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Plant Morphology, Paradermal Anatomy, and Leaves Metabolite Profiles of *Rhododendron multicolor* Miq. from Cibodas Botanic Garden, West Java, Indonesia

Devy Janisca Shalihi[1], Neni Murniati [1], Wiguna Rahman[2], Prima Wahyu Kusuma Hutabarat[3], and Intani Quarta Lailaty[2*]

[1]Department of Biology Education, University of Bengkulu
(Bengkulu, Indonesia, 38371)
[2]Research Center for Applied Botany, National Research and Innovation Agency (BRIN)
(Cibinong, West Java, Indonesia)
[3]Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN)
(Cibinong, West Java, Indonesia)

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*__Corresponding author__: Intani Quarta Lailaty; **e-mail**: inta009@brin.go.id

ABSTRACT. *Rhododendron multicolor Miq. exhibits distinctive morphoanatomical characteristics and various bioactive compounds with medicinal and horticultural potential. Numerous studies indicated Rhododendron species are widely utilized as medicinal and ornamental plants, owing to their diverse phytochemical profiles and distinctive morphoanatomical characteristics. However, comprehensive studies of the morphoanatomy and leaves metabolite profile of R. multicolor are still limited globally, as well as its minimal utilization by local communities, highlighting a significant knowledge gap. Therefore, this study aimed to analyze the morphology, paradermal anatomy, and metabolite profile of young leaves and mature leaves of R. multicolor. Morphoanatomy characteristics were analyzed descriptively, anatomical features were analyzed using specific formulas, and metabolite profiling was analyzed using qualitative phytochemicals and gas chromatography-mass spectrometry (GC-MS). The result shows that R. multicolor is a shrubby plant characterized by funnel-shaped flowers in a vibrant red hue, along with narrowly elliptic, scaly leaves. The scales are stellate lobed irregularly with lower density than stomata; the epidermis is polygonal to irregular that has a higher density compared to stomata. The results of the qualitative phytochemical test of Rhododendron multicolor are that it contains phenols, flavonoids, Mayer alkaloids, Bouchardat alkaloids, Dragendorf alkaloids, tannins, and saponins. Based on GC-MS analysis of young leaves and mature leaves of R. multicolor, a total of 31 metabolite compounds from 21 compound groups were identified, with the major compound being squalene from the terpenoid group, which has the potential to be an antioxidant, anticancer, antibacterial, antifungal, antitumor, and cardioprotective.*

Keywords: *Morphoanatomy, metabolite compounds, Rhododendron multicolor Miq, Vireya, Cibodas Botanic Garden.*

INTRODUCTION

Rhododendron is one of the largest genera in the Ericaceae family, where many of their species have a high horticultural value and medicinal properties. *Rhododendron* is widespread across the world and has been cultivated as ornamental plants. It is a great choice for ornamental use in gardens and landscapes due to its eye-catching foliage and vibrant blossoms [1, 2, 3, 4]. Besides as an ornamentals in China, around 25 species of *Rhododendron* is widely used as traditional medicine for the treatment of respiratory diseases, pain, bleeding, inflammation, arthralgia, rheumatism, and pain [5]. In Nepal, *Rhododendron arboreum* is used as a drug with low side effects. *Rhododendron* is used as an antiviral drug and antibiotic activity [6].

The diversity of *Rhododendron* in Indonesia ranks second across the world after China [7]. There are approximately 200 species of *Rhododendron* that grow and spread from Sumatra to Papua, some of which are endemic plants to Indonesia (Vireya group). The Cibodas Botanic Garden has 14 species of *Rhododendron*, six species come from the Vireya group. One of the species that attracts attention is *Rhododendron multicolor* Miq. from Bengkulu [8]. *Rhododendron multicolor* was collected from Kaur Regency, Bengkulu City, on November 8, 2023 [9].

R. multicolor remains underexplored potential, a consequence of both insufficient scientific inquiry and its underutilization within local contexts. Several species of *Rhododendron* are still unfamiliar and have not been adopted by the local people or the wider community in general

[10]. However, there are many *Rhododendron* species that have been studied, especially regarding their morphoanatomy and metabolite profiles, showing uniqueness of morphoanatomy and bioactivity of metabolite compounds. For instance, the studied *R. sessilifolium* has unique trumpet-shaped flowers [11], *R. macgregoriiae* from Papua has striking bright yellow flowers [12], *R. retusum* leaves have flavonoid compounds that show bioactivity such as antibacterial, antiviral, antidiabetic, analgesic, and spasmolytic [13].

Therefore, this research aimed to analyze the morphology, paradermal anatomy, and metabolite compounds of *R. multicolor* in Cibodas Botanic Garden (CBC) to evaluate its phytochemical and horticultural potential for improving its potential as a medicinal and ornamental plant, contributing to scientific advancement.

RESEARCH METHODS

Plant Morphology Observation of *Rhododendron multicolor*

The *R. multicolor* morphology was observed directly and used stereo microscope then documented on each part. Furthermore, the results were described descriptively compared to a literature study from Rhododendrons of Subgenus Vireya's book and Plant Morphology book. The samples were obtained from plants collection in the Cibodas Botanic Garden. Parameters observed based on the morphological characterization are *R. multicolor*'s stature, habitat, height, leaves (type, shape, arrangement, texture, color, position, length, diameter, vein type, edge type, apex and base type, scales type, and scales and stomata distribution), buds (shape, apex and base type, texture, length, diameter, and color), flowers (type, shape, arrangement, growth direction, position, quantity in one pedicle, texture, color, length, diameter, blooming period, color of pistils and stamens, stamens height and shape, and texture and color of petioles), bracts (shape, texture, length, and diameter), fruits (type, shape, apex and base type, texture, position, length, diameter, color, tail's type and length, and pedicles color), seeds (type, position, quantity, texture, shape, and seed tail's type), stems (type, shape, texture, growth direction, color, and position), and root type.

Paradermal Leaves Anatomy Observation of *Rhododendron multicolor*

The paradermal anatomy of young leaves and mature leaves of *R. multicolor* were examined using the replica method with nail polish. Healthy young leaves and mature leaves were selected for preparation. The leaves were cleaned with tissue, then applying clear nail polish topcoat on the abaxial and adaxial. After the nail polish has dried, cover it with tape, and then the tape is pulled slowly to produce the impression of stomata, scales, and epidermises. The impressions were mounted placed on glass objects and observed under an OptiLAB light microscope (Olympus CX23) at 4x, 10x, and 40x magnifications. Measurements were performed using Image Raster 3.0 software, with five replicates for each parameter. Observations included epidermal characteristics (shape, number, size, and location), scales (shape, number, size, structural characteristics, and location), and stomata (shape, number, size, location, stomatal index, and stomatal density). Stomatal density (SD) and stomatal index (SI) were calculated using established formulas [17].

$$SD = \frac{\Sigma \text{ Stomata}}{\text{Field Area}}$$

$$IS = \frac{\Sigma \text{ Stomata}}{\Sigma \text{ Stomata} + \Sigma \text{ Epidermis}} \times 100$$

Field area on the magnification area of 10x the adaxial and abaxial mature leaves are 0.77 mm. Field area on the magnification area of 40x the adaxial and abaxial mature leaves are 0.05 mm.

Metabolite Profile Analysis of *Rhododendron multicolor*

The metabolite profile of young leaves and mature leaves of *R. multicolor* were observed using methanol maceration 1:10 and Gas Chromatography (GC) Agilent 7890B and mass spectrometer (MS) 5977A(MSD). In maceration technique, sample preparation involved drying young leaves and mature leaves in an oven at 40°C until a constant weight was achieved, followed by grinding the dried material into a fine powder. The simplicia were dissolved using methanol 1:10 (1 gram simplicial:10 ml methanol) in glass bottles covered by aluminum foil, then shaken using a shaker for 24 hours. Then, the extracts were filtered using Whatman filter paper. The filtrations were concentrated to obtain a crude extract. The crude extracts were transferred into 1.5 ml tubes and stored at a temperature of 4°C for GC-MS analysis. GC-MS' merk was Agilent Merk and type was 19091S-433:93.92873 DB-5MS UI 5% Phenyl Methyl Silox. Dimention was 0°C—325°C (325°C):30 m x 250 μm x 0.25 μm. The volume of the sample was injected for analysis as much as 1 μL. The initial temperature was set at 40°C and the post run temperature at 300°C, then ran for 1 minute. GC-MS analysis results are Retention Time (RT), compound name, formula, area, and match score. All of the compounds were selected based on a match score above 85. Then, the selected compounds were identified and recorded according to the biological potential and group compounds based on standardized literature. Data of RT, compound name, area, match score, compound group, and biological potential were collected using Microsoft Excel.

RESULTS AND DISCUSSIONS

Plant Morphology of *Rhododendron multicolor*

R. multicolor is a shrubby plant with taproot that has a height of 118.25 cm ±16,997, a habitat in the terrestrial, and no glandular hairs (Figures 1A & 2).

Leaves of *Rhododendron multicolor*

R. multicolor's leaves are simple, coriaceous, with prominent midvein both side, narrowly elliptic in shape, size of young leaves 4.4 cm ±0.05 × 0.96 cm ±0.057, size of mature leaves 20.5 cm ±0.15 × 2.23 cm ±0.25, a whorled arrangement with five leaves, entire margin, obtuse leaves base with leaves edge meeting at the base on the same side, upper leaves surface glabrous and glossy (*nitidus*), lower leaves surface glaucous, leaves color red when young with the adaxial leaves redder than the abaxial and turn green when mature with the adaxial leaves greener than the abaxial, brown scales can be seen directly on the abaxial leaves and sometimes in petioles. scales occur sparcely, the epidermis on some sides can be seen directly, but the stomata cannot be seen directly, but the stomata cannot be seen directly (Figures 1D, 2A, 2B, & 3A). Finely scaly petioles that can be seen directly with a sparse scale-spreading type. Petioles are without hairs, slender, cylindrical in shape, and located at the edges (*basilicas*) (Figure 2C & 2E).

Buds of *Rhododendron multicolor*

R. multicolor's buds are elliptical with the conical apex upwards and pointed. The base is bluntly rounded, with bracts apex spreading, a scaly surface at the apex and hairless, reddish-yellow-green in color, flat edges, glaucous surface, smooth texture, measuring 2.9 cm ±0.1 x 1.6 cm ±0.96, and surrounded by simple white hairs at the apex (Figures 1C, 2D, & 3F).

Flowers of *Rhododendron multicolor*

R. multicolor's flowers are complete inflorescences composed of multiple florets, *funnel-shaped*, arranged in a circle (*cyclic*), flower arrangement to the base of the flower forms a structure like a cup, grows upwards (*perigynous),* half-hanging flower position, flowers located on the apex of the branches (*terminal*), actinomorphic symmetrical flower shape, number of flowers 3-6 in one peduncle, smooth, and slightly fleshy flower texture, striking red flowers, flower corolla classified as polypetalous with five non-overlapping petals (*valvate),* flower size 4 cm ±0.2 x 2.2 cm ±0.2, rounded at the base, and red with yellow pistils head color, pistils bare, stamens 7-10 of unequal length (*dimorphic*), located under the ovaries and pistils (*apical*), and stamens red. The pedicel is cylindrical, finely textured without hair and scale, striking red, and fleshy (Figures 1B & 3B). The bracts originating from the bud have an elliptical shape, flat edges, smooth texture, cream-colored, measures 2.16 cm ±0.28 x 0.6 cm ±0.1, scaly at the apex, and without hair (Figures 1C, 2D, & 3C).

Fruits of *Rhododendron multicolor*

R. multicolor's fruits are *dehiscent* fruit, cylindrical elongated capsule, blunt apex and base, smooth and shiny fruit surface, thick and fleshy fruit skin. The exocarp is not separate, the position of the fruit at the end of the stalk, the size of the fruits are 1.76 cm ±0.208 x 0.4 cm ±0.1, the texture of the fruits are smooth, the fruit and its pedicle is green when young and turned dark maroon when ripe, pedicel is 1.7 cm ± 0.25 (Figure 1E & 3D).

Seeds of *Rhododendron multicolor*

R. multicolor's seeds are albumin seeds, the position of the seeds spread in capsules in large quantities and small in size such as dust, fine seeds texture, round seed shape, tailless, and seeds length are 0.17 cm ±0.02 (Figures 1F & 3E).

Stems of *Rhododendron multicolor*

R. multicolor's stems are woody stems (*ligneous*), cylindrical in shape, with a smoky surface (*saber*), and grooved (*sulcatus*), monopodial stems branching, clear stems structure, perpendicular (*erectus*) direction of growth towards the direction of light (*phototropism*), multi-branched, green when young and brown when mature, located at ground level, and neither scaly nor hairy (Figures 1A & 2F).

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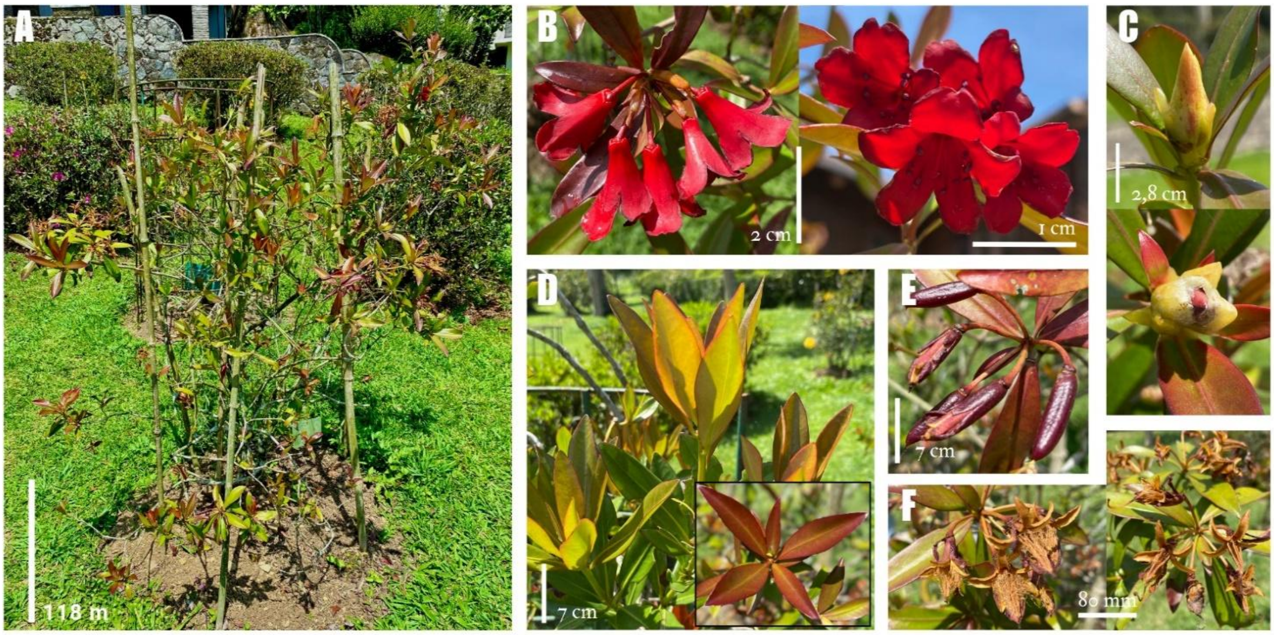

Figure 1. Morphology of *Rhododendron multicolor* in Cibodas Botanic Garden. Note: (A) habit, (B) flowers, (C) buds, (D) leaves, (E) fruits, and (F) seeds.

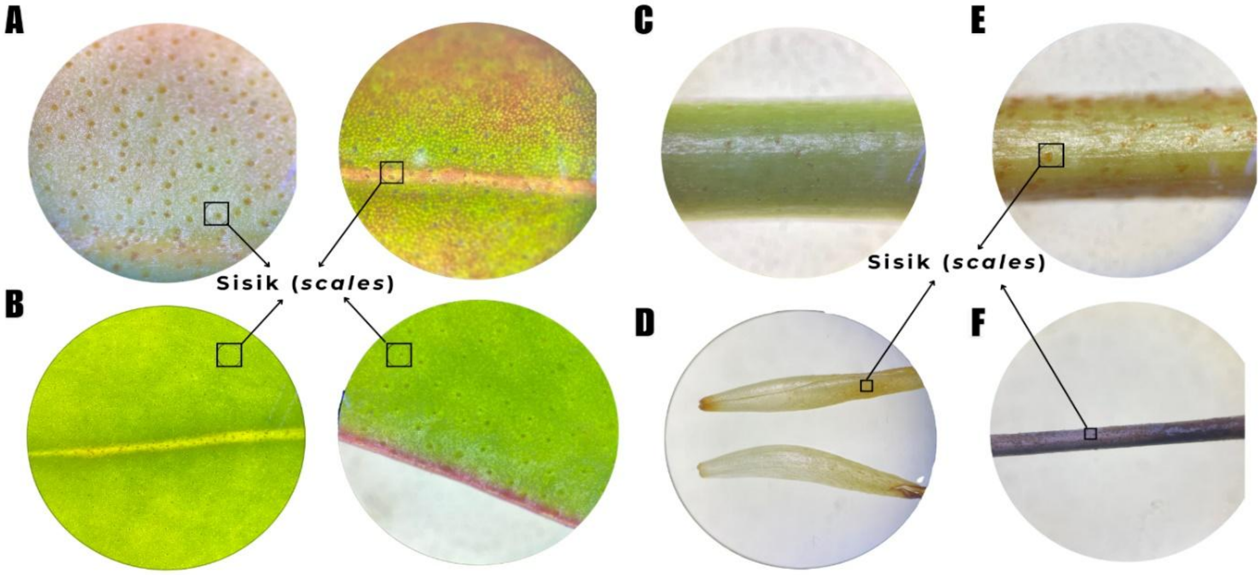

Figure 2. Morphology of *Rhododendron multicolor* on a stereo microscope. Note: (A) young leaves, (B) mature leaves, (C) petiole, (D) bracts, (E) pedicle, and (F) stem.

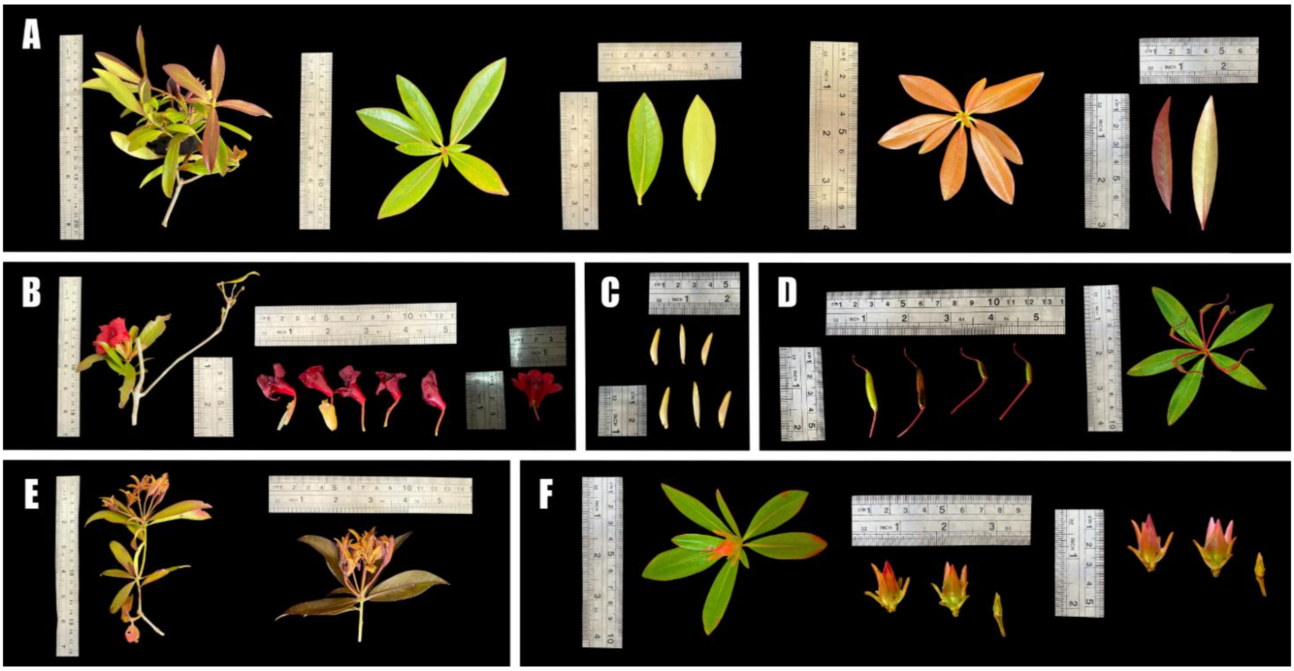

Figure 3. Measurement of *Rhododendron multicolor*. Note: (A) leaves, (B) flowers, (C) bracts, (D) fruits, (E) seeds, and (F) buds.

Paradermal Leaves Anatomy of *Rhododenron multicolor*

R. multicolor exhibits polygonal to irregular epidermal cells of varying sizes. The epidermal cells are tightly arranged, yellowish-green, and the number of epidermal cells are more numerous on the abaxial than on the adaxial (Figures 4 & 5). The epidermal cells are often slightly larger than the stomata based on figures 4C and 5C, resulting in a relatively low stomatal density. The size of epidermal cells significantly influences the number of stomata visible in the field of view; smaller epidermal cells are associated with higher stomatal density. Due to smaller epidermal cells providing wider space for stomata in one unit area with smaller stomata size without overlapping each other [14]. Moreover, environmental factors that influence epidermal cells will also influence stomatal density as an effort to adapt to the environment, such as when gas exchange efficiency occurs in an environment with limited CO^2 availability and lack of water [15].

Stomata of *R. multicolor* are found only on the abaxial surface of young leaves and mature leaves (hypostomatic) (Figures 3 & 4). Stomata are usually found on the abaxial surface of the leaves [16]. Stomata are gaps on the epidermis that are bound by two specialized cells called guard cells and neighboring cells [17, 18, 19]. *R. multicolor's* stomata are paracytic, greenish yellow in neighboring cells, pink on the outside of the guard cells and purple on the inside, when the stomatal cells open there is a green stomatal pore. The placement of the stomatal cells is spread out and sparse (not dense), with the size of the stomatal cells varying, most of which appear smaller than the epidermal cells. The average size of *R. multicolor's* stomata is medium resulting in a relatively medium density based on Figure 4C & 5C and Table 1 & 2. According to research, each plant has different stomata sizes, influenced by internal factors, such as genetic characteristics and external factors in the form of the environment where the plant is located [16, 20]. The size of the stomata is often correlated with the stomatal density, so small stomata give high density, and large ones give low density [21]. The density and index of stomatal cells in young leaves are higher than stomatal cells in mature leaves (Figures 3 & 4), it is influenced by internal and external factors. The internal factors may be due to the presence of young leaves in a phase of rapid growth and development. The higher density of stomata in young leaves allow for higher gas exchange for photosynthesis [22]. Increased stomatal density helps young leaves manage water loss effectively. As mature leaves, they may develop mechanisms to reduce water loss, resulting in lower stomatal density. As young leaves enlarge, more stomata are needed so that the stomatal density is maximum. As leaves reach full size and begin to age, the stomatal density tends to stabilize or decrease [23]. In addition, young leaves may also be more exposed to environmental conditions such as light intensity and humidity, which can affect stomatal development. For example, higher light conditions often correlate with increased stomatal density to optimize photosynthesis under favorable conditions [24]. As leaves mature, physiological changes occur that may result in fewer stomata being required for effective gas exchange due to changes in leaves structure and function. Mature leaves may have developed thicker cuticles or other adaptations that reduce the need for high stomatal density while still maintaining adequate gas exchange rates [25]. Therefore, changes in the size, number, and type of stomatal distribution can occur due to internal and external factors (light intensity, air temperature, and soil pH) [26, 27].

Scales of *R. multicolor* are stellate lobed irregularly shaped, broad marginal flange with a small center, lobes fused with tapered ends, yellow in the lobe, the center of the scales is brown small circle, and concave inwards with the veins of the scales protruding straight from the middle circle to the edges. The scales are located above the epidermises, more clearly visible on the abaxial & the positions are spread with the sparse scales spread type (Figures 3 & 4, Tables 1 & 2). Scales are small-sized leaves modifications that play an important role in protection, transpiration reduction, aesthetics, taxonomy identification, and nutrient or energy reserves to support plant growth in certain phases [28].

Owing to the distinctive structure of the epidermises, stomata, and scales, *R. multicolor* can adapt to its environment. *Rhododendron* can adapt easily to various environmental conditions [4].

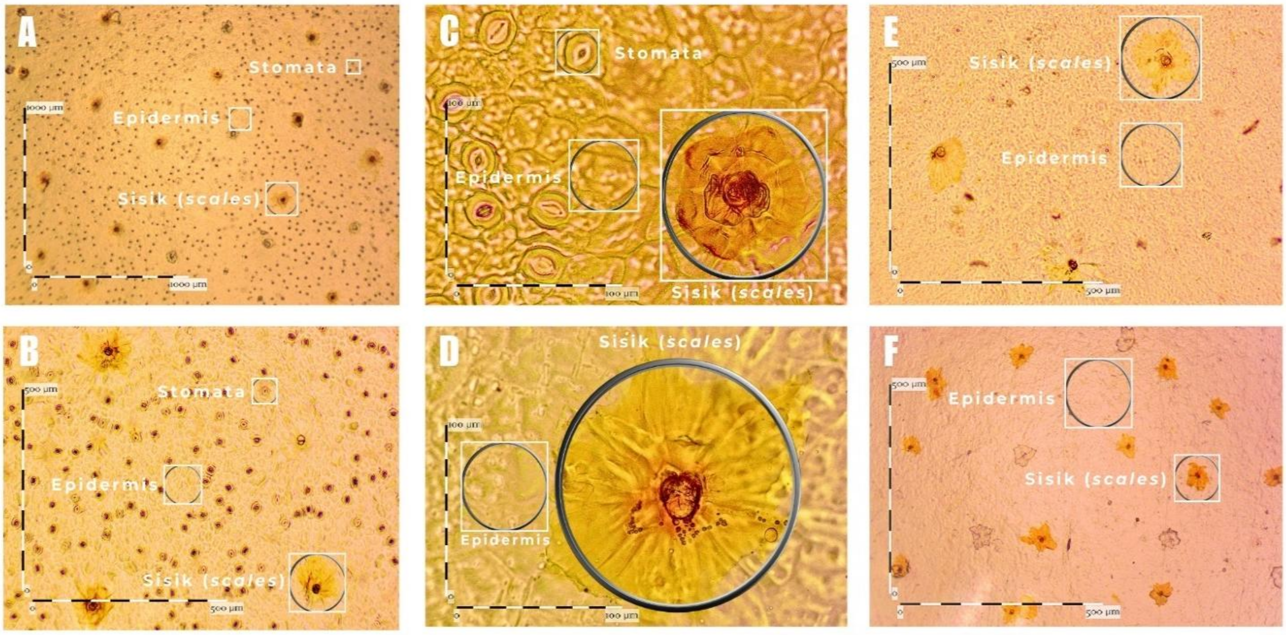

Figure 4. Anatomy of young leaves of *Rhododendron multicolor* on a light microscope. Note: (A) magnification 4x adaxial, (B) magnification 10x abaxial, (C) magnification 40x abaxial, (D) magnification 40x adaxial, (E) magnification 10x adaxial, and (F) magnification 4x adaxial.

Table 1. Anatomy of young leaves of *Rhododendron multicolor*

Cell	Parameter	Adaxial		Abaxial	
		10x magnification	40x magnification	10x magnification	40x magnification
Epidermis	Shape	Polygonal to irregular with varying sizes			
	Quantity	530 ±7	30.6 ±7.23	888.3 ±9.71	52 ±13.22
	Size (length, µm)	34.77 ±5.64	31.24 ±4.98	31.28 ±8.51	13.18 ±2.33
	Size (width, µm)	38.60 ±10.00	32.49 ±3.38	22.34 ±4.33	12.55 ±3.48
Stomata	Quantity	No stomata are visible	No stomata are visible	150 ±15	6 ±2
	Stomata Index			14,43 ±1.31	10,22 ±4.03
	Stomata density			194,8 ±19.48	120 ±40
	Size (length, µm)			27.70 ±2.01	10.04 ±0.77

Cell	Parameter	Adaxial		Abaxial	
		10x magnification	40x magnification	10x magnification	40x magnification
	Size (width, µm)			27.11 ±2.85	9.11 ±0.39
Scales	Quantity	4 ±1	1 ±0	5 ±1	1 ± 0,00
	Shape	Stellate lobed irregularly			
	Size (length, µm)	104.02 ±61.45	135.69 ±17.80	117.15 ±24.12	87.82 ±10.20
	Size (width, µm)	119.54 ±73.14	122.65 ±16.39	119.74 ±11.79	80.56 ±25.75

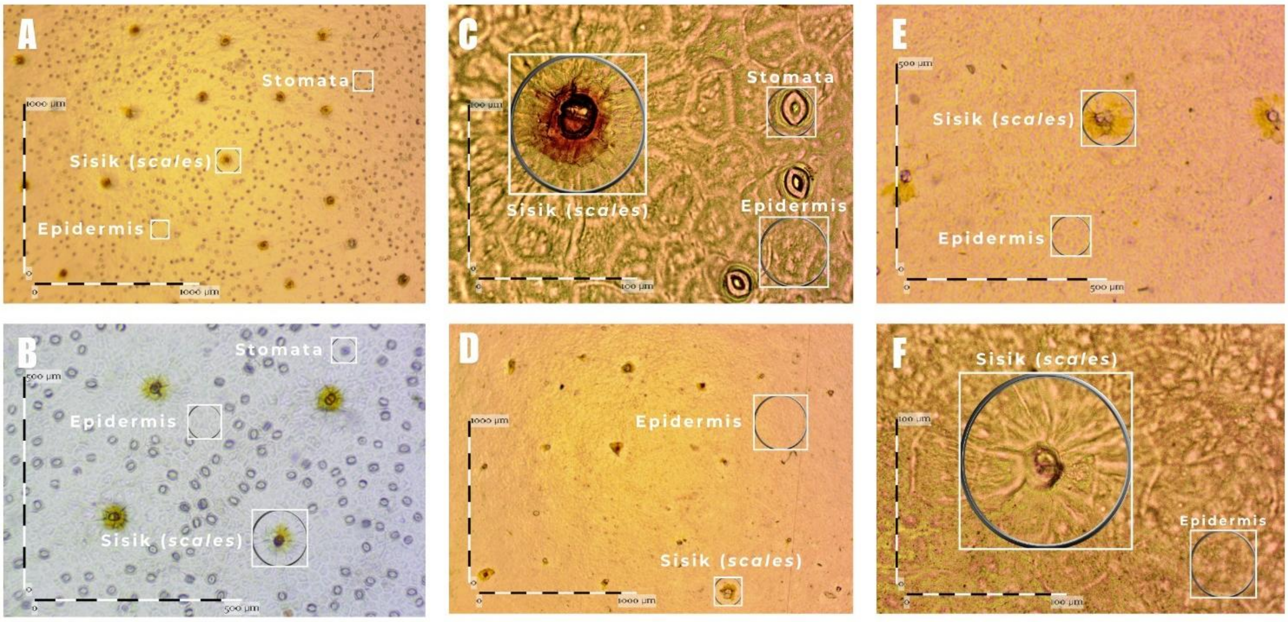

Figure 5. Anatomy observation of mature leaves of *Rhododendron multicolor* on a light microscope. Note: (A) magnification 4x abaxial, (B) magnification 10x abaxial, (C) magnification 40x abaxial, (D) magnification 4x adaxial, (E) magnification 10x adaxial, and (F) magnification 40x adaxial.

Table 2. Anatomy observation of mature leaves of *Rhododendron multicolor*

Cell	Parameter	Adaxial		Abaxial	
		10x magnification	40x magnification	40x magnification	40x magnification
Epidermis	Shape	Polygonal to irregular with varying sizes			
	Quantity	772 ±5	22.3 ±5.50	747 ±7.21	36.6 ±0.57
	Size (length, µm)	34.39 ±12.46	36.85 ±7.39	37.38 ±3.64	37.92 ±7.26
	Size (width, µm)	32.10 ±9.67	35.70 ±6.27	36.07 ±10.84	32.85 ±6.12
Stomata	Shape	Elips			
	Quantity	No stomata are visible	No stomata are visible	134 ±13.74	3 ±2.08
	Stomata Index			15,19 ±1.36	11,13 ±1.36
	Stomata density			174,02 ±17.85	93,3 ±41.63
	Size (length, µm)			25.69 ±2.36	24.20 ±2.39
	Size (width, µm)			21.56 ±2.88	23 ±0.76
Scales	Quantity	3.3 ±0.57	1 ± 0	3.3 ±0.57	1 ±0
	Shape	Stellate lobed irregularly			

Cell	Parameter	Adaxial		Abaxial	
		10x magnification	40x magnification	40x magnification	40x magnification
	Size (length, µm)	104.28 ±13.78	138.49 ±6.61	68.08 ±9.41	126.55 ±39.89
	Size (width, µm)	104.73 ±11.95	143.65 ±21.79	70.92 ±14.67	148.45 ±32.02

Metabolite Compounds of *Rhododendron multicolor*

Metabolite profiles are correlated with specific morphological and anatomical changes under stress conditions caused by alterations in metabolite profiles [29]. For instance, petal color correlates with flavonoid content in *R. pulchrum* [30], flower color is determined by metabolic pathways in *R. dauricum* [31], varying anthocyanin content significantly influences flower color in *R.* triflorum [32], petal anatomy may influence the accumulation of protective metabolites associated with defense mechanisms in *Rhododendron* species [33], and scales are known to contain flavonoids and other secondary metabolites, so the anatomical structure and quantity of the scales may influence the accumulation of these compounds [34], based on Figure 4 & 5, *R. multicolor* also have quite a lot of scales and unique structure.

Metabolite compounds of *R. multicolor* were analyzed by qualitative phytochemicals and GC-MS. Qualitative phytochemical test is preliminary test to determine the content of metabolite compounds of samples in general by observing changes in color and formation (alkaloids, flavonoids, tannins, and terpenoids) or the presence of foam (saponins) for positive results. The results of this study (Table 3) are in accordance with various previous studies that show *Rhododendron* is abundant in phenolic compounds, such as flavonoids that are known to have antioxidant activity [35, 36, 37]. *Rhododendron* contain many secondary metabolites, such as flavonoids, saponins, and tannins [38]. For instance, studies on *R. arboreum* and *R. przewalskii* reveal similar phytochemical constituents, such as flavonoids and tannins, which contribute to their medicinal properties [39, 40]. However, the unique combination and concentration of these compounds in *R. multicolor* may distinguish it from other species. The specific profile of *R. multicolor* could highlight its unique therapeutic potential.

Table 3. Results of qualitative phytochemical tests of *Rhododendron multicolor*

Compound Leaves	Young Leaves	Mature Leaves
Phenol	++	+
Flavonoid	++	+++
Alkaloid Mayer	+++	+++
Alkaloid Bouchardat	+++	+++
Alkaloid Dragendorf	+++	+++
Tannin	+++	+++
Saponin	+	+++

Note: (-) = negative, (+) = weak positive, (++) = strong positive, (+++) = very strong positive

Metabolite compound tests were performed on young leaves and mature leaves extracts of *R. multicolor* to determine the compounds that contribute to its biological potential through extraction & GC-MS. Extraction of metabolite compounds of *R. multicolor* with methanol solvents (polar solvents) to extract polar metabolite compounds, such as flavonoids, alkaloids, saponins, terpenoids, and tannins. The choice of extraction technique depends on the nature of the sample, the type of compound to be analyzed, and the sensitivity required. Maceration extraction is carried out to separate compounds from simplicial or their mixtures [41, 42]. Factors that affect extraction include time, temperature, solvent type, material comparison, and particle size [43, 44]. The longer the maceration time, the more cells break down and the active ingredients are dissolved [45]. GC-MS is then carried out to identify and measure various volatile compounds with high volatility and sensitivity, either targeted or untargeted [46]. It can give rise to each separate compound's molecular mass, elemental composition, functional group, molecular geometry, and spatial isomerism [47].

GC-MS analysis identified a total of 31 metabolite compounds in *R. multicolor* extract with match scores exceeding 85. This included 14 compounds from young leaves and 17 compounds from mature leaves, categorized into 9 distinct groups of young leaves and 12 distinct groups of mature leaves. Seven compounds and eight group compounds were common between young and mature leaves (Table 4 & 5 and Figures 7 & 8). The GS-MS Chromatogram can be seen on Figures 6 & 7. *R. multicolor* has abundant metabolite compounds in antimicrobial, cancer therapy, anti-inflammatory, antioxidant, heart health, cardiovascular, strengthens the immune systems, and manages metabolic disorders, antiaging, drug formulation, as well as control of pregnancy and sexually transmitted diseases, so it has the potential to be a medicinal plant (Table 4 & 5). Moreover, *R.*

multicolor leaves have also been identified as having potential as chemicals and additives, larvicides, insecticides, fungicides, bioacharides, bioherbicides, biostimulants, fuel components, biodiesel, materials and polymers, stability and preservatives, pheromone components and insect attractants, chemical derivatives to increase the volatility of compounds, and radio fluorination (Table 4 & 5). The variation of bioactive compounds in plants is caused by several factors, including differences in geographical location, place altitude, plant location, seasonal changes, and physiological factors (genetics, plant nutrition, and maturity stage) [48, 49, 50]. Temperature and CO^2 also affect the production of secondary metabolites, the higher the temperature and CO^2 levels, the higher the production of secondary metabolites produced [51].

Based on Table 6, it is known that five of the six major compounds of *R. multicolor* come from the terpenoid group, which has antioxidant, anticancer, antifungal, antibacterial, and antiviral activities [51], except for the group of hydrocarbon compounds that do not have potential in the health sector. Moreover, the major compound is squalene. Squalene is predominantly found in the unsaponifiable fraction of various plant oils. Research has shown that the concentration of squalene in plants is strongly influenced by factors such as the plant species, growth location, growth period, and the extraction techniques used to isolate squalene from other compounds [52]. Additionally, many plant-derived terpenes possess remarkable bioactive properties, making them useful in applications such as antimicrobials, antibiotics, dietary supplements, flavorings, and repellents. Squalene is classified as a triterpene with significant nutritional and medicinal benefits, offering promising potential for pharmaceutical applications. Squalene's bioactive property and applications are cardioprotectors applied via intravenous injection and orally consumption to cholesterol control, antioxidant applied topically emulsions, oral administration, antibacterial and antifungal applied on cream topical, oral medication, anticancer applied on preventive and chemotherapeutic substances; drugs and vaccines (emulsions, conjugates), detoxifying applications on nutritional supplements [53], antioxidant, cardioprotective, reduce atherosclerosis [54], antitumor, and chemoteraphy [55].

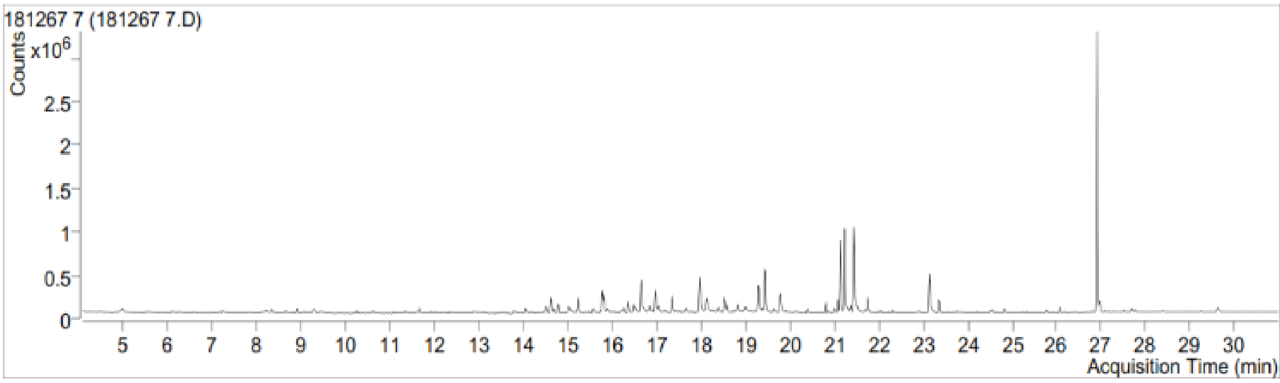

Figure 6. GS-MS chromatogram of *Rhododendron multicolor*'s young leaves

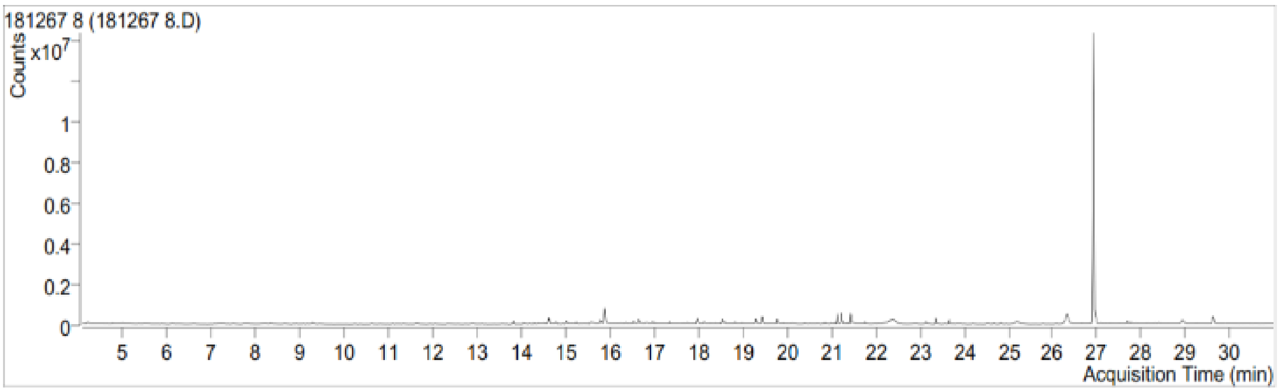

Figure 7. GS-MS chromatogram of *Rhododendron multicolor*'s mature leaves

Table 4. GC-MS analysis results of methanol extract of *Rhododendron multicolor*'s young leaves

RT	Wide Area	Compound	Group	Match Score	Biological Potency
11.6752	73156	Dodecane, 5 methyl-	Alkana	86.4	Antioxidant [56]
14.6327	216745	2,6-Difluorobenzoic acid, 4-nitrophenyl ester	Ester	88.6	Anticancer, anti-inflammatories, & intermediates in the synthesis of organic compounds [57, 58]

RT	Wide Area	Compound	Group	Match Score	Biological Potency
15.7866	360854	2,2,4-Trimethyl-1,3-pentanediol di-sobutyrate	Ester	90.4	Plasticizer & additive formulations [59, 60, 61]
15.8227	290544	Diethyl Phthalate	Phthalate	90.9	Teratogenic agents, aspirin, plasticizers, insecticides, mosquito repellents, cosmetics, and stabilizers in solid rocket propellants [62, 63]
17.9765	810866	Loliolide	Terpenoids (Monoterpen oids)	90.6	Antidiabetic, therapeutic, antioxidant, phytoestrogen, & osteoporosis prevention [64, 65, 66]
19.4431	823523	Hexadecanoic acid, methyl ester	Ester	92.6	Antibacterial, antibiotics, excipients in drug formulations, emollients, improving the texture and taste of cosmetic formulations, & biofuels [67, 68, 69]
19.7913	72870	Dibutyl phthalate	Phthalates	90.9	Plasticizers, solvents, fragrances, emollients, & coatings [70, 71]
21.1406	1090540	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Ester	95.6	Anti-inflammatory, antioxidant, lowers cholesterol levels, provides essential fatty acids, ingredients in cosmetic formulations to improve hydration and skin texture, anti-melanogenesis, & insect repellents [72, 73, 74]
21.2330	1694744	Oxirane, tetradecyl-	Hydrocarbon	86.9	Anticancer, drug synthesis, compound stability, & insecticide [75, 76, 77, 78]
21.233	1979954	Phytol	Terpenoids (Diterpenoid s)	96.7	Anticancer, antimicrobial, antioxidant, anti-inflammatory, & carbon monoxide reduction [79, 80, 81]
23.1444	905515	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-Ethylhexyl ester	Ester	93.2	Ingredients in sunscreen to minimize DNA photodamage & pharmaceutical and cosmetics formulations [82, 83]
26.9091	5039801	1,5,9-Undecatriene, 2,6,10-trimethyl-, (Z)-	Hydrocarbon	87	Antimicrobial, organic synthetic, flavoring, and fragrance [84, 85,86, 87]
26.9091	5267007	Squalene	Terpenoids (Triterpenoi ds)	97.4	Adjunct therapy for cancer & cholesterol synthesis [88]
29.6286	108513	Dl-alpha-Tocopherol	Heterocyclic	88.8	Antioxidants, increase *superoxide dismutase* (SOD) activity, reduce malondialdehyde (MDA) levels, increase motility and spermatozoa viability, & supplementation [89]

Notes: The data were summarized based on the match score >85

Table 5. Results of GC-MS analysis of methanol extract of *Rhododendron multicolor's* mature leaves

RT	Wide Area	Compound	Group	Match Score	Biological Potency
4.2161	268885	2,3-Butanediol, [S-(R*,R*)]-	Vic-diols	88.6	Antimicrobials, therapeutics, formulations in drugs and cosmetics, solvents and intermediates in chemical reactions, flavoring agents, biostimulants, larvicides, components in rocket fuel systemss, & components in the production of synthetic rubber [90, 91, 92, 93]
14.6321	371860	2,6-Difluorobenzoic acid, 4-nitrophenyl ester	Ester	89.9	Antivirals, radiofluorins, & enzyme inhibitors [94, 95]
15.7875	260261	2,2,4-Trimethyl-1,3-pentanediol di-isobutyrate	Ester	88.4	Solvent, raw materials in the chemical industry, additives, paint formulations, coatings, components in cosmetics, &

RT	Wide Area	Compound	Group	Match Score	Biological Potency
					emulsifying and binding agents [96, 97, 98, 99]
15.8234	182283	Diethyl Phthalate	Phthalates	89.9	Teratogenic agents, aspirin, plasticizers, improving the texture of cosmetics, insect repellents, insecticides, & aroma stabilizers [100, 101]
18.5332	260172	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpenoids	85	Antimalarial, therapeutic, synthetic precursors of vitamin E and vitamin K1, bioherbicide, CO gas reducer, & modulates transcription in cells via PPAR-alpha transcription factor and retinoid X receptor (RXR) [102, 103, 104, 105]
19.4421	456044	Decanoic acid, methyl ester	Ester	85.8	Anti-inflammatory, antimicrobial, emollient, cosmetic and pharmaceutical formulations, & biodiesel [106, 107]
19.4480	516184	Hexadecanoic acid, methyl ester	Ester	90.3	Antibacterial, anti-inflammatory, antifibrotic, therapeutic, additives to stabilize fats and oils, help slow down autooxidation of unsaturated fatty acids, lubricants and plasticizers, modulators in biological systemss, emollient and stabilizer, improve the texture and stability of creams and lotions, chemical intermediates in surfactants production, emulsifier, & cleaning products formulation [108, 109, 110]
21.1405	564626	11,14,17-Eicosatrienoic acid, methyl ester	Lipids	92.4	Anti-inflammatory, nutritional supplements, dietary supplements, & cosmetic formulations to improve skin hydration and skin barrier [111, 112, 113]
21.1405	581185	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Fatty acid	86	Anti-inflammatory, therapeutic, treatment of nervous disorders, insect attractants, dietary supplements to improve cardiovascular health, reduce inflammation, improve heart health, cosmetic formulations to improve skin hydration, & biodiesel [114, 115, 116]
21.2321	1021997	Phytol	Terpenoids (Diterpenoids)	93.8	Anxiolytic, anti-inflammatory, antiproliferative, antimicrobial, vitamin E and K1 synthesis bases, schistosomes drug, cosmetic and cleaning products formulation, emollient, fragrance & flavoring components [117, 118, 119, 120, 121, 122]
23.1457	155839	2-Ethylhexyl trans-4-methoxycinnamate	Ester	86.2	Anti-aging, ingredients in sunscreen, foundation, lotion, & lip balm to minimize DNA photodamage, pharmaceuticals formulations, & coatings [123, 124, 125, 126]
23.1455	145798	Cinoxate	Ester	86.8	Novel UV filtering agents, cosmetic formulations aimed at reducing the risk of skin cancer, & protecting keratinocytes from damage [127, 128, 129, 130]
23.3604	383631	4,8,12,16-Tetramethylheptadecan-4-olide	Lipids	86.7	Anti-inflammatory, antioxidant, & cosmetic formulations [131, 132, 133, 134, 135]
23.6585	267608	Hexanedioic acid, dioctyl ester	Ester	92.1	Plasticizing material, paint and coating formulation, & chemical derivatives [130, 136]

RT	Wide Area	Compound	Group	Match Score	Biological Potency
26.3205	2022820	Alpha-Amyrin	Terpenoids (Triterpenoids)	87.7	Adjunct therapy for cancer, intermediate metabolites in cholesterol synthesis, anti-inflammatory, antibacterial, skin protection, & diabetes [137, 138, 139]
26.9231	27421735	Squalene	Terpenoids (Triterpenoids)	97.3	Metabolites intermediate in cholesterol synthesis, providing skin protection, & adjunct cancer therapy [140, 141]
29.6194	913658	Dl-alpha-Tocopherol	Vitamin E	92.5	Antioxidants, nutritional supplements, improve spermatozoa motility and vitality, maintain healthy skin, & help maintain skin moisture and elasticity [142, 143]

Notes: The data were summarized based on the match score >85

Table 6. Compounds identified by GC-MS analysis of *Rhododendron multicolor*

Organ	Solvent	Number of Peaks	Major Compounds	Group	Wide Area
Young leaves	Methanol	14	Squalene	Terpenoids	5271986
			1,5,9-Undecatriene, 2,6,10-trimethyl-, (Z)-	Hydrocarbon	5039801
			Phytol	Terpenoids (diterpenoid)	1979954
Mature leaves		17	Squalene	Terpenoids	27421735
			Alpha-Amyrin	Terpenoids (triterpenoids)	2022820
			Phytol	Terpenoids (diterpenoid)	1021997

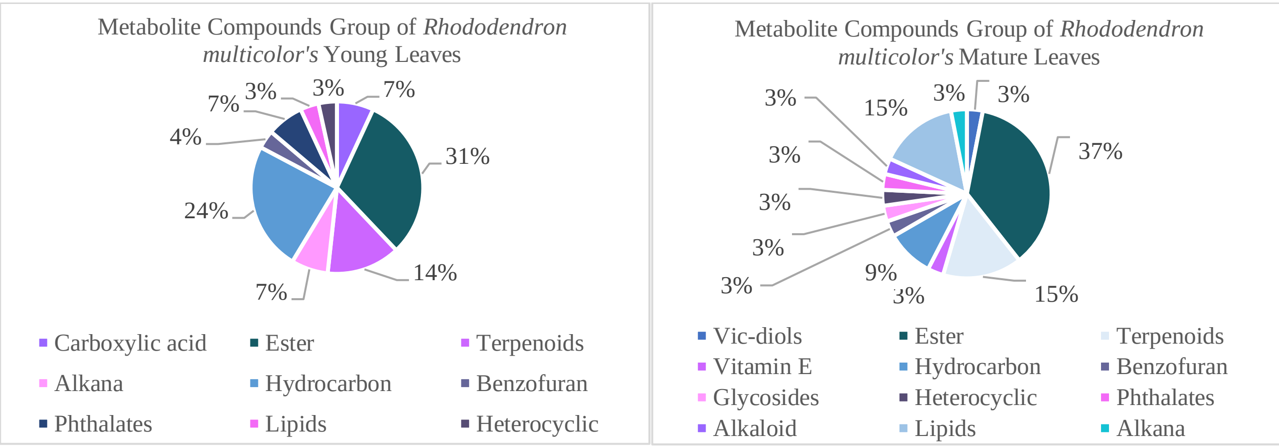

Figure 8. Young leaves and mature leaves compound groups of *Rhododendron multicolor*

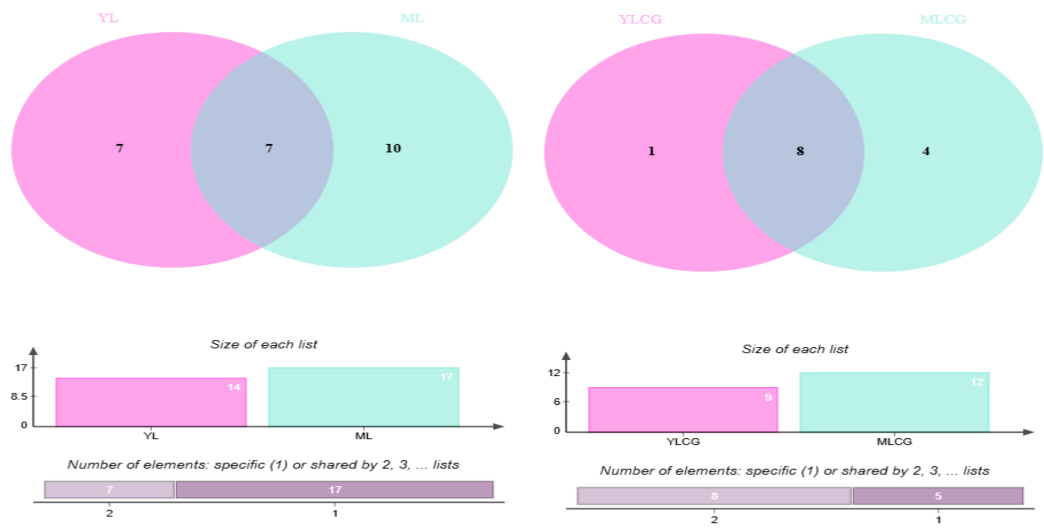

Figure 8. Young leaves (YL) and mature leaves (ML) compound groups of *Rhododendron multicolor*

CONCLUSION

Rhododendron multicolor Miq. has a distinctive morphoanatomy and abundant metabolite compounds. *R. multicolor* has a funnel shape of flower, scaly lanceolate leaves, scaly stems, taproot, stellate lobed irregularly of scales, polygonal to irregular epidermis, and varying sizes of stomata, which are only found on the abaxial surface. The density of epidermises are higher than stomata and stomatal density is higher than the scales. GC-MS analysis identified 31 metabolite compounds with scores above 85, consisting of 14 metabolite compounds of young leaves and 17 metabolite compounds of mature leaves, and 9 groups compounds of young leaves and 12 groups compounds of mature leaves. The major compound is squalane from the terpenoid group, which has the potential to be an antioxidant, anticancer, antibacterial, antifungal, antitumor, and cardioprotective.

For future research can conduct comparative morphoanatomy studies across various *Rhododendron* species using advanced microscopy. Analyze paradermal sections of leaves to assess the arrangement of palisade and spongy mesophyll, which may influence photosynthetic efficiency and drought resistance. Perform comprehensive metabolomic profiling using LC-MS. Investigate the effects of seasonal changes on the metabolite profile, particularly flavonoids and anthocyanins related to flower color and health benefits. Correlate morphological and metabolite data with ecological performance to understand their role in species adaptability. As well as utilize findings to guide conservation efforts and promote sustainable cultivation practices that preserve genetic diversity within *Rhododendron* species.

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