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The morphoanatomy, histochemistry, and phytochemistry of the leaves and fruits of *Rhodomyrtus tomentosa*

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Abstract. *Kuntorini EM, Sari SG, Fariani R. 2023. The morphoanatomy, histochemistry, and phytochemistry of the leaves and fruits of* Rhodomyrtus tomentosa. *Biodiversitas 24: 98-105. Rhodomyrtus tomentosa* (Ait.) Hassk is native to Southeast Asia. The entire plant has been used in traditional Vietnamese, Chinese, and Malaysian medicine for a long time. Bioactive substances are abundant in ripe fruits. However, few studies have examined phytochemical changes and distribution throughout leaf and fruit growth. This study evaluated the morphoanatomy, histochemical, and phytochemical properties of *R. tomentosa* leaves and fruits. The cross-sectional anatomical structures observed in the leaves included the epidermis, mesophyll, and carrier bundle, while the fruit structures observed included a pericarp layer with an exocarp/outer layer, mesocarp, and endocarp. Flavonoids, tannins, terpenoids, and alkaloids were found in the leaf's adaxial and abaxial epidermis, mesophyll, xylem, phloem, midrib parenchyma, secretory cavities, and trichomes. They were also distributed throughout the fruit's exocarp, mesocarp, endocarp, secretory cavities, xylem, phloem, trichomes, and seeds. Young leaves contained the highest total flavonoid concentration (196 ± 1.77 mg QE/g), green fruit had the highest total phenol concentration (97.70 ± 18.15 mg GAE/g), old leaves contained the highest total alkaloid concentration (13.22 ± 0.98%), and red fruit had the highest total tannin concentration (1.66 ± 0.15 mg GAE/g).

Keywords: Flavonoids, fruits, histochemistry, Karamunting, leaves, *Rhodomyrtus tomentosa*

INTRODUCTION

Rhodomyrtus tomentosa (Aiton) Hassk., a prolific evergreen shrub in the Myrtaceae family in southern and southeastern Asia, from India, east through southern China, the Philippines, and south to Malaysia and Sulawesi, has rose-pink blooms and dark purple edible bell-shaped fruits. In the Indonesian language, it is known as "*karamunting*", while in English, it is most commonly called "rose myrtle". This plant favors natural open light and adapts quickly to a range of soil conditions. Moreover, it requires little maintenance and is rarely affected by pests and illnesses. Most parts of this plant (leaves, roots, buds and fruits) have been used in traditional medicines in Vietnam, China and Malaysia for a long time. In the traditional medicine of Vietnam, the unripe fruits of the plant are utilized to treat diarrhea or dysentery and ripe ones are used to stimulate the immune system (Zhao et al. 2019). Wounds, heartburn, abscesses, and gynecopathy have been treated with *R. tomentosa*. In China, they use it as a medicine to treat urinary tract infections (Hamid et al. 2017a). Modern pharmacological studies discovered that *R. tomentosa* has antibacterial (Zhao et al. 2018), anticancer (Zhang et al. 2016), anti-inflammatory (Na-Phatthalung et al. 2018), and antioxidant (Hamid et al. 2017b) effects. In Indonesia, *R. tomentosa* roots and leaves treat diarrhea, stomachaches, and postpartum recovery. Most of these effects match typical *R. tomentosa* usage (Zhao et al. 2019). Epidemiological studies have shown that eating fruits and vegetables may protect against chronic diseases such as cardiovascular disease, type 2 diabetes, and cancer, as well as other health benefits (Del Rio et al. 2013). Phytochemicals, particularly phenolics, are responsible for fruit and vegetable health benefits. According to recent findings, *R. tomentosa* berries contain over 19 phenolics, including anthocyanins, phenolic acids, flavonols, ellagitannins, and stilbenes. Due to their nutritional value and taste, *R. tomentosa* berries are a regional favorite (Zhao et al. 2017).

Anatomical studies are essential taxonomic indicators, including determining where compounds are secreted and accumulated in a medicinal species' vegetative and generative organs and are important in the quality control of medicinal plants. Pharmacognosy studies of macroscopic and microscopic characteristics are needed for medicinal raw material authentication and for quality assessment in drug standardization. Among the various alternative plant medicinal sources, accurate plant authentication is required (Raghvendra et al. 2021). Various taxa of Myrtaceae have been the subject of anatomical studies (Ferreira et al. 2011); however, there has been no histochemical anatomical study of the leaves and fruit of *R. tomentosa*.

Histochemical studies are needed to reveal the typical pattern of distribution of secondary metabolites in plant tissues. Secondary metabolites are distributed in plant tissues based on their functionality. At the cellular level, secondary metabolites can be located in specific secretory tissues or are distributed throughout the tissue's cells,

including in the parenchyma of the transport bundle (Nugroho 2017).

Histochemical analyses of fruit development can help explain how metabolites affecting color and flavor are synthesized and accumulated during plant organ development. Histological studies can assess the morphological and anatomical details of *R. tomentosa* leaves and fruits. However, studies have not usually addressed secondary metabolite localization in this species. Particular staining and procedures can reveal metabolites, including pectin, lipids, lignin, proteins, phenolic compounds, tannins, starch grains, essential oils, rubber, and terpenoids (Vio-Michaelis et al. 2020). Unequivocal botanical characterizations are needed to identify medicinal plants correctly. Thus, the objective of this present study was to evaluate the leaves' and fruits' morphoanatomy, histochemistry, and phytochemistry of a sample of identified *R. tomentosa* collected in South Kalimantan, Indonesia.

MATERIALS AND METHODS

Plant materials

The 2nd and 10th leaves down from the stem tip and the green, red, and purple fruits of *R. tomentosa* were collected from wild plants in Banjarbaru, South Kalimantan, Indonesia (3°29′0″S, 114°52′0″E) (Figure 1). Three replicate samples of leaves and fruit were collected from three different plants. Herbarium Bogoriense at the Indonesian Institute of Sciences in Bogor, Indonesia, identified the plant specimen.

Procedures

Morphoanatomy slides preparation

Retamales et al. (2014) described the transverse sections of leaves and fruit using paraffin embedding with safranin staining. In the present study, minor adjustments were made to the immersion duration in dehydration and dealcoholization. Three replicates of fruits and leaves from three different plants were fixed in FAA (Formalin 40%: Glacial acetic acid: Alcohol 70%, 5:5:90) solution for 24 hours. These replicates were then dehydrated, stored in 70% ethanol, and embedded in paraffin. Sections 8 to 10 μm thick were obtained on a rotatory microtome, dyed with Safranin, and mounted in Entelan to serve as permanent slides.

Histochemical test

Fresh, fully developed leaves (from the central nerve region and the blade) were cross-sectioned and subjected to the histochemical test. Hand-cut sections were treated with a dilute 10% aqueous acidic $FeCl_3$ (Sigma-Aldrich, Germany) solution containing a small quantity of Na_2CO_3 (Sigma-Aldrich, Germany) solution mounted in clove oil for phenolic compounds, which were identified as dark green or black color in tissues (Trimanto et al. 2018). Flavonoids were identified using Wilson's reagent. Each segment was soaked in citric acid:boric (Prolabo) (5:5 w/w) in 100 mL absolute ethanol for 15 minutes, mounted in glycerine water and inspected under a light microscope (Olympus, Tokyo, Japan). A yellow color signifies the presence of flavonoids (Pratiwi et al. 2020). With 5% copper acetate ($Cu_2(CH_3COO)_4$), terpenoids stained yellow-brown, indicating their presence in cells or tissues (Pratiwi et al. 2020). $FeCl_3$ (0.5% - 1% $FeCl_3$ in 0.1 N HCL) stained tannins blackish green, suggesting their existence (Robil and Tolentino 2015). Wagner's reagent was used to detect alkaloids, and any red-brown staining of tissues or cells indicated the presence of alkaloid compounds (Pratiwi et al. 2020). The material was photographed using OptiLab and a light microscope (Olympus, Tokyo, Japan).

Crude extract preparation

Young leaves (2nd - 6th down from the tip) and old leaves (7th - 12th down from the tip) were picked from the shoot of *R. tomentosa* plants, while harvested green, red, and purple fruits were dried in an oven at 40°C before being powdered at room temperature. A sample of 500 g of each powdered material was macerated for 24 hours in 1000 mL of ethanol (SmartLab, Indonesia). After 24 hours, the solvent was discarded and replenished thrice (Kusuma et al. 2016). Finally, extracts from the same samples were pooled, mixed, filtered, and dried using a rotary evaporator. The extract yield was calculated by this equation: (weight of ethanol extract/weight of dried leaves or fruits) × 100%.

Total phenolic compounds

Folin-Ciocalteu colorimetry was used to determine the triplicate samples' total phenolic contents (TPC) (Roy et al. 2018). The samples' extract absorbances were measured with a UV-Vis spectrophotometer (spectrophotometer UV 1800-Shimadzu, Japan) at 760 nm, and the total phenol content was calculated using the standard gallate acid (Merck, Germany) at mg GAE/g dry weight.

Figure 1. Aerial part of *Rhodomyrtus tomentosa* (Aiton.) Hassk

Total flavonoids content

Total flavonoid content was determined using AlCl colorimetry. A 1 mL sample of the extract was mixed in a test tube with 4 mL of distilled water and with 0.3 mL of 5% sodium nitrite (Sigma-Aldrich, Germany). After 5 minutes, 0.3 mL of 10% aluminum chloride (Sigma-Aldrich, Germany) was added and stirred. After 5 minutes, 2 ml of sodium hydroxide (1 M) was added, and distilled water was added until the volume was precisely 10 mL. The identical method used for the earlier measurement was used to gauge the sample's extract absorbance at 510 nm. Finally, the total flavonoid content was determined using a quercetin standard curve (Sigma-Aldrich, Germany) at mg QE/g dry weight (Roy et al. 2018).

Total tannin content

The Folin-Ciocalteu method was used to calculate the total tannin content. The following ingredients were added to 0.1 mL of sample extract: 7.5 mL of distilled water, 0.5 mL of 10% F-C reagent, and 1 mL of 35% sodium carbonate solution (Sigma-Aldrich, Germany). Then, using distilled water, the volume was increased to 10 mL. After being mixed, the mixture was left at room temperature for 30 minutes. The sample's extract absorbance was determined at a wavelength of 725 nm. Finally, the total tannin content was determined using the standard gallate acid (Merck, Germany) at mg GAE/g dry weight (Roy et al. 2018).

Total alkaloids content

2.5 g of the sample extract was diluted in 50 mL of 10% acetic acid (in ethanol). After being shaken with a magnetic stirrer for 4 hours, the solution was filtered. After that, the filtrate was evaporated. Then, ammonium hydroxide (Sigma-Aldrich, Germany) was drip-applied until an alkaloid precipitate formed. The precipitate-filtering filter paper was weighed before usage. After that, the residue was filtered and cleaned with an ammonium hydroxide 1% solution. The filter paper containing the precipitate was oven dried for 30 minutes at 60°C. After the residue had cooled, it was weighed until a constant weight was obtained. The alkaloid yield was calculated by the weight percentage of the precipitated alkaloid obtained against the initial weight of the extract. (Alasa et al. 2017).

Data analysis

The results from three replications were used to calculate the means and standard deviations (mean ± SD). Using IBM SPSS statistics 21 and Microsoft Excel®, the quantitative data were statistically evaluated using a one-way analysis of variance, with significant differences set at $p < 0.05$. When a statistically significant difference was detected, the analysis of variance was followed by an LSD post hoc test.

RESULTS AND DISCUSSION

Morphoanatomy of *R. tomentosa* leaves and fruits

Young and old *R. tomentosa* leaves have similar epidermal, mesophyll, and transport bundle structures. The adaxial and abaxial epidermis comprises the epidermis. Compared to the abaxial epidermis, the adaxial is larger. The mesophyll consists of palisade tissue and spongy, single-layer palisade tissue grouped compactly. The leaves are dorsiventral in shape, having palisade tissue on the adaxial side. The leaf stalk's carrier bundle network is made up of phloem and xylem. The leaf bone has parenchyma tissue as filling and collenchyma under the epidermis as reinforcement. Trichomes cover the leave's lower epidermis, while a thin cuticle covers the upper epidermis (Figure 2).

The fruit grows from fertilization. The growth of fruit components, such as fruit skin (pericarp), seed coat, endosperm, and embryo, follows fruit development. *R. tomentosa* fruit is a bacca/buni fruit, which has three layers (exocarp, mesocarp, and endocarp). The thin outer layer (exocarp) is rather stiff and the thick inner layer (mesocarp), soft and runny, is the edible part (Hamid et al. 2017a). The cross-section of green, red, and purple fruit displays a fruit pericarp layer with an exocarp/outer layer, middle mesocarp, and endocarp. (Figure 3). The stiff exocarp has a polyhedral epidermis and several sclerenchyma layers. The edible mesocarp has big, watery parenchyma cells. The endocarp cells are thin-walled. The 1.5 mm seeds have 6 pseudo-locules with thin false septa (Hermanto et al. 2013; Hamid et al. 2017a).

Histochemical tests

Histochemical tests measure the secondary metabolites of the plant. Histochemical tests use qualitative analysis to detect compounds without measuring their concentration (Dhale 2011). Secondary metabolite components such as phenols, flavonoids, tannins, terpenoids, and alkaloids were found in *R. tomentosa*'s leaves and fruits at specific locations or, more generally, in the tissues. The secondary metabolites were detected histochemically. After the application of reagents, the identified compounds showed specific color changes (Figure 4).

Histochemical tests on young and old leaves, green, red, and purple fruit indicated positive reactions to flavonoid compounds (4f-4j) and phenols (Figure 4k-4o). Young and old leaves had phenolic, and flavonoid compounds in the adaxial and abaxial epidermis, mesophyll, xylem, phloem, midrib parenchyma, secretory space, and trichomes. Fruit phenolic and flavonoid compounds were found in the exocarp, mesocarp, endocarp, secretory space, xylem, phloem, trichomes, and seeds.

Figure 4p-4t shows alkaloid compounds in young and old leaves in the adaxial and abaxial epidermis, mesophyll, xylem, phloem, midrib parenchyma, secretory space, and trichomes. In green and red fruit, alkaloids were found in the endosperm, exocarp, mesocarp, endocarp, secretory space, xylem, phloem, trichomes, and seeds.

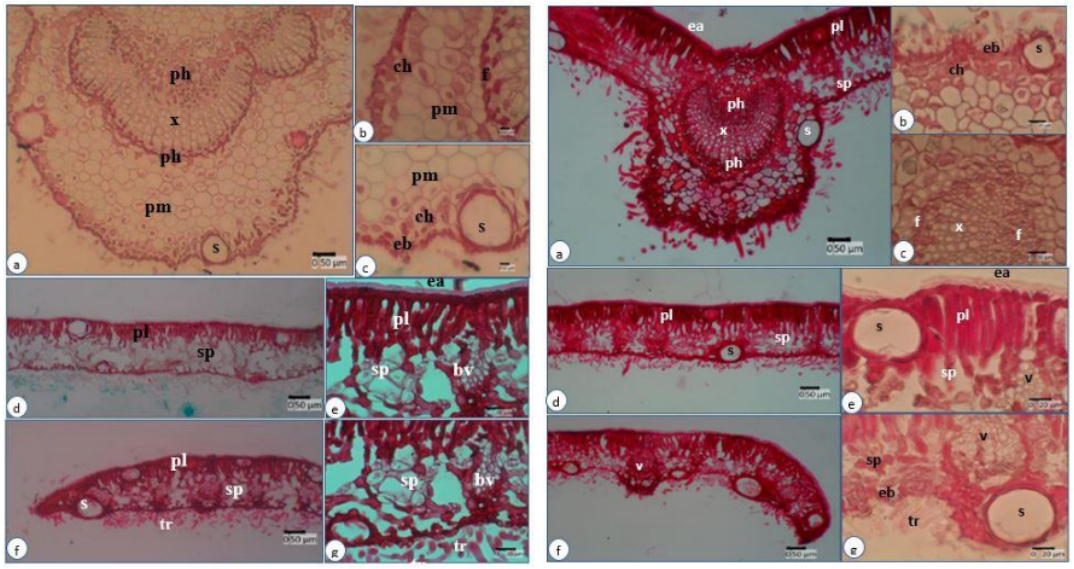

Figure 2. Cross-section of (A) young leaves, (B) old leaves *of R. tomentosa;* palisade mesophyll (pl), sponge mesophyll (sp), parenchyma midrib (pm), secretory cavity (sc), trichomes (tr), abaxial epidermis (eb), adaxial epidermis (ea), collenchyma (ch), vascular bundle (v), phloem (f), xylem (x)

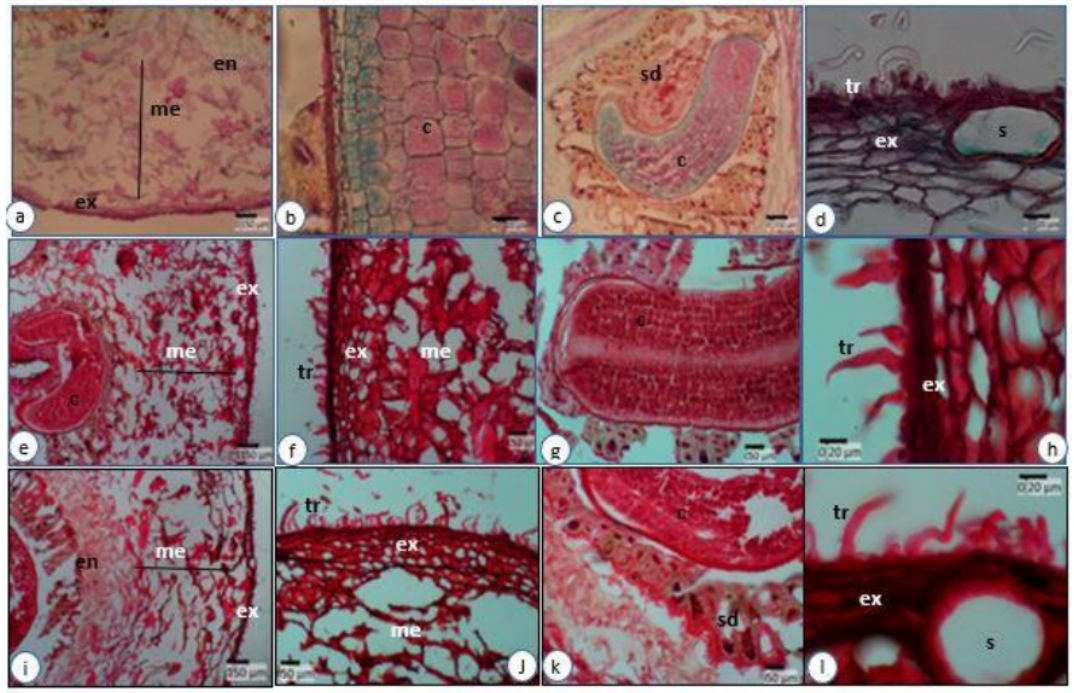

Figure 3. Cross-section of green fruit (a-d), red fruit (e-h) and purple fruit (i-l) of *R. tomentosa;* exocarp (ex) mesocarp (me), endocarp (en), seed (sd), secretory cavity (s), trichomes (tr)

Figure 4u-4y shows tannin compounds in young and old leaves in the adaxial and abaxial epidermis, mesophyll, xylem, phloem, midrib parenchyma, secretory cavity, trichomes, and druse crystals. Tannin compounds were found in the exocarp, mesocarp, endocarp, secretory cavity, xylem, phloem, trichome, and seeds in the green and red fruit but not in the purple fruit.

Figure 4z-4d1 shows terpenoid compounds in young and old leaves in the adaxial and abaxial epidermis, mesophyll, xylem, phloem, midrib parenchyma, secretory cavity, trichomes, and druse crystals. Terpenoid compounds were found in the exocarp, mesocarp, endocarp, secretory cavity, xylem, phloem, trichomes, and seeds. Terpenoid compounds were not found in the endocarp of purple fruit.

Phytochemistry

The results of phytochemical measurements showed that the highest total flavonoid levels were in young leaves at 196.0 mg QE/g, followed by green fruit and old leaves, 164.625 mg QE/g, and 134.875 mg QE/g, followed by flavonoid levels in red and purple fruits at 84.625 mg QE/g and 55.75 mg QE/g, respectively. The highest measured total phenol content was 97.70 mg/mL recorded in green fruit, followed by old leaves at 59.30 mg/mL, young leaves at 54.40 mg/mL, red fruit at 19.40 mg/mL, and the lowest, in purple fruit at 17.23mg/mL. The highest total alkaloid content was recorded in old leaves at 13.23%, green fruit at 9.28%, red fruit at 7.80%, purple fruit at 7.05%, and young leaves, the lowest at 5.64%. For total tannin content, the measurements showed the highest value in red fruit at 1.658 mg GAE/g, followed by purple fruit at 1.402 mg GAE/g, young leaves at 0.943 mg GAE/g, old leaves at 0.880 mg GAE/g, and the lowest tannin content in green fruit at 0.804 mg GAE/g. (Table 1).

Discussion

According to research by Al-Edany and Al-Saadi (2012) on the anatomical characteristics of several Myrtaceae species, the upper surface of the epidermal tissue on the leaves of the Myrtaceae family is covered by a variable-thickness cuticle layer. Young and old leaves of *R. tomentosa* have one layer of epidermal tissue on the upper surface (adaxial). The epidermal tissue's cells are square and closely packed. Al-Edany and Al-Saadi (2012) found that the cells of Myrtaceae leaf epidermal tissue are square or rectangular and uniseriate.

Kantachot et al. (2007) examined the leaves of 28 species in 12 genera of the Myrtaceae family and found that *R. tomentosa* had dorsiventral leaves, which have palisade tissue only on one side. The cells in the upper epidermis are thicker than those in the lower epidermis. The leaves of *R. tomentosa* do not have a hypodermis. The lower epidermis of the leaves has unicellular trichomes and anomocytic stomata. In the cross-section of the leaf stalk and petiole, vascular tissue forms a U-shaped pattern. Drusen-shaped Ca oxalate crystals are seen in leaf bone maternal parenchyma tissue.

The leaves of *R. tomentosa* have palisade/pole and spongy mesophyll tissue. The palisade parenchyma is found beneath the upper epidermis. The cells that comprise the palisade parenchyma tissue are formed like pillars, appear tightly packed, and are just one layer thick. There are many chloroplasts in this palisade parenchyma tissue. The spongy tissue is located beneath the palisade tissue. The cells that make up the spongy tissue are formed like branches, appear loosely organized, and are uneven. Spongy parenchyma tissue contains chloroplasts, although less than palisade/pillar tissue. According to Al-Edany and Al-Saadi (2012), Myrtaceae leaves' mesophyll tissue has 1 to 2 layers of palisade tissue and several rows of up to 15 layers of compact and loose spongy tissue or 2 layers of palisade tissue on the adaxial surface, and spongy tissue on the abaxial surface.

Rhodomyrtus tomentosa leaves have bicollateral vascular bundles consisting of xylem bundles flanked by phloem. Ferreira et al. (2011) describe the bundle of vessels in *Psidium guineense* Sw. (family Myrtaceae) leaves that include xylem flanked by phloem. The xylem and phloem function as a delivery system for vascular plants. The primary function of the xylem is to transport water and solutes, while the primary function of the phloem is to distribute photosynthesis products.

Rhodomyrtus tomentosa leaf epidermal tissue on the abaxial side has unicellular trichomes. Kantachot et al. (2007) identified abundant unicellular and uniseriate trichomes on the leaves of Myrtaceae species such as *Decaspermum parviflorum*, *Melaleuca cajuputi*, *Psidium guajava*, *Rhodamnia dumetorum*, *Tristaniopsis burmanica* var. *rufescens*, and *R. tomentosa*. Trichomes from modified epidermal cells reduce evaporation from the leaves and minimize animal disturbances (Nugroho 2017).

Table 1. Total amount of compounds in ethanolic extracts of leaves and fruits of *R. tomentosa*

Sample	Total flavonoids (mgQE/g)	Total phenolics (mgGAE/g)	Total alkaloids (%)	Total tannins (mgGAE/g)
Young leaves	196 ± 1.77^{c}	54.40 ± 0.09^{b}	5.64 ± 0.20^{a}	0.94 ± 0.74^{a}
Old leaves	134.88 ± 6.89^{c}	59.30 ± 16.73^{b}	13.22 ± 0.98^{d}	0.88 ± 0.01^{a}
Green fruits	164.63 ± 2.65^{d}	97.70 ± 18.15^{c}	9.28 ± 0.33^{c}	0.80 ± 0.30^{a}
Red fruits	84.63 ± 2.30^{b}	19.40 ± 0.47^{a}	7.80 ± 0.97b^{c}	1.66 ± 0.15^{c}
Purple fruits	55.75 ± 0.71^{a}	17.23 ± 4.20^{a}	7.05 ± 0.41a^{b}	1.40 ± 1.20^{b}

Note: The data presented are the mean ± standard deviation. One-Way ANOVA and LSD test with = 0.05 were used to evaluate data. Numbers followed by different superscript letters in the same column show significantly different results

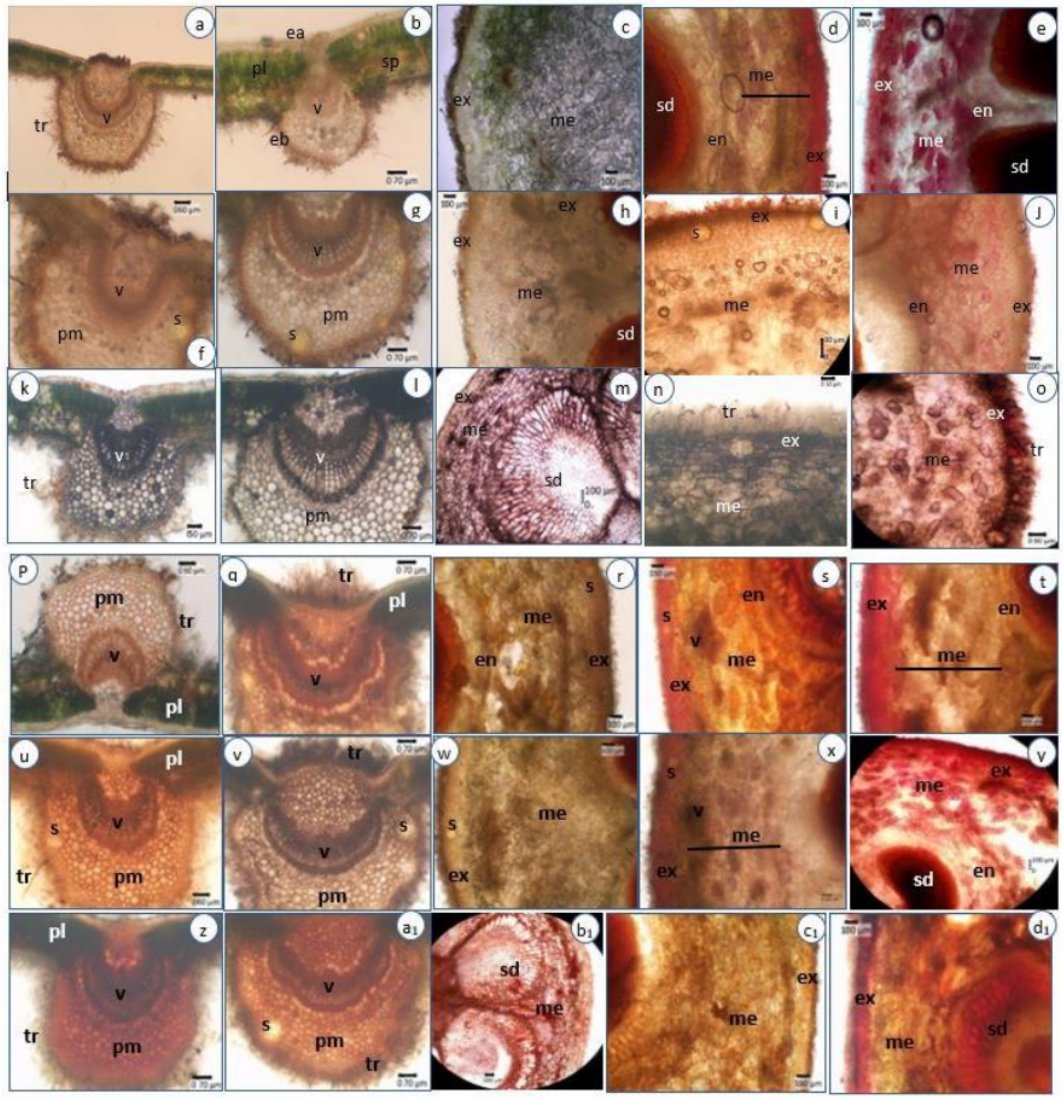

Figure 4. Transverse sections of histochemically stained leaves and fruits of *R. Tomentosa*. (a-e) Free-hand section of a young leaf without staining; (f-j) the positive reaction to Wilson's reagent indicating the presence of flavonoid in leaves and fruits; (k-o) the positive reaction to ferric chloride indicating the presence of phenolics in leaves and fruits: (p-t) the positive reaction to Wagner's reagent indicating the presence of alkaloid in leaves and fruits; (u-y) the positive reaction to $FeCl_3$ indicating the presence of tannin in leaves and fruits; (z-d_1) the positive reaction to $Cu_2(CH_3COO)_4$ 5% indicating the presence of terpenoid in leaves and fruits: palisade mesophyll (pm), sponge mesophyll (sm), parenchyma midrib (pm), secretory cavity (s), trichomes (tr), druse crystal (dc), abaxial epidermis (eb), adaxial epidermis (ea) exocarp (ex) mesocarp (me), endocarp (en), seed (sd) vascular bundle (v)

Retamales et al. (2014) found *Myrceugenia rufa* (Myrtaceae) fruit anatomy similar to *R. tomentosa*. The ripe fruit's pericarp has three zones: exocarp, mesocarp, and endocarp. The exocarp cells are irregular and plano-convex. The cuticle is thin and covered in hair. The mesocarp comprises 7-8 layers of large and isodiametric parenchyma cells with thin walls. Multiple and large secretory cavities are observed throughout this tissue. The endocarp is a delicate tissue that surrounds the seed.

Histochemical test results by Agustina et al. (2016) recorded flavonoids and other phenolics in the stem, leaf, and flower-fruit-seed organs of *Acalypha indica*. Phenols in

Acalypha wilkesiana are also distributed in the xylem in stem organs, leaf mesophyll, and in the flower-fruit seed.

Flavonoids can be found in every plant organ, including the leaves, roots, wood, flowers, nectar, and seeds. Flavonoids can be stored in cell walls, vacuoles, and glandular trichomes. Lipophilic compounds generated by glandular trichomes include terpenoids, lipids, waxes, and flavonoid aglycones. Flavonoids concentrate in cells and are not present in the intercellular space, resulting in a symplastic distribution (Petrussa et al. 2013).

According to Cartea et al. (2011), phenolic compounds are widespread phytochemicals. Structure-wise, they are simple phenols, phenolic acids, hydroxycinnamic acid derivatives, and flavonoids. Flavonoids (flavonols and anthocyanins) and hydroxycinnamic acids are plants' most abundant and diversified polyphenols. Flavonoids are the most prevalent phenolics and are found throughout all plants. Flavonoids in the leaf and fruit epidermis protect against UV radiation and pigment, stimulate nitrogen-fixing nodules, and fight disease.

The same study found positive reactions to reagents that detect phenolics in leaf mesophyll cells, pith parenchyma, and stem cortical parenchyma cells of *Adhatoda zeylanica* (Acanthaceae), *Ruta graveolence* Linn. (Rutaceae), and *Vitex negundo* Linn. (Verbenaceae) (Dhale 2011).

Alkaloids are heterocyclic nitrogen-containing chemicals utilized in medicine. Alkaloids protect plants from insects and herbivores, detoxify toxins, and store nitrogen. Many products consumed by humans contain alkaloids, such as coffee, tea, cocoa, tobacco, areca nut, and marijuana. Alkaloids have neuroprotective, blood pressure-lowering, pain-relieving, antibacterial, anticancer, and antimalarial effects (Carqueijeiro et al. 2013). Alkaloids detected in *R. tomentosa's* leaves and fruits support its historic medicinal usage in Singapore. In China, the leaves were traditionally used as a painkiller, the roots for stomach ulcers, and the seeds for improved digestion and for snake bites. In Indonesia, the leaves were used to cure wounds. In traditional Vietnamese medicine, the plant's raw fruit treats diarrhea and dysentery, while the ripe ones boost the immune system (Zhao et al. 2019).

According to Ferreira et al. (2011), histochemical tannin assays on *Psidium guineense* Sw (Myrtaceae) leaves indicated positive results in the epidermis, parenchyma, vascular bundles, and secretory cavities. These tannins in *R. tomentosa* leaves and fruits help treat diarrhea and dysentery. Ferreira et al. (2011) added that Myrtaceae leaves are used to treat diarrhea and intestinal infections due to their astringent tannin content. Tannins protect plants, which is why epidermal cells have them. Myrtaceae plants have tannins in their vascular systems also.

The meroterpenoids of *R. tomentosa* are antibacterial and antifungal. Myrtaceae secondary metabolites like meroterpenoids have been extensively explored as antibiotics. Thus, *R. tomentosa* could prevent microbial deterioration and extend food shelf life as a biocontrol agent and natural preservative (Zhao et al. 2019). Meroterpenoid compounds have been isolated and identified from various other Myrtaceae species, including *Eucalyptus tereticornis* (Liu et al. 2018) and *Leptospermum brachyandrum* (Zou et al. 2018).

Belwal et al. (2019) reported that flavonoid content declined throughout fruit ripening in *Rubus ellipticus*, *Myrica esculenta*, *Prunus persica*, blueberry (*Vaccinium corymbosum*), and blackberry fruit, as did Lin et al. (2020) in blueberries. Flavonoid levels vary by organ type. Hou et al. (2021) found that phenols and flavonoids drop from 5.38 mg RE/g in young citrus fruits to 0.69 mg RE/g in ripe fruit. The same trend was seen in grapes and lemons (Zhu et al. 2020). This phenomenon may be produced by many enzymes involved in flavonoid synthesis, such as 1,2-rhamnosyltransferase and flavanone-7-O-glucosides, which accelerate biosynthesis in the early growth period. Insufficient flavonoid aglycones may explain the decrease in flavonoids during citrus fruit ripening.

Lai et al. (2013) found a decrease in total phenol levels during *R. tomentosa* fruit ripening. The same pattern of declining phenol levels during fruit ripening occurs in other Myrtaceae plants, such as Brazilian cherry *Eugenia uniflora* L. (Celli et al. 2011). Belwal et al. (2019) further explain the accumulation and degradation of distinct phenolic compounds during fruit ripening connected to their biosynthesis pathways, which are mainly regulated by enzyme expression and various genetic and environmental variables. Elmastaş et al. (2017) added that phenol compounds are phenylpropanoid metabolic pathway intermediates. Enzymes convert aromatic amino acids into polyphenols. Enzyme activity affects the production of these molecules. Genetic diversity, pH, light, temperature, and climatic change affect enzyme activity. Fruit enzyme activity might vary depending on plant growth and ripening.

The anatomical characteristics reported in this study contribute to the identification of *R. tomentosa* and support quality control tests. Some features may stand out, such as hairy leaves with curved edges; epidermal cells with ripples and wall projections: and secretory cavities with dimensions that go beyond the height of the palisade parenchyma. Characters that can contribute to accuracy in taxonomic classification include the cross-sectional anatomical structure of leaves, including the epidermis, mesophyll, carrier bundle, secretory cavity, and trichomes, while fruit characteristics include a pericarp layer with an exocarp/outer layer, mesocarp, and endocarp. In this study, flavonoids, tannins, terpenoids, and alkaloids were found in the leaf's adaxial and abaxial epidermis, mesophyll, xylem, phloem, midrib parenchyma, secretory cavity, and trichomes. They were distributed throughout the fruit's exocarp, mesocarp, endocarp, secretory cavity, xylem, phloem, trichomes, and seeds. Young leaves contained the highest total flavonoid concentration (196 ± 1.77 mg QE/g), green fruit had the highest total phenol concentration (97.70 ± 18.15 mg GAE/g), old leaves contained the highest total alkaloid concentration (13.22 ± 0.98%), and red fruit had the highest total tannin concentration (1.66 ± 0.15 mg GAE/g).

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REFERENCES

Agustina N, Purwestri YA, Nugroho LH. 2016. Antioxidant activity and histochemical analysis of *Acalypha indica* L. and *Acalypha wilkesiana* Muell. Arg. vegetative and generative organs. Intl J Pharmacogn Phytochem Res 8 (10): 1657-1662.

Alasa AN, Anam S, Jamaluddin. 2017. Analyze total level of secondary metabolites from ethanol extracts of "Tamoenju" leaves (*Hibiscus surattensis* L.). KOVALEN 3 (3): 258-268. DOI: 10.22487/j24775398.2017.v3.i3.9334.

Al-Edany TY, Al-Saadi SAAM. 2012. Taxonomic significance of anatomical characters in some species of the family Myrtaceae. Am J Plant Sci 3: 572-581. DOI: 10.4236/ajps.2012.35069.

Belwal T, Pandey A, Bhatt ID, Rawal RS, Luo Z. 2019. Trends of polyphenolics and anthocyanins accumulation along ripening stages of wild edible fruits of Indian Himalayan region. Sci Rep 9 (1): 1-11. DOI: 10.1038/s41598-019-42270-2.

Carqueijeiro I, Noronha H, Duarte P, Gerós H, Sottomayor M. 2013. Vacuolar transport of the medicinal alkaloids from *Catharanthus roseus* is mediated by a proton-driven antiport. Plant Physiol 162 (3): 1486-1496. DOI: 10.5772/intechopen.78510.

Cartea ME, Francisco M, Soengas P, Velasco P. 2011. Phenolic compounds in brassica vegetables. Molecules 16 (1): 251-280. DOI: 10.3390/molecules16010251.

Celli GB, Pereira-Netto B, Beta T. 2011. Comparative analysis of total phenolic content, antioxidant activity, and flavonoids profile of fruits from two varieties of brazilian cherry (*Eugenia uniflora* L.) throughout the fruit developmental stages. Food Res Intl 44: 2442-2451. DOI: 10.1016/j.foodres.2010.12.036.

Del Rio D, Rodriguez-Mateos A, Spencer JPE, Tognolini M, Borges G, Crozier A. 2013. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic disease. Antioxid Redox Signal 18 (14): 1818-1892. DOI: 10.1089/ars.2012.4581.

Dhale DA. 2011. Histochemical investigation of some medical plants. Adv Res Pharm Biol 1 (2): 147-154.

Elmastaş M, Ayşe D, Nusret G, Ümit D, Mehmet G. 2017. Changes in flavonoid and phenolic acid contents in some *Rosa* species during ripening. Food Chem 235: 154-159. DOI: 10.1016/j.foodchem.2017.05.004.

Ferreira P, Mendes C, Reis S, Rodrigues C, Oliveira D. 2011. Morphoanatomy, histochemistry and phytochemistry of *Psidium guineense* Sw. (Myrtaceae) leaves. J Pharm Res 4 (4): 942-944.

Hamid AH, Mutazah SSR, Yusoff MM. 2017a. *Rhodomyrtus tomentosa:* a phytochemical and pharmacological review. Asian J Pharm Clin Res 10: 10-16. DOI: 10.22159/ajpcr.2017.v10i1.12773.

Hamid, HA, Mutazah R, Yusoff MM, Karim NAA, Razis AFA. 2017b. Comparative analysis of antioxidant and antiproliferative activities of *Rhodomyrtus tomentosa* extracts prepared with various solvents. Food Chem Toxicol 108: 451-457. DOI: 10.1016/j.fct.2016.10.004.

Hermanto C, Luh N, Indriani P, Hadiati S. 2013. Keragaman dan Kekayaan Buah Tropika Nusantara. IAARD Press. [Indonesian]

Hou JX, Liang L, Su MY,Yang TM, Mao XJ, Wang YX. 2021. Variations in phenolic acids and antioxidant activity of navel orange at different growth stages. Food Chem 360: 1-7. DOI: 10.1016/j.foodchem.2021.129980.

Kantachot C, Chantaranothai P, Thammathaworn A. 2007. Contributions to the leaf anatomy and taxonomy of Thai Myrtaceae. Nat Hist J Chulalongkorn Univ 7 (1): 35-45.

Kusuma IW, Ainiyati N, Suwinarti W. 2016. Search for biological activities from an invasive shrub species rose myrtle (*Rhodomyrtus tomentosa*). Nusant Biosci 8 (1): 55-59. DOI: 10.13057/nusbiosci/n080110.

Lai TNH, Herent MF, Quetin-Leclercq J, Nguyen TBT, Rogez H, Larondelle Y, Andre CM. 2013. Piceatannol, a potent bioactive stilbene, as major phenolic component in *Rhodomyrtus tomentosa*. Food Chem 138 (2-3): 1421-1430. DOI: 10.1016/j.foodchem.2012.10.125.

Lin Y, Huang G, Zhang Q, Wang Y, Dia VP, Meng X. 2020. Ripening affects the physicochemical properties, phytochemicals and antioxidant capacities of two blueberry cultivars. Postharvest Biol Technol 162:1-8. DOI: 10.1016/j.postharvbio.2019.111097.

Liu H, Feng MY, Yu Q, Yan H, Zeng Y, Qin XJ, He L, Liu HY. 2018. Formyl phloroglucinol meroterpenoids from *Eucalyptus tereticornis* and their bioactivities. Tetrahedron 74 (13): 1540-1545. DOI: 10.1016/j.tet.2018.02.020.

Na-Phatthalung P, Teles M, Voravuthikunchai SP, Tort L, Fierro-Castro C. 2018. Immunomodulatory effects of *Rhodomyrtus tomentosa* leaf extract and its derivative compound, rhodomyrtone, on head kidney macrophages of rainbow trout (*Oncorhynchus mykiss*). Fish Physiol Biochem 44 (2): 543-555. DOI:10.1007/s10695-017-0452-2.

Nugroho LH, 2017. Struktur dan Produk Jaringan Sekretori Tumbuhan. Gadjah Mada University Press, Yogyakarta. [Indonesian]

Petrussa E, Braidot E, Zancani M, Peresson C, Bertolini A, Patui S, Vianello A. 2013. Plant flavonoids-biosynthesis, transport and involvement in stress responses. Intl J Mol Sci 14 (7): 14950-14973. DOI: 10.3390/ijms140714950.

Pratiwi DR, Yohana CS, Diah R. 2020. Localization of alkaloid and other secondary metabolites in *Cinchona ledgeriana* Moens: anatomical and histochemical studies on fresh tissues and cultured cells. HAYATI J Biosc 27 (1): 1-7. DOI: 10.4308/hjb.27.1.1.

Raghvendra KD, Satish D, Ashwini KD. 2021. Pharmacognostical and qualitative phytochemical study of *Glinus oppositifolius* (Linn.) Aug. DC. : an important ethnobotanical plant. GIS Sci J 8 (7): 2105-19.

Retamales H, Serra MT, Scharaschkin T. 2014. Anatomical studies of the flower, fruit and seeds of *Myreugenia rufa* (Myrtaceae). Bol Mus Nac Hist Nat 63: 89-100.

Robil JLM, Tolentino VS. 2015. Histological localization of tannins at different developmental stages of vegetative and reproductive organs in *Medinilla magnifica* (Melastomataceae). Flora: Morpho Distrib Funct Ecol 217: 82-89. DOI: 10.1016/j.flora.2015.10.003.

Roy A, Krishnan ML, Bharadvaja N. 2018. Qualitative and quantitative phytochemical analysis of *Centella asiatica*. Nat Prod Chem Res 6 (4): 323-326. DOI: 10.4172/2329-6836.1000323.

Trimanto, Dwiyanti D, Indriyani S. 2018. Morfologi, anatomi dan uji histokimia rimpang *Curcuma aeruginosa* Valeton dan Zipj. Jurnal Ilmu-Ilmu Hayati 17 (2): 123-133. DOI: 10.14203/beritabiologi.v17i2.3086. [Indonesian]

Vio-Michaelis S, Feucht W, Gómez M, Hadersdorfer J, Treutter D, Schwab W. 2020. Histochemical analysis of anthocyanins, carotenoids, and flavan-3-ols/proanthocyanidins in *Prunus domestica* L. fruits during ripening. J Agric Food Chem 68: 2880-2890. DOI: 10.1021/acs.jafc.9b01954.

Zhang YB, Li W, Zhang ZM, Chen NH, Zhang XQ, Jiang JW, Wang GC, Li YL. 2016. Two new triterpenoids from the roots of *Rhodomyrtus tomentosa*. Chem Lett 45 (3): 368-370. https://doi.org/10.1246/cl.151188.

Zhao GH, Zhang RF, Liu L, Deng Y, Wei Z, Zhang Y, Ma Y, Zhang M. 2017. Different thermal drying methods affect the phenolic profiles, their bioaccessibility and antioxidant activity in *Rhodomyrtus tomentosa* (Ait.) Hassk berries. LWT-Food Sci Technol 79: 260-266. DOI: 10.1016/j.lwt.2017.01.039.

Zhao LY, Liu HX, Wang L, Xu ZF, Tan HB, Qiu SX. 2018. Rhodomyrtosone B, a membrane-targeting anti-MRSA natural acylgphloroglucinol from *Rhodomyrtus tomentosa*. J Ethnopharmacol 228: 50-57. DOI: 10.1016/j.jep.2018.09.011.

Zhao ZF, Wu L, Xie J, Feng Y, Tian J, He X, Wang L, Wang X, Zhang Y, Wu S, Zheng X. 2019. *Rhodomyrtus tomentosa* (Aiton.): A review of phytochemistry, pharmacology and industrial applications research progress. Food Chem 309: 1-10. DOI: 10.1016/j.foodchem.2019.125715.

Zhu C, Zhou X, Long C, Du Y, Li J, Yue J, Pan S. 2020. Variations of flavonoid composition and antioxidant properties among different cultivars, fruit tissues and developmental stages of citrus fruits. Chem Biodivers 17(6): 1-23. DOI: 10.1002/cbdv.201900690.

Zou ZX, Tan GS, Huang Q, Sun HH, Huo LQ, Zhong WQ, Zhao LY, Liu HX, Tan HB. 2018. Brachyanins A-C, pinene-derived meroterpenoids and phloroglucinol derivative from *Leptospermum brachyandrum*. Fitoterapia 130: 184-189. DOI: 10.1016/j.fitote.2018.08.026.

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