23917/bioeksperimen.v8i1.18086. [Indonesian]
- Stanisci A, Bricca A, Calabrese V, Cutini M, Pauli H, Steinbauer K, Carranza ML. 2020. Functional composition and diversity of leaf traits in subalpine versus alpine vegetation in the Apennines. *AoB Plants* 12 (2): plaa004. DOI: 10.1093/aobpla/plaa004.
- Ștefănescu B-E, Călinoiu LF, Ranga F, Fetea F, Mocan A, Vodnar DC, Crișan G. 2020. Chemical composition and biological activities of the nord-west romanian wild bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.) leaves. *Antioxidants* 9 (6): 495. DOI: 10.3390/antiox9060495.
- Svanberg I, Ståhlberg S. 2021. Wild European dewberry, *Rubus caesius* L. (fam. Rosaceae), in Sweden: From traditional regional consumption to exotic dessert at the Nobel Prize banquet. *J Ethnic Foods* 8: 37. DOI: 10.1186/s42779-021-00114-3.
- Tong Y-H, Huang Y-S, Ye X-H, Cai Z-Y, Xia N-H. 2020. *Vaccinium napoense*, a new species of *V.* sect. *Conchophyllum* (Ericaceae) from Guangxi, China. *Nordic J Bot* 38 (12): e02773. DOI: 10.1111/njb.02773.
- Tong Y-H, Zhu Y-Y, Ye X-H, Ye X-E, Yang C-Z, Xia N-H. 2021. *Vaccinium zhangzhouense*, a new species endemic to Fujian, China. *Nordic J Bot* 39 (7): e03091. DOI: 10.1111/njb.03091.
- Torres-Guevara FÁ, Ganoza-Yupanqui ML, Mantilla-Rodríguez E, Suárez-Rebaza LA, Bussmann RW. 2023. Ethnobotany of fruit species native to paramos and cloud forests of Northern Peru. *Ethnobot Res Appl* 25: 1-15. DOI: 10.32859/era.25.10.1-15.
- Vilkičkyte G, Raudone L. 2021. Phenological and geographical effects on phenolic and triterpenoid content in *Vaccinium vitis-idaea* L. leaves. *Plants* 10 (10): 1986. DOI: 10.3390/plants10101986.
- Wang L, Wang J, Tan X, Fang C. 2020. Analysis of NOx pollution characteristics in the atmospheric environment in Changchun City. *Atmosphere* 11(1). DOI: 10.3390/ATMOS11010030
- Yu F, Groen TA, Wang T, Skidmore AK, Huang J, Ma K. 2017. Climatic niche breadth can explain variation in geographical range size of alpine and subalpine plants. *Intl J Geogr Inf Sci* 31 (1): 190-212. DOI: 10.1080/13658816.2016.1195502.
- Yulyana A, Chaidir C, Simanjuntak P, Sulastri L, Abdillah S. 2023. The water fraction of Cantigi (*Vaccinium varingiaefolium* Bl. Miq.) fruits demonstrate the highest antimetabolic syndrome properties on enzyme assay. *Pharmacia* 70: 587-594. DOI: 10.3897/pharmacia.70.e109333.
- Yulyana A, Winarno H, Kosasih K. 2016. Karakterisasi ekstrak daun cantigi (*Vaccinium varingiaefolium* Miq.). *Jurnal Sains dan kesehatan* 1 (5): 276-283.
- Zehnder T, Lüscher A, Ritzmann C, Pauler CM, Berard J, Kreuzer M, Schneider MK. 2020. Dominant shrub species are a strong predictor of plant species diversity along Subalpine pasture-shrub transects. *Alp Bot* 130: 141-156. DOI: 10.1007/s00035-020-00241-8.
- Zhang L, Liu P, Li L, Huang Y, Pu Y, Hou X, Song L. 2019. Identification and antioxidant activity of flavonoids extracted from xinjiang jujube (*Ziziphus jujube* Mill.) leaves with ultra-high pressure extraction technology. *Molecules* 24 (1): 122. DOI: 10.3390/molecules24010122.
- Zhou Y, Yang M, Tai Z, Jia J, Luan D, Ma X. 2022. Carbohydrates and secondary compounds of alpine tundra shrubs in relation to experimental warming. *BMC Plant Biol* 22: 482. DOI: 10.1186/s12870-022-03851-y.
- Zoratti L, Jaakola L, Häggman H, Giongo L. 2015. Anthocyanin profile in berries of wild and cultivated *Vaccinium* spp. along altitudinal gradients in the Alps. *J Agric Food Chem* 63 (39): 8641-8650. DOI: 10.1021/acs.jafc.5b02833.