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# Morphoanatomy and Screening Metabolites Profile of *Pararuellia napifera* (Zoll.) Bremek. & Nann.-Bremek. (*Gempur Batu*) at Different Altitudes

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## ABSTRACT

*Pararuellia napifera* (Zoll.) Bremek. & Nann.-Bremek. (*Gempur Batu*) is a wild plant that can thrive in tropical areas and is traditionally used to treat nephrolithiasis. Therefore, this study aimed to explore the morphological, anatomical, and secondary metabolite profiles of *P. napifera* that grows at different altitudes. This study was conducted using a purposive sampling survey method in the lowlands (<400 m.a.s.l) and highlands (>800 m.a.s.l) in Banyumas, Central Java. The results show that *P. napifera* at both altitudes has a shrub habitus with fibrous roots, creeping round stems, alternated simple leaves, *verticillaster* compound flowers, and oval-shaped fruits (*foliculus*) with blackish-brown color flattened round seeds. *P. napifera* in lowland regions exhibit greater morphological characteristics compared to those in highland areas, with an average height of 18.5 cm in contrast to 12.64 cm. The habitus of *P. napifera* in lowland regions also indicates larger stem diameters and leaf sizes. Anatomical analysis of the roots stems, and leaves of *P. napifera* from both lowland and highland regions reveals a consistent tissue arrangement, although differences are observed in the structure of the transport tissues. Meanwhile, the result of phytochemical screening shows that the roots and leaves extract contains flavonoids, alkaloids, terpenoids, tannins, and saponins.
**Keywords:** Anatomy; Flavonoid; Medicinal plant; Morphology; Phytochemistry; Ruellia

## INTRODUCTION

The genus *Pararuellia* syn. *Ruellia* has 250 species of flowering plants generally distributed in tropical countries (Ahmad et al., 2017). Most species are perennial herbs or shrubs, with small trees being rare. Opposite, simple leaves are common, though some species exhibit a rosette form. Flowers are trumpet-shaped, either solitary or compound, and display a range of corolla colors, including blue, purple, pink, red, yellow, and white (Ezcurra, 2008). Some species of this genus have potential as medicine, with various types of secondary metabolites such as flavonoids, lignans, coumarins, alkaloids, terpenoids, sterols, phenol glycosides, phenyl ethanoids, megastigmane glycosides, and benzoxazinoid glucosides (Samy et al., 2015). The diverse group of secondary metabolite compounds can be utilized in the production of drugs to treat various diseases, including flu, asthma, fever, bronchitis, high blood pressure, eczema, diabetes, anti-plasmolytic, anti-inflammatory, antimicrobial, analgesic, gonorrhea, syphilis, skin diseases, etc. (Afzal et al., 2015).

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*Pararuellia napifera* (Zoll.) Bremek. & Nann.-Bremek. syn *Ruellia napifera* Zoll. (*Gempur Batu*) is one of the species of Pararuellia that is also believed to have potential as a medicinal substance, but there is no scientific investigation and data to prove it. *P. napifera* has the habitus of: shrubs approximately 15 cm tall; roots occasionally with nodose thickening; rosette single leaves; spatulate or obovate leaf shapes; and 7 mm trumpet-shaped bisexual flowers with purplish-white corollas (Nugroho, 2016). *P. napifera* has potential as a source of natural antioxidants and antimicrobials, overcoming cardiovascular risk factors, helping protein metabolism, and PPAR alpha gene expression in diabetics (Center, 2015). *P. napifera* is a cosmopolitan herbaceous that is found in tropical regions such as Indonesia both in lowlands and highlands. Meanwhile, the altitude is directly related to environmental conditions such as temperature, light intensity, and humidity (Christian, 2016). Altitude influences the interactions between biotic and abiotic factors, including temperature, humidity, light intensity, rainfall, and soil nutrient content that encourage plant adaptation (Ping et al., 2013a).

Adaptation in plants is related to the metabolic processes that can cause differences in morphological structure, anatomy, physiology, and reproductive patterns of plants (Montesinos-Navarro et al., 2011)(Ping et al., 2013). Plants in lowland areas often exhibit taller growth, longer roots, and broader leaves compared to their highland counterparts (Jenabiyan et al., 2014) (Yuliani et al., 2015). These differences in the anatomical characteristics of high and lowland plants can be observed from the epidermal tissue layer, cuticle, mesophyll, vascular tissue, density, and stomatal index (Tiwari et al., 2013). Environmental temperature influences the enzymatic systems that are essential for plant metabolism (Beusekom et al., 2015). Light intensity influences the density and index of stomata that correlate with the processes of respiration, photosynthesis, and transpiration expressed in plant height, length and number of roots, stem and root diameter, number and surface area of leaves, flowering time, stomatal density and epidermal structure. The existence of incompatible environmental conditions can encourage plants to adapt by increasing secondary metabolism higher than primary metabolism (Fukui et al., 2017).

*P. napifera* has received limited scientific attention, with its potential applications largely underexplored. To date, there has been no comprehensive research focusing on its morphology, anatomy, or secondary metabolite profiles. In this context, performing an in-depth study on these aspects is crucial. Therefore, this study aims to determine the morphological and anatomical characteristics as well as secondary metabolite profiles of *P. napifera.* growing at different altitudes. By thoroughly exploring these attributes, the plant's potential can be effectively harnessed and developed for broader applications.

## MATERIALS AND METHODS

### Materials

In this study, the materials used included *P. napifera* plants obtained from lowlands and highlands. This study was conducted using *a* purposive sampling survey method in lowlands (<400 m.a.s.l) and highlands (>800 m.a.s.l) in Banyumas District and the surrounding areas. Morphological observations of *P. napifera* plants included the roots, stems, leaves, flowers, fruits, and seeds. The anatomical structure was examined using preserved preparations prepared through the embedding method. Materials used for anatomical preparations are alcohol, xylol, paraffin, and safranin. While secondary metabolite profiles were collected from the roots and leaves. Materials for phytochemical screening are Cyanidin reagent, $H_2SO_4$, Dragendorff reagent, $FeCl_3$ 10%, Liberman Burchard reagent, and HCl.

### Morphological character observation

*P. napifera* plants obtained were then observed and measured. The observation parameters included (1) the habitus; (2) the plant height; (3) the root, *i.e.* its type, length, and diameter; (4) the stem, *i.e.* its length, diameter, shape, type, growth direction, color, and stem surface; (5) the leaf, *i.e.* its length, width, type, and shape; (6) the flower, *i.e.* its number, type, color, location, shape, and floral formula; (7) the fruit, *i.e.* its length, width, shape, color, and type; and (8) the seed, *i.e.* its diameter, shape, and color.

### Anatomical preparation and observation

The preparation of leaf, stem, and root tissue solutions was carried out by using the *Sass Embedding Method* that had been modified (Sass, 2011). First, fixate the samples for about 24 hours, and then dehydrate the prepared solutions using graded alcohol (70%-80%-96%-100%). Furthermore, dealcoholize the prepared solutions with alcohol and xylol (3:1; 1:1; and 1:3). Then, the prepared solutions were added with xylol:paraffin (1:1). Embed the prepared solutions in paraffin and slice each with 10-15 µm in thickness. Samples were colored with safranin and then mounted the prepared solutions. The samples were observed using a light microscope at 40x and 100x magnification.

### Screening of phytochemical profile

Phytochemical profile analyses were carried out by drying all parts of the *P. napifera* organ and then macerating it using ethanol for three days. The extracts were tested for flavonoids, alkaloids, tannins, terpenoids, and saponins. Phytochemical screening was carried out using a color test with reagents using the methods of Harborne with modifications (Harborne, 1984).

A flavonoid test was conducted from 1 g of extract reacted with 1 mL of Cyanidin reagent. The test results were positive for flavonoids if there was a color change to orange, red, green, or yellow. The alkaloid test of 1 mL of extract was reacted with concentrated $H_2SO_4$ and Dragendorf reagent. It tested positive for alkaloids if there was a change in color to

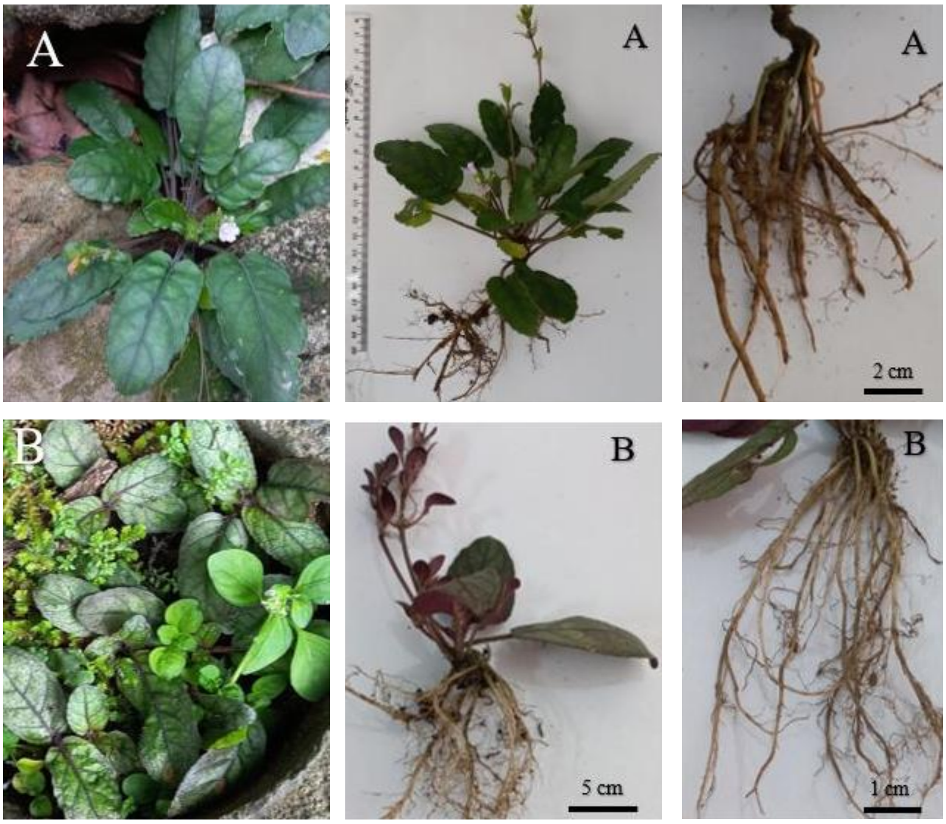


**Figure 1. Comparison of habitus and morphological characters of *Pararuellia napifera* (Zoll.) Bremek. & Nann.-Bremek. plants that grow in lowlands (A) and highlands (B)**

light yellow (+), yellow (++), and brown (+++). The tannin test was carried out with 1 mL extract reacted with $FeCl_3$ 10% and tested positive if a dark green or blue color was formed. A terpenoid/steroid test was performed with 1 mL of extract reacted with Liberman Burchard reagent. The test results were positive for terpenoids if there was a change in color to red or yellow, while positive for steroids if there was a change in color to blue or purple. The Saponin test was carried out with 1 mL of extract added by 2 mL of hot distilled water then shaken, and after the foam appeared, HCl was added. It was positive for saponin if the foam remained, *i.e.* with a foam height category of 1 cm (+), 2-4 cm (++), and ≥ 5 cm (+++).

### Data analysis

The data of morphological characters (roots, stems, leaves, flowers, fruits, and seeds), anatomical characters (roots, stems, and leaves), and phytochemical profiles were analyzed descriptively to determine the differences in *P. napifera* growth in lowlands and highlands.

## RESULTS

### Morphology and Anatomy of *Pararuellia napifera* (Zoll.) Bremek. & Nann.-Bremek. At Different Altitudes

*P. napifera* at both altitudes had the same morphological characteristics qualitatively, and they both had a shrub habitus with fibrous roots (Figure 1). The shape of the stems was round (*teres*), woody type with creeping growth direction (creeping along the ground and roots coming out from the nodes); its surface was smooth with a hairy texture, and was dark green to brown. The leaves were simple ones with phyllotaxis alternate, dark green color was on the upper surface and light green or purplish green color was on the lower one; its shape was oval (*ovatus*), some had *obtuse* with *acutus* ends or *obtuse* with *cordate* ends, it had pinnate leaf veins (*penninervis*), *crenatus* leaf margins with hairy surface and also thin leaf flesh membrane (*membranaceus*) (Figure 2). There was also *flos terminalis* in which compound flowers of *verticillaster* type appeared at the tip of the leaf. It appeared with sessile attachment, purplish-white

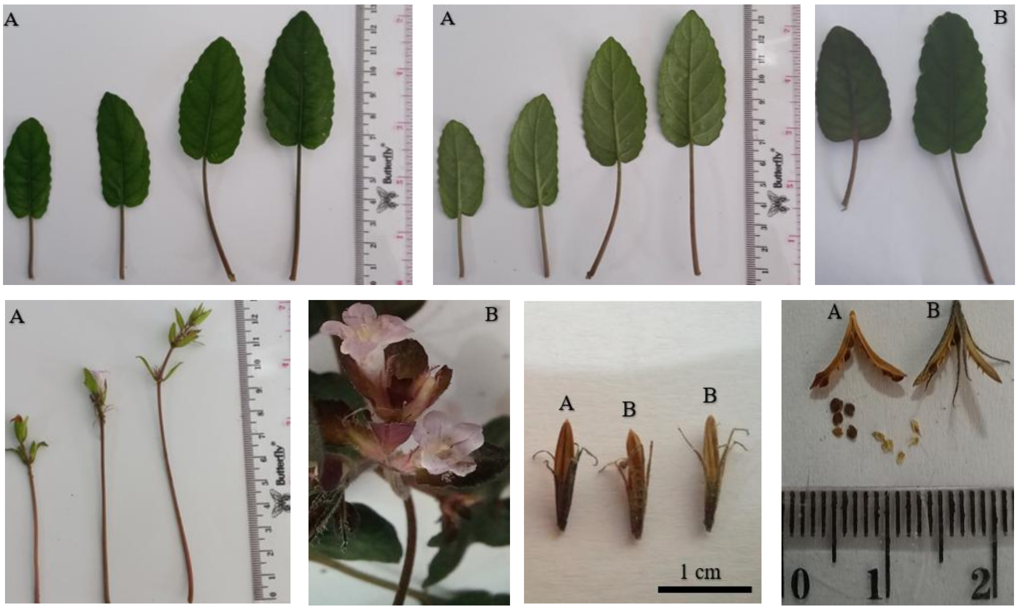


**Figure 2. The diversity of leaves morphology, flower structure, fruit shape, and seeds of *Pararuellia napifera* (Zoll.) Bremek. & Nann.-Bremek. that grow in lowlands (A) and highlands (B)**

corolla color, and multiple symmetry attached petals (*sympetalus gamopetalus*) in the shape of a small trumpet which has the floral formula *[C(5)A2+2]G1. The oval-shaped fruit was green when young and brown when old (*foliculus*). The seeds are flat and round with a blackish-brown color.

The observation showed that *P. napifera* growing in the lowlands had a larger size than in the highlands (Figure 3). The average height of the species grown on lowlands was 18.5 cm, compared to 12.64 cm on highlands. The number and length of roots from both altitudes were not different, while the diameter of roots growing in the lowlands was greater (*i.e.* 0.3 cm) than the ones in the highlands (*i.e*. 0.1 cm). The stems of *P. napifera* growing at low altitudes were averagely longer (*i.e.* 2.07 cm) than at high altitudes (*i.e.* 0.47 cm). The average number of leaves in the lowlands (15.55) was more than in the highlands (9) with almost the same blade size, while the stalks were longer in the lowlands (4.66 cm) than in the highlands (2.06 cm). The number of flowers produced by one individual in the lowlands was an average of 6.3, while in the highlands was 4.5 with almost the same size of fruits and seeds.

The anatomical structure of the roots, stems, and leaves of *P. napifera* had almost the same tissue arrangement at both altitudes (Figure 4). The results of cross-sections of the roots at low and high altitudes were composed of a layer of epidermal tissue, cortex, and vascular tissue with polyarch-stele type. The difference could be seen in the number of root hairs that grew laterally on the roots of *P. napifera* in the lowlands compared to the highlands. The roots of *P. napifera* that grew at high altitudes had a thicker epidermal tissue structure. The cells in the cortex tissue were larger than at low altitudes. In cross-sectional anatomy, the stem had a layer of epidermis, cortex, and transport tissue. The difference in stem anatomy structure could be seen in the thickness of epidermal tissue in the highlands that was thicker than the lowlands. Leaf tissue structure consisted of epidermis, parenchyma consisting of palisade and sponges, as well as transport tissues. The difference in leaf anatomy was visible in the epidermal tissue of leaves that grew at high altitudes where it had thicker cells than the ones in the lowlands.

### Phytochemical Profile of *Pararuellia napifera (Zoll.) Bremek. & Nann.-Bremek.* at Different Altitudes

The phytochemical analyses were carried out by the maceration process of leaves and root simplisia of *P. napifera* using ethanol. The results of the phytochemical screening of *P. napifera* in leaves and root extracts showed positive flavonoids, alkaloids, terpenoids, tannins, and saponins (Table I). The highest contents were alkaloid and saponin from leaf extracts that grew in lowlands. Subsequently, root extracts were also positive for the five classes of phytochemical

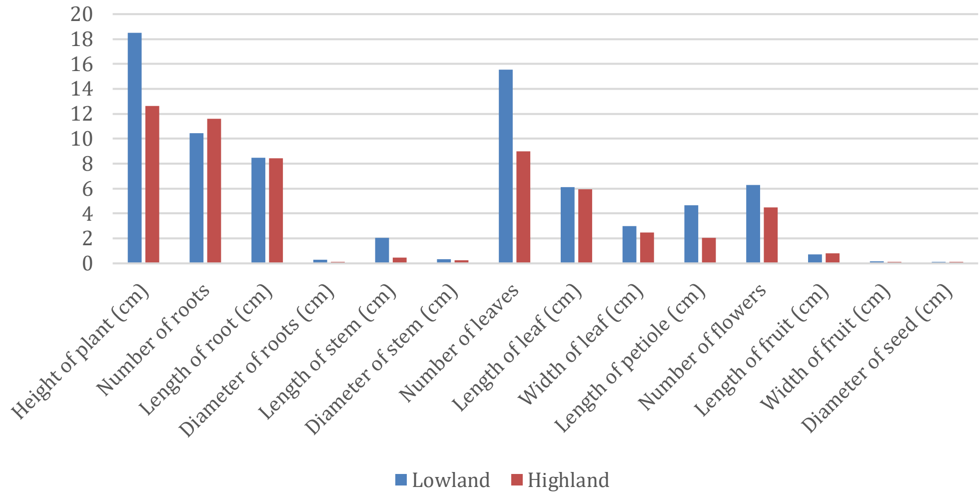


**Figure 3. The comparison of morphological characters between *Pararuellia napifera* (Zoll.) Bremek. & Nann.-Bremek. that grow in lowlands and highlands quantitatively**

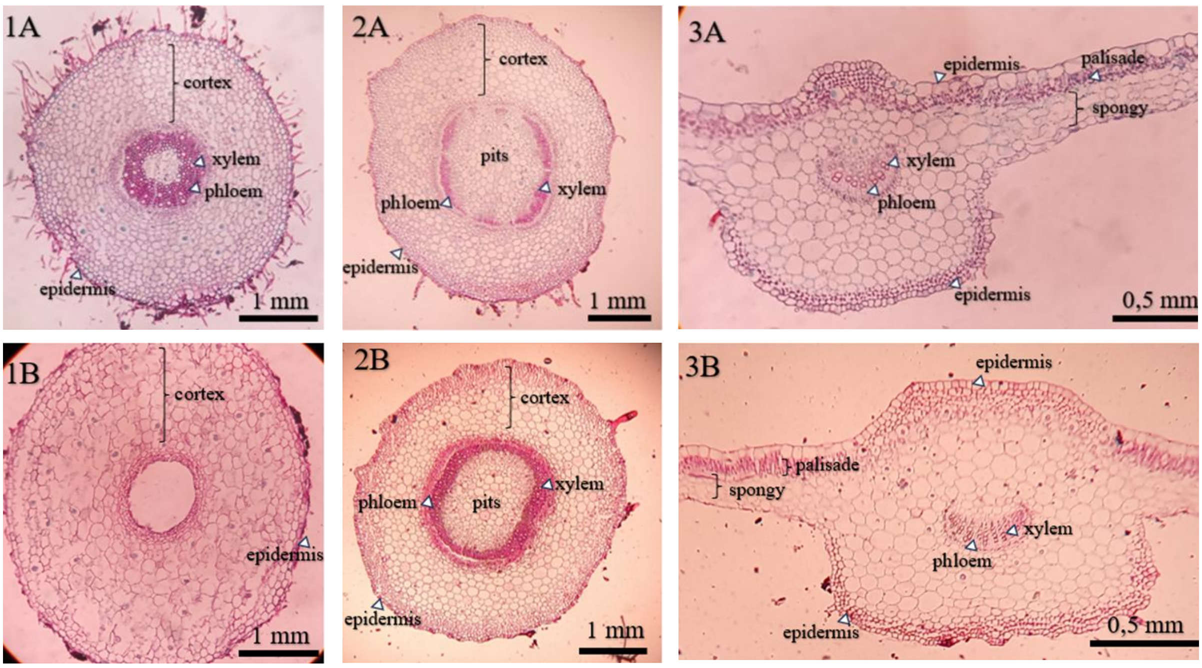


**Figure 4. The comparison of the anatomical structure of roots (1), stems (2), and leaves (3) of *Pararuellia napifera (Zoll.) Bremek. & Nann.-Bremek.* that grow in lowlands (A) and highlands (B)**

compounds but the content was lower than the leaves.

The screening results of flavonoid, tannin, and terpene contents in the leaves and root extracts of *P. napifera* from lowlands and highlands showed similar contents. The screening results of alkaloid and saponin of root extract from both lowland ls and highlands areas showed the same results, while the leaves extract showed different results. The leaves extract from the lowlands area had more concentrated alkaloid and saponin contents when compared to the highlands. These differences were indicated by the brown color of the alkaloid extract (+++) and the height of the foam produced by the saponin extract (≥ 5 cm, +++).

## DISCUSSION

The morphological characteristics of *P. napifera* in the lowlands were higher than the one's highlands. The species obta*ined* from the lowlands had a larger habitus, taller plants, larger

**Table I. The phytochemical profile of leaves and roots extracts of *Pararuellia napifera* (Zoll.) Bremek. & Nann.-Bremek. that grow at different altitudes**

| Altitude | Organs | Phytochemical compounds | | | | |
|---|---|---|---|---|---|---|
| | | Flavonoids | Alkaloids | Tannins | Terpenes | Saponins |
| lowland (<400 masl) | Leaves | ++ | +++ | + | + | +++ |
| | Roots | + | + | + | + | + |
| highland (>800 masl) | Leaves | ++ | ++ | + | + | ++ |
| | Roots | + | + | + | + | + |

Note: Indicates the presence (+) low content; (++) medium content; (+++) high content

roots, and stem diameters, more leaves with more intense colors, larger leaves sizes, and more flowers than in the highlands (Figure 3). Morphologically, it showed that the lowland was a suitable place for the growth of *P. napifera.* The difference in size was a form of adaptation of *P. napifera* to different environmental conditions. Lowlands were optimal places for the growth of *P. napifera*, while highlands could cause growth to be inhibited or distorted. Factors in the growing environment strongly influence the photosynthesis and metabolic processes of plants. In the lowlands, the environmental conditions was suitable for the metabolism of *P. napifera*, thereby, it could take place optimally. In addition, *P. napifera* could also grow with optimal morphological characteristics. Most plants that grew at higher altitudes had shorter, smaller leaves, and more trichomes. Smaller leaves' surface area affected the absorption of carbon dioxide as a source of plant energy, allowing the size of the plant to also be smaller. The existence of trichomes with a greater number of plants in the highlands played a role in maintaining moisture and protecting plants from intense sunlight (Hashim et al., 2020).

The anatomical structure of *P. napifera,* which grew in the lowlands and highlands, had the same tissue structure. The tissue structure of root stems, and leaves consisted of epidermis, ground tissue (*parenchyma*), and transport tissues (*xylem* and *phloem*). The distinguished anatomical structure of the two altitudes was in the epidermal tissue. Epidermal tissue in the roots stems, and leaves of *P. napifera* growing in the highlands had a larger cell size, therefore the epidermal tissue layer appeared thicker than the lowlands. This condition was a way for plants to adapt to highlands that had lower temperatures. In general, plant adapt in the same way. At high altitudes, *Fritillaria unibracteata* had significantly reduced plant height and reduced leaf area (Xu et al., 2020). In *Vicia unijuga* A. Braun, *Medicago sativa* L. and *Onobrychis viciifolia* Scop growing at low altitudes had ir-regular epidermal cell shapes, spongy mesophyll tissue, and increased leaves thickness (Zhou et al., 2023). These changes could be caused by UV intensity and low temperature therefore through metabolism plants made adaptations as a defense response. Altitude created a different climate in the environment. Fluctuations in climate change at high altitudes were also common. The differences in environmental conditions of different altitudes included temperature, humidity, light intensity, air pressure, wind speed, and rainfall. Each of these factors forms environmental conditions that directly and indirectly contribute to the development and survival of plants. Lowlands are characterized by lower humidity and higher temperature, while highlands exhibit higher humidity and lower temperatures. As altitude increases, the air becomes cooler and drier. Plants at high altitudes are subjected to various environmental stressors, including high UV exposure, extreme temperature fluctuations, strong winds, and variable humidity conditions. Additionally, higher altitudes are associated with reduced air pressure, lower concentrations of carbon dioxide and oxygen, and decreased water and nutrient availability (Roupioz et al., 2016). Carbon is one of the most important compounds for the sustainability of all plant species. To survive in these conditions, plants would adapt by changes to their morphology, anatomy, and physiology. The adaptation that appeared morphologically was from the size of the plant being smaller in the highlands compared to the lowlands. Anatomical adaptation could be seen from the size of the epidermal tissue of *P. napifera* in the highlands, which was thicker than the lowlands.

The results of this analysis indicated that the height of the growing area could not be categorized as “a stress factor” for the production of secondary metabolites of *P. napifera.* The screening results showed that the height at which it grow descriptively influenced the alkaloid and saponin contents of *P. napifera.* The physiological and biochemical responses of plants reflect their adaptation to environmental conditions. *P. napifera,* a cosmopolitan wild plant, shows high tolerance and grows naturally in suitable environments without cultivation. Apart from adaptation to the environment, *P. napifera*, which grows in the lowlands has more optimal plant morphological characteristics than the highlands. *P. napifera* in the lowlands has the highest plant height, number, and size of leaves compared to the highlands, but the phytochemical profile is not descriptively different.

The number and size of leaves are some of the factors that can influence the photosynthesis process of plants. Michaletz et al., (2016) explained that leaves size is one of the determinants of leaf thermoregulation because it influences the leaf energy balance between the solar radiation heating and the transpiration cooling processes through leaf tissue. The thermoregulation of wider leaves can support the activity of photosynthetic enzymes; thereby the production of secondary metabolites such as flavonoids, alkaloids, and polyphenols is more optimal. However, environmental conditions such as high light intensity and high temperature of where it grows can also limit the efficiency of photosynthesis, which can affect the content of secondary metabolites produced. Subsequently, secondary metabolite production has certain limitations related to supporting environmental factors. The alkaloid and saponin contents of *P. napifera* are more concentrated in lowland plants, aligning with their larger and more abundant leaves.

Some of the findings showed that total phenols, flavonoids, and bioactive compounds such as gallic acid, ascorbic acid, and quercetin showed a positive correlation with altitude ($p < 0.01$), while the tannin, taxol, and flavanol contents of the *Taxus wallichiana* plant did not show any correlation to the height at which it grows (Adhikari et al., 2022). The flavonoid (1.529 ± 0.167% w/w) and phenolic (1.65 ± 0.006% w/w) contents showed that the *Phylanthus niruri* sample taken at the altitude of 243 m.a.s.l was significantly higher than the one at the altitude of 772 m.a.s.l (1.180 ± 0.041% w/w) and 104 m.a.s.l (0.973 ± 0.105% w/w) (Wahyuni et al., 2023). Studies on strawberries at the altitudes of 0 – 1000 m.a.s.l and 1000 – 2000 m.a.s.l had not identified any significant differences in phenol content and antioxidant activity (Guevara-Terán et al., 2023). The total average of phenolics and flavonoids in *Sphagnum junghuhnianum* extracts of Mount Alab and Mount Kinabalu populations at the altitude range of 1,800-2,300 m.a.s.l did not differ significantly (Majuakim et al., 2014). The accumulation of bioactive compounds in plants is influenced by environmental conditions, but as long as these conditions do not become overly stressful, the content of secondary metabolites remains stable. Environmental conditions are greatly influenced by several factors including rainfall, humidity, temperature, light intensity, wind speed, and nutrients in the soil (Ping et al., 2013b). These factors will influence the metabolic processes in plants, both primary and secondary. Theoretically, secondary metabolites will be higher if plants are in extreme environmental conditions that provide stress to the plant. The presence of stress will force plants to respond to the environment through the production of secondary metabolites, which are known to be a form of plant defense and adaptation, although their function for plants is not yet known with certainty (Fukui et al., 2017).

## CONCLUSION

In conclusion, *P. napifera*, which grows in the lowlands, has a taller plant habitus, stem size, and number of leaves, as well as several flowers. Subsequently, root, stem, and leaf tissue of *P. napifera* grown in lowlands and highlands have almost the same anatomical characteristics, the difference is in the thickness of the tissue. *P. napifera* in the highlands has thicker tissue than the lowlands and a higher secondary metabolite content than the highlands. Alkaloids and saponins are the highest compounds from leaves extracts that grow in lowlands.

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