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PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE METHANOL EXTRACT OF RHODODENDRON RENSCHIANUM FLOWERS, ENDEMIC TO KELIMUTU NATIONAL PARK, INDONESIA, USING UV–VIS SPECTROPHOTOMETRY

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Abstract

Rhododendron renschianum is an endemic Indonesian species characterized by bell-shaped flowers with bright red petals and a yellowish base. This study aimed to document the presence of and quantify major phytochemical groups in the methanolic extract of R. renschianum flowers. Phytochemical screening yielded positive results for triterpenoids, tannins, flavonoids, alkaloids, and quinones. However, quantitative analysis using UV–Vis spectrophotometry indicated an apparent triterpenoid content of 2685.48 ± 123.49 mg UA/g, highlighting a limitation of UV–Vis analysis for investigating reactive compounds such as triterpenoids. The remaining compounds, in ascending order of content, were flavonoids, alkaloids, tannins, and quinones, with values of 5.98 ± 0.70 mg QE/g, 90.09 ± 67.09 mg CAF/g, 109.24 ± 1.44 mg TA/g, and 130.93 ± 30.19 mg HQ/g, respectively. These findings add to the baseline data for the initial investigation of the secondary metabolite content of this endemic species and provide a preliminary basis for further pharmacological research.

Keywords: *Kelimutu lake; Phytochemical screening;* Rhododendron renschianum*; UV-Vis quantification*

Introduction

Flowers are specialized reproductive organs that often have a distinct and richer profile of secondary metabolites than vegetative organs such as leaves or stems [1–3]. These metabolites, particularly phenolic pigments, flavonoids, and volatile compounds, are essential for ecological interactions, including pollinator attraction and environmental defense [4,5]. Because these bioactive compounds govern unique floral traits, including pigmentation driven by anthocyanins and carotenoids [6–9], floral extracts are of significant interest in natural product chemistry. Consequently, phytochemical profiling of these organs serves as a critical first step in discovering novel chemotypes and understanding the

broader chemical ecology of plants [4,10,11].

Within this context, the genus *Rhododendron* exhibits extensive morphological and chemical diversity, with several species well documented for their flavonoid, tannin, and phenolic contents [12–16]. However, phytochemical investigations remain notably limited for *Rhododendron renschianum*, an endemic Indonesian species restricted to the mountainous habitat of Kelimutu National Park in Ende Regency, East Nusa Tenggara [17]. Distinguished by its prominent orange-red flowers, *R. renschianum* presents a unique morphological phenotype that suggests a distinct underlying metabolite profile [18]. Furthermore, its highly restricted geographical distribution, particularly its adaptation to an active volcanic crater environment characterized by elevated sulfur levels [19], provides a unique ecological variable that may influence its secondary metabolite biosynthesis [20]. Despite its ecological significance and the established phytochemical relevance of the broader *Rhododendron* genus, the specific chemical composition of *R. renschianum* flowers remains entirely uncharacterized. Exploring this species is critical for understanding whether its unique ecological niche translates into a distinct phytochemical profile compared with previously investigated *Rhododendron* species.

Addressing this knowledge gap is essential not only for expanding the chemotaxonomic understanding of the genus but also for supporting the conservation and biochemical utilization of endemic genetic resources. This is particularly urgent given the recent ecological pressures on the species, as evidenced by its reported disappearance from the Eka Karya Bali Botanical Garden in 2024, where it had been growing since 2011 [21,22]. Therefore, the objective of this study was to perform a preliminary qualitative and semi-quantitative phytochemical characterization of the methanolic flower extract of *R. renschianum*, specifically by evaluating the presence of flavonoids, tannins, saponins, alkaloids, terpenoids, steroids, and quinones.

Experimental section

Materials

R. renschianum flowers were collected in June 2024, when they were in full bloom (see Figure 1). Sampling at this stage can yield higher amounts of isolated metabolites than sampling at other stages. This is because, during full bloom, the resulting color and aroma attract animals and insects, thereby increasing the production of secondary metabolites in response to external stimuli or threats [8,26]. Samples were collected from five trees in the same habitat around the crater of Nua Muri Lake. Research in the Kelimutu National Park area was carried out under permit SI.12/T.40/TU/KSA.5.4/B/06/2024 for entry into the conservation area and was accompanied by a botanist. The taxonomy of *R. renschianum* is as follows [24]:

Kingdom : Plantae
Phylum : Tracheophyta
Class : Magnoliopsida
Order : Ericales
Family : Ericaceae
Genus : *Rhododendron*
Species : *R. renschianum* Sleumer

The materials used in this study included caffeine (Sigma-Aldrich), ursolic acid, saponin, and quercetin (Sigma-Aldrich). Other reagents obtained from Merck included hydroquinone, Folin–Ciocalteu reagent, methanol, tannic acid, 97% H_2SO_4, Na_2SO_4, CH_3COOH, Na_2CO_3, 32% HCl, CH_3COOK, $AlCl_3$, bromocresol green (BCG) indicator, phosphate buffer, and NaOH. In addition, 95% ethanol was obtained from One Med, chloroform from Fulltime, and anisaldehyde from Loba Chem.

Instruments

The instrument used for compound identification and quantification was a GENESYS 40 Vis/50 UV–Vis spectrophotometer.

Procedure

Sample preparation

R. renschianum flowers were thoroughly washed with distilled water to remove surface debris and contaminants. The plant material was subsequently air-dried at room temperature in the shade, avoiding direct sunlight, until a constant weight was achieved. The dried samples were then ground into a fine powder and passed through a 60-mesh sieve to ensure uniform particle size for subsequent extraction.

Maceration

A 10.0 g aliquot of powdered flower material was macerated in 100 mL of methanol at a solid–solvent ratio of 1:10 (w/v) at ambient temperature for 24 hours. Afterward, the suspension was filtered to remove particulate matter, and the filtrate was transferred onto glass plates and evaporated to dryness at room temperature, yielding viscous residues. Methanol was selected as the solvent based on the study by Hayat et al. (2020), who compared four organic solvents: ethanol, methanol, ethyl acetate, and petroleum ether. Methanol produced a higher total content of secondary metabolite compounds than the other solvents [25]. The extraction process was repeated approximately five times, and each extract was collected for further investigation.

Qualitative and Quantitative Analyses

Qualitative and quantitative analyses were performed by adapting and combining established phytochemical protocols from the literature to assess saponins, quinones, flavonoids, tannins, terpenoids, and alkaloids. Quantitative determinations were carried out using UV–Vis spectrophotometry with appropriate calibration standards and concentration calculations based on the Beer–Lambert law [26–29].

Phytochemical Screening

Saponin.

A total of 0.3 g of extract was added to 5 mL of hot water, shaken vigorously for 10 seconds, and left to stand for 10 minutes. Next, three drops of 1% HCl were added. Positive results were indicated by the formation of stable foam.

Quinone.

The extract filtrate was placed on a spot plate, and two to three drops of 1 N NaOH were added. A color change to red indicated the presence of quinone compounds.

Flavonoid.

The extract was placed on a spot plate, and concentrated HCl and Mg powder were then added. The formation of a yellow, orange, or red color indicated that the sample contained flavonoids.

Tannin.

The extract was dissolved in 10 mL of hot water for 5 minutes and then transferred to a spot plate. A 1% $FeCl_3$ solution was added dropwise. The formation of a dark blue or blackish-green color indicated the presence of tannin compounds.

Alkaloid.

A total of 2 g of extract was added to 5 mL of HCl. The mixture was brought to a boil while being shaken and then filtered. The filtrate was tested using three specific reagents on a spot plate: Mayer's reagent formed a white precipitate, Wagner's reagent produced a brown precipitate, and Dragendorff's reagent formed an orange precipitate.

Steroid and Triterpenoid.

A total of 0.3 g of extract was dissolved in 15 mL of ethanol and placed on a spot plate. The solution was treated with three drops of anhydrous acetic acid and one drop of concentrated H_2SO_4. Steroids were indicated by blue coloration, whereas red coloration indicated the presence of triterpenoid compounds.

A B

Figure 1. *R. renschianum* trees (A) and *R. renschianum* flower (B).

Quantitative Test

Quinone.

A 1000 ppm hydroquinone stock solution was prepared by dissolving 0.050 g of hydroquinone in 1.0 mL of 4 N HCl and 25.0 mL of 96% ethanol, followed by heating and stirring until a homogeneous solution was obtained. The solution was filtered through filter paper containing 1.0 g of anhydrous Na_2SO_4 into a 50.0 mL volumetric flask and diluted to the mark with 96% ethanol. Working standards of 0, 20, 40, 60, 80, and 100 ppm were prepared by appropriate dilution of the stock solution. The absorption maximum, λmax, was determined by scanning a 60 ppm standard from 200 to 400 nm using ethanol as the blank. All standards were subsequently measured at the determined λmax to construct a calibration curve. For sample analysis, 0.025 g of *R. renschianum* extract was mixed with 1.0 mL of 4 N HCl and 25.0 mL of 96% ethanol, heated and stirred until homogeneous, then filtered through filter paper containing 1.0 g of Na_2SO_4 into a 25.0 mL volumetric flask and diluted to the mark with ethanol. The sample absorbance was measured at λmax, and the total quinone content was calculated by linear regression against the calibration curve. The results are reported as the mass of quinone per mass of extract.

Flavonoid.

A 1000 ppm quercetin stock solution was prepared by dissolving 0.050 g of quercetin in 50.0 mL of methanol and mixing until fully dissolved. Working standards of 0, 20, 40, 60, 80, and 100 ppm were prepared by serial dilution of the stock solution in methanol. For complexation, each standard and blank was treated with 1.0 mL of 2% $AlCl_3$ and 1.0 mL of 120 mM potassium acetate (CH_3COOK), vortexed to homogeneity, and incubated at room temperature for 1 hour. The absorption maximum, λmax, was determined by scanning a 60 ppm standard from 400 to 800 nm using methanol as the blank. All standards were subsequently measured at the determined λmax to construct a calibration curve by linear regression. For sample analysis, 1.0 mL of *R. renschianum* extract was mixed with 1.0 mL of 2% $AlCl_3$ and 1.0 mL of 120 mM CH_3COOK, vortexed until homogeneous, incubated for 1 hour, and measured at λmax. Total flavonoid content was calculated from the calibration curve and expressed as the mass of quercetin equivalent per mass of extract.

Tannin.

A 1000 ppm tannic acid stock solution was prepared by dissolving 0.050 g of tannic acid in 50.0 mL of methanol and mixing until fully dissolved. Working standards of 0, 20, 40, 60, 80, and 100 ppm were prepared by appropriate dilution of

the stock solution in methanol. For color development, each standard was treated by combining 15.0 mL of deionized water and 1.0 mL of Folin–Ciocalteu reagent, mixing thoroughly, and allowing the mixture to react for 8 minutes at room temperature. Then, 3.0 mL of sodium carbonate solution was added, and the mixture was homogenized. The absorption maximum, λmax, was determined by scanning the 60 ppm standard from 400 to 800 nm using methanol as the blank. The absorbance of all standards was subsequently measured at the determined λmax to construct a calibration curve by linear regression. For sample analysis, 1.0 mL of *R. renschianum* extract was mixed with 15.0 mL of deionized water and 1.0 mL of Folin–Ciocalteu reagent, vortexed until homogeneous, and allowed to stand for 8 minutes. After the addition of 3.0 mL of sodium carbonate and thorough mixing, the reaction mixture was incubated at room temperature for 2 hours to complete color development. The sample absorbance was measured at λmax, and the total tannin content was calculated from the calibration curve by linear regression. The results are expressed as tannic acid equivalents per unit mass of extract.

Alkaloid.

A 1000 ppm caffeine stock solution was prepared by dissolving 0.050 g of caffeine in 50.0 mL of deionized water and mixing until fully dissolved. Aliquots of 0.2, 0.4, 0.8, and 1.0 mL of the stock solution were transferred to separate extraction vessels. Each aliquot was combined with 5.0 mL of phosphate buffer and 5.0 mL of BCG indicator, then subjected to liquid–liquid extraction with chloroform three times using 3.0, 3.0, and 4.0 mL portions. The combined chloroform extracts were transferred to a 10.0 mL volumetric flask and diluted to the mark with chloroform. Absorbance was measured at λ = 470 nm using chloroform as the blank. Absorbance values from the standard aliquot series were used to construct a caffeine calibration curve. For sample analysis, 0.050 g of *R. renschianum* extract was dissolved in 5.0 mL of 2 N HCl, and the solution was filtered. A 1.0 mL aliquot of the filtrate was collected and washed three times with 10.0 mL portions of chloroform. The remaining aqueous filtrate was neutralized with 0.1 N NaOH, after which 5.0 mL of phosphate buffer and 5.0 mL of BCG indicator were added. The mixture was extracted with chloroform three times using 3.0, 3.0, and 4.0 mL portions. The combined chloroform extracts were transferred to a 10.0 mL volumetric flask and diluted to the mark with chloroform. Sample absorbance was measured at λ = 470 nm [30], and the alkaloid concentration was determined by linear regression against the caffeine calibration curve. Total alkaloid content is reported as caffeine equivalents per unit mass of extract.

Triterpenoid.

A 1000 ppm ursolic acid stock solution was prepared by dissolving 0.050 g of ursolic acid in 50.0 mL of methanol and mixing until fully dissolved. Working standards of 0, 20, 40, 60, 80, and 100 ppm were prepared by appropriate dilution of the stock solution in methanol. The absorption maximum, λmax, was determined by scanning a 60 ppm standard from 200 to 400 nm using methanol as the blank. The absorbance of all standards was then measured at the determined λmax and used to construct a calibration curve by linear regression. For sample analysis, 0.050 g of *R. renschianum* extract was dissolved in 50.0 mL of methanol and mixed until homogeneous. The sample absorbance was measured at the previously determined λmax. Because triterpenoids lack strong chromophores and primarily absorb in the low-UV region, direct spectrophotometric measurement is highly susceptible to matrix interference from other co-extracted UV-absorbing metabolites. Consequently, to account for this analytical limitation, the calculated values are strictly defined as apparent triterpenoid concentrations. This apparent concentration was estimated from the calibration curve and reported as apparent ursolic acid equivalents per unit mass of extract, serving as a preliminary semi-quantitative estimate that requires future chromatographic validation.

Data Analysis

The outcomes of the qualitative assays are summarized in tabular form, where a positive response (+) denotes the presence of secondary metabolites and a negative response (-) indicates their absence. To evaluate the efficiency of the maceration process, the extraction yield was calculated prior to quantification using the following equation:

Yield (%) = $(W1/W2)$ x 100

where W_1 represents the mass of the concentrated viscous extract (g), and W_2 represents the initial mass of the dried plant material (g).

Quantitative determination of metabolite levels was performed using the following equation:

$$Total\ content = (C.V.Df)/m$$

where C represents the extract concentration (mg/mL), V represents the sample volume (mL), Df represents the dilution factor, and m represents the mass of the viscous extract (g).

The concentration was derived from the Beer–Lambert law using mean absorbance values. These absorbance values were fitted to the calibration curve equation:

y = bx + a, where y denotes the measured absorbance, x is the solution concentration (ppm), b is the slope of the regression line, and a is the y-intercept.

All results are expressed as mean ± standard deviation (SD). The SD, limit of detection (LOD), and limit of quantitation (LOQ) were calculated according to standard statistical procedures to ensure analytical reliability [31,32]:

$$SD = \sqrt{((\sum(y - y')\text{^}2)/(N - 2))}$$
$$LOD = (3\ x\ SD)/b$$
$$LOG = (10\ x\ SD)/b$$

It should be noted that, although UV–Vis spectrophotometry provides an accessible and rapid preliminary assessment of metabolite classes, the method has inherent analytical limitations regarding specificity. Assays that rely on the low-UV region are susceptible to matrix interference from co-extracted compounds, which can lead to overestimation. Consequently, the reported values represent apparent, semi-quantitative contents that provide a baseline for future validation using more specific chromatographic techniques, such as HPLC or LC–MS.

Results and Discussion

Saponin.

Based on the saponin test results, no stable foam was observed in the test sample (see Figure 2). This suggests that *R. renschianum* flowers are likely to contain no saponins (see Table 1). Other *Rhododendron* species reported to lack saponins in their flower extracts include *R. anthopogon* and *R. arboreum* from India [35,36]. The absence of saponins may be related to the solvent used during maceration; several researchers have found that using different solvents for the same sample can yield different test results for the presence of secondary metabolites [37,38]. This was demonstrated by Kashyap and Anand (2016), who reported that *R. arboreum* flowers macerated in ethanol showed positive results for saponins, although with a relatively low total content of 0.11 ± 0.009 mg/g [39]. Nevertheless, the absence of saponins in flower extracts may also be attributed to the absence of abiotic stress in *R. renschianum* flowers. Saponins begin to be produced by plants during pathogen attack, so plant parts that are not attacked may have very low levels of saponins, whereas attacked tissues may contain higher levels [38]. This finding is consistent with previous research showing that saponins accumulate in the parenchymal cells of vegetative organs, including roots, stems, and leaves [39].

Table 1. Results of qualitative and quantitative tests of secondary metabolites in *R. renschianum* flowers.

Secondary Metabolite Compound	Qualitative Test	Quantitative Test	Limit of Detection (LOD) (ppm)	Limit of Quantitation (LOQ) (ppm)
Saponin	-	0	0	0
Tannin (mg TA/g); λmax = 786 nm	+	109.24 ± 1.44	13.89	46.28
Alkaloid (mg CAF/g); λmax = 470 nm				
Mayer	-	90.09 ± 67.09	25.28	84.93
Wagner	+			
Dragendorff	+			
Quinone (mg HQ/g); λmax = 293 nm	+	130.93 ± 30.19	2.47	8.24
Steroid	-	0	0	0
Triterpenoid (mg UA/g); λmax = 208 nm	+	2685.48 ± 123.49	2627.03	8920.12
Flavonoid (mg QE/g); λmax = 426 nm	+	5.98 ± 0.70	25.48	84.93

Figure 2. Saponin screening result.

Tannin.

The tannin screening results showed a darkening of the sample color, indicating the presence of tannins in *R. renschianum* flowers (see Figure 3). This discoloration resulted from the formation of complex compounds through a reaction between tannins and $FeCl_3$ [42]. Quantitative tests using UV–Vis spectrophotometry at 786 nm, with tannic acid as the standard, yielded R^2 = 0.9879 and a linear regression equation of y = 0.0049x + 0.0348 (see Figure 4A), with LOD and LOQ values of 13.89 ppm and 46.28 ppm, respectively. The total tannin content after a maceration time of 24 hours was 109.24 ± 1.44 mg TA/g (see Table 1), where TA denotes tannic acid equivalents per gram of *R. renschianum* flower extract. The value obtained in this study was higher than that reported by Bharathy et al. (2024), in which the total tannin contents of water and ethanol extracts of *R. arboreum* were 49.84 ± 0.65 mg TA/g and 37.42 ± 0.77 mg TA/g, respectively [31], and higher than the methanol extract value of 51.57 ± 0.03 mg TA/g [34].

This elevated tannin accumulation could be an adaptive response to oxidative stress induced by the sulfur-rich volcanic environment of the Kelimutu crater. Although further ecological studies are required to confirm this hypothesis, a similar case has been reported in which tannin accumulation was associated with sulfur-rich environments [41].

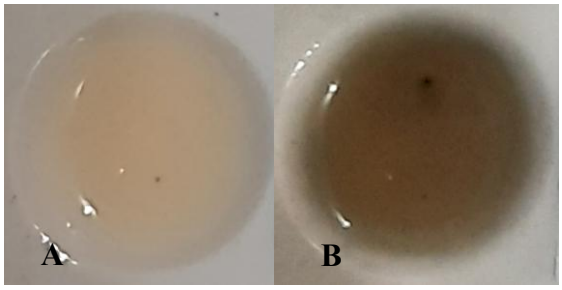
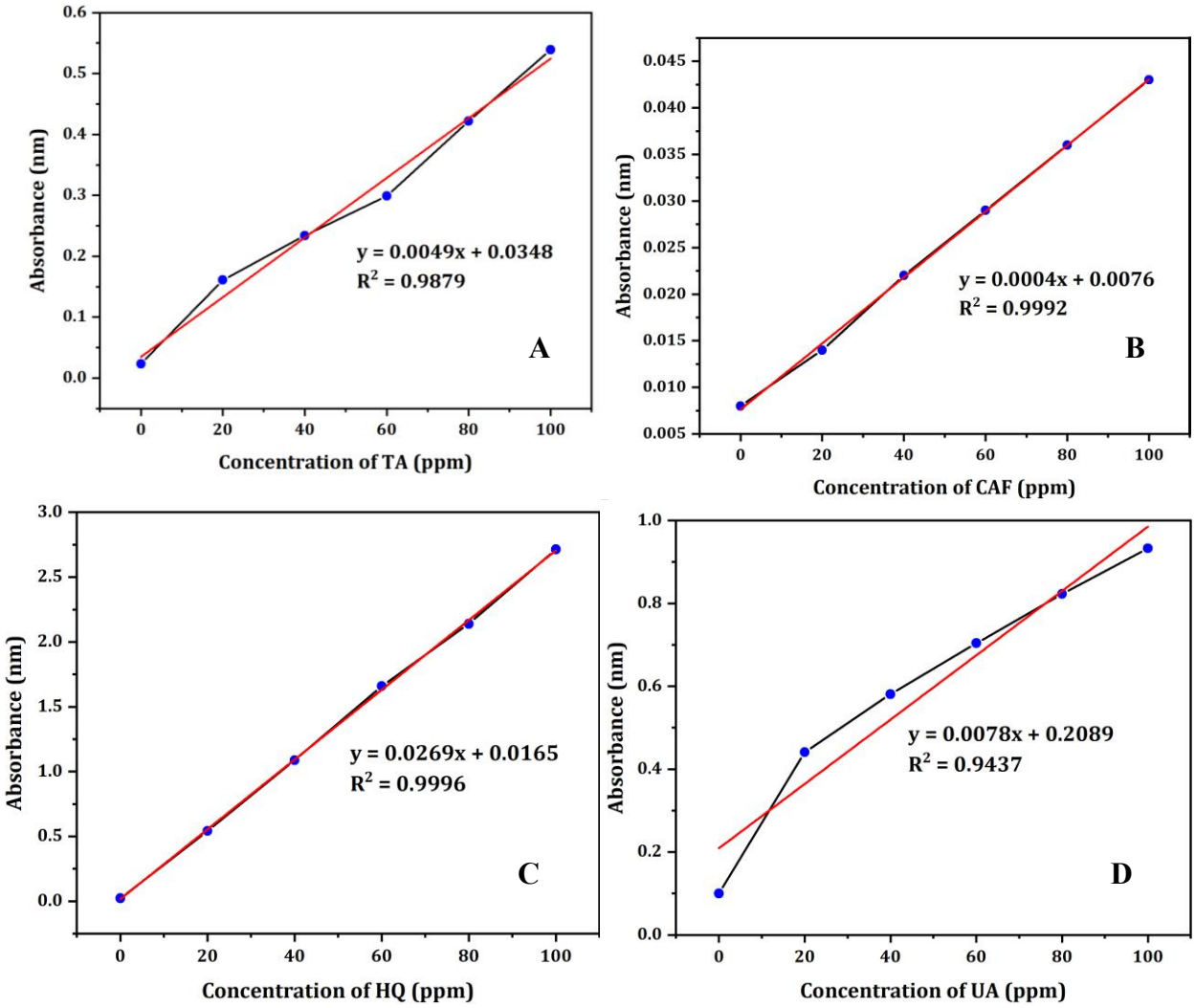


Figure 3. Methanol extract (A) and tannin screening result (B).



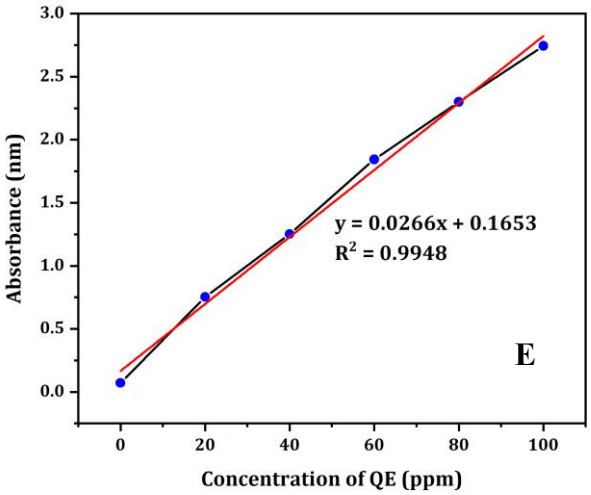


Figure 4. Standard curves: tannin using tannic acid (A), alkaloid using caffeine (B), quinone using hydroquinone (C), triterpenoid using ursolic acid (D), and flavonoid using quercetin (E).

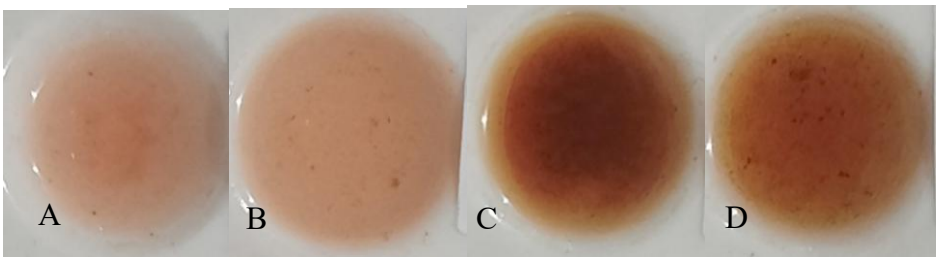


Figure 5. Alkaloid screening results using various reagents: sample (A), Mayer's reagent (B), Wagner's reagent (C), and Dragendorff's reagent (D).

Alkaloid.

Qualitative alkaloid screening required the use of multiple reagents to minimize false negatives and accommodate the structural diversity of this compound class [43,44]. Although Mayer's reagent yielded no discoloration, negative results have also been reported in *R. arboreum* with red and pink flowers, as well as in *R. campanulatum* and *R. anthopogon* [35]. Wagner's and Dragendorff's reagents produced positive brown and orange-brown precipitates, respectively (see Figure 5). Quantitative evaluation at 470 nm yielded an excellent regression fit, with y = 0.0004x + 0.0076 and R^2 = 0.9992 (see Figure 4B), as well as LOD and LOQ values of 25.48 ppm and 84.93 ppm, respectively. However, the calculated total alkaloid content was 90.09 ± 67.09 mg CAF/g (see Table 1). The exceptionally large standard deviation indicates substantial analytical variability and limited precision in this specific assay. This extreme fluctuation likely stems from the low specificity of spectrophotometric methods for complex crude extracts. At 470 nm, significant matrix interference from non-alkaloid nitrogenous compounds or co-extracted floral pigments can severely skew absorbance readings. Therefore, although the apparent concentration is higher than the 0.03 ± 0.002 mg/g reported for *R. arboreum* [39], it must be interpreted critically as a preliminary apparent concentration rather than a definitive absolute baseline.

Quinone.

Observations of the qualitative test results indicated a positive response for quinone, with the sample changing color to red (see Figure 6). This discoloration occurs due to the oxidation of phenols into highly

reactive quinones, which then undergo polymerization to form colored pigments [46]. In addition, testing with a UV–Vis spectrophotometer yielded an R^2 value close to 1 (0.9996), with a standard curve equation of y = 0.0269x + 0.0165 (see Figure 4C). The total quinone content of the methanol extract of *R. renschianum* flowers was 130.93 ± 30.19 mg HQ/g, with HQ representing hydroquinone equivalents per gram of sample. Another *Rhododendron* species reported to contain quinones is *R. chrysanthum* [45].

Steroid dan Triterpenoid.

Steroid screening (see Figure 7) was negative, with no blue-green color change in the sample, whereas triterpenoids showed a positive result, with a color change to red-brown. The findings of this study expand the list of *Rhododendron* species reported to contain triterpenoids, following previous reports on *R. arboreum*, *R. luteum*, *R. brachycarpum* G. Don, *R. ferrugineum*, *R. latoucheae*, *R. multicolor*, and *R. cowanianum* [48–53]. Crucially, the quantitative estimation using UV–Vis spectrophotometry at 208 nm yielded an implausibly high apparent value of 2685.48 ± 123.49 mg UA/g.

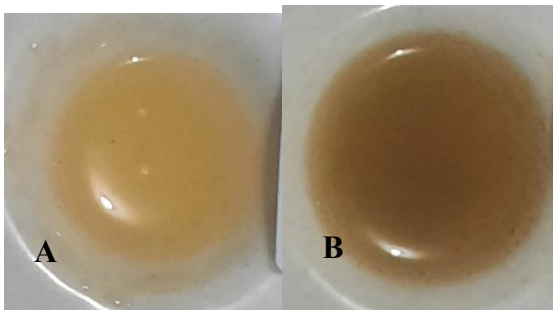


Figure 6. Methanol extract (A) and quinone screening result (B).

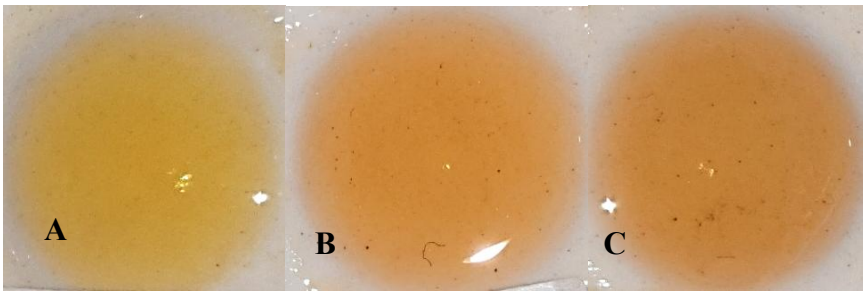


Figure 7. Steroid and triterpenoid screening results: sample (A), steroid test (B), and triterpenoid test (C).

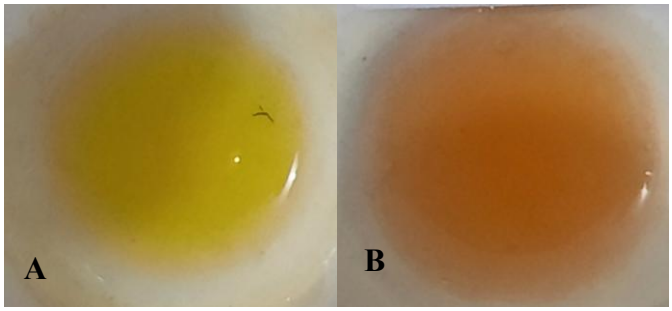


Figure 8. Methanol extract (A) and flavonoid screening result (B).

This specific quantitative result must be interpreted with extreme caution. Because triterpenoids lack strong, distinct chromophores, measuring absorbance in the deep-UV region at 208 nm is inherently problematic for crude extracts; virtually all unsaturated organic molecules and uncharacterized secondary metabolites absorb strongly at this wavelength, leading to substantial overestimation due to matrix interference [52]. This analytical unreliability is clearly reflected in the suboptimal linearity, with R^2 = 0.9473, and the exceptionally high LOD and LOQ values of 2627.03 ppm and 8920.12 ppm, respectively, calculated from the calibration curve y = 0.0078x + 0.0289 (see Figure 4D). Consequently, these numerical values cannot be considered chemically definitive. They serve solely as a preliminary qualitative indicator, underscoring the necessity for selective derivatization or advanced chromatographic validation, such as GC–MS or HPLC, to accurately quantify triterpenoids in future studies [55].

Flavonoid.

Flavonoids are the most abundant group of secondary metabolites in plants and are the main natural pigments affecting the color of *Rhododendron* flowers, along with anthocyanins [56,57]. Based on phytochemical screening tests, the methanol extract of *R. renschianum* flowers underwent a reddish-orange color change (see Figure 8), indicating that the flowers were positive for flavonoids. This discoloration results from the formation of benzopyrylium salts, which are red due to the reduction of polyhydroxy groups of flavonols by magnesium in hydrochloric acid [58]. UV–Vis observations at 426 nm, using quercetin as the standard, yielded an R^2 value of 0.9948, a standard curve equation of y = 0.0266x + 0.1653 (see Figure 4E), an LOQ of 84.93 ppm, and an LOD of 25.48 ppm. Based on Table 1, the total flavonoid content obtained was 5.98 ± 0.70 mg QE/g. This value was relatively low compared with the flavonoid contents reported for *R. arboreum* and *R. myrtifolium* flowers, which were 33.25 ± 0.89 mg RE/g and 8.58 ± 0.19 mg CE/g, respectively [39.59].

Conclusion

Phytochemical screening of the methanol extract of *R. renschianum* flowers indicated the presence of tannins, alkaloids, triterpenoids, quinones, and flavonoids. Meanwhile, saponins and steroids were not detected in this study. Further studies using different solvents and extraction methods are recommended to assess their effects on metabolite content in the samples. The quantitative results varied among metabolite classes, with a very high apparent total triterpenoid content of 2685.48 ± 123.49 mg UA/g. This specific quantitative result must be interpreted with extreme caution. Because triterpenoids lack strong, distinct chromophores, measuring absorbance in the deep-UV region at 208 nm is inherently problematic for crude extracts. Therefore, future studies should use alternative solvents and more selective analytical methods. Among the remaining quantified metabolites, quinones showed the highest content, followed by tannins, with values of 130.93 ± 30.19 mg HQ/g and 109.24 ± 1.44 mg TA/g, respectively. Furthermore, the total alkaloid content was 90.09 ± 67.09 mg CAF/g, and the total flavonoid content was 5.98 ± 0.70 mg QE/g.

Ultimately, this study provides preliminary qualitative and semi-quantitative phytochemical information on the secondary metabolite profile of *R. renschianum* flowers. By establishing this baseline chemical composition, these findings can support and guide future pharmacological investigations, as related *Rhododendron* species have been widely reported to possess diverse biological activities.

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