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Research article

Anatomical and histochemical responses of Senggani (*Melastoma malabathricum* L.) grown in different soil types

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Abstract

Importance of the work: Senggani (*Melastoma malabathricum* L.) grows in various habitats and contains several secondary metabolites, offering several benefits. However, no published study has investigated the anatomical characteristics and distribution of secondary metabolites related to plant adaptation at the tissue level in Senggani grown in different soil types.

Objectives: To analyze anatomical characteristics and tissue distribution of secondary metabolites of Senggani grown in different soil types (Mediterranean, latosol and regosol).

Materials and Methods: Anatomical slide preparation was carried out using a paraffin embedding method, while a histochemical technique was used to observe the distribution of phenolics, terpenoids and alkaloids.

Results: The Mediterranean soil type produced the highest stem periderm thickness and leaf trichomes index, with the lowest epidermal thickness and number of stem xylem vessels. The latosol had the highest root cortex thickness, root xylem vessel diameter, sponge thickness and leaf xylem vessel diameter. Furthermore, plants grown on the regosol had the highest root stele diameter, stem xylem vessel number, stomatal size and leaf trichome density. Qualitatively, the Mediterranean and regosol soil types produced a higher distribution of phenolics, terpenoids and alkaloids compared to the latosol. The predominant site for secondary metabolites in roots was in the parenchyma of the phloem and xylem. In stems, these compounds were concentrated in xylem vessels and distributed in the trichomes, epidermis and mesophyll, as well as in the parenchymal cells of midribs and vascular tissues from leaves.

Main finding: *M. malabathricum* could grow in Mediterranean, latosol and regosol soils. Plants grown in the latosol soil had better anatomical characteristics but a reduced secondary metabolite distribution compared to the Mediterranean and regosol soils.

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Introduction

The genus *Melastoma*, which belongs to the family *Melastomataceae*, comprises 99 accepted species found in Seychelles, tropical and subtropical Asia, and the west Pacific (Plants of the World Online, 2024). Among these species, Senggani (*Melastoma malabathricum* L.) is a hardy, adaptable plant that thrives in various habitats, such as roadsides, riverbanks, secondary forests, and grasslands (Muhaemin, 2008). It is abundant in humid tropical climates across Southeast Asia, flourishing in environments including rice fields, highlands, and mountainous regions (Tambaru, 2017). In pharmacology, Senggani has been used widely as a medicinal material (Jofrry et al., 2012), due to its contents of various secondary metabolites, such as alkaloids, flavonoids, sterols, tannins and glycosides (Ferdous et al., 2018), which have been reported to have certain physiological benefits as analgesics and antimicrobials (Mustapha et al., 2017), as well having antipyretic, anti-inflammatory and antibacterial properties (Noviyanti and Linda, 2020).

Generally, plant growth is influenced by soil type, along with water and nutrient availability that are often unpredictable (Lynch, 2018). The soil type may affect anatomical chracteristics (Tufaila, 2014). In Indonesia, there are 7 soil types, including Mediterranean, latosol and regosol classes (Anonim, 2023).

Mediterranean soil is included in the alfisol order (Yulianto and Purnama, 2019), typically forming in karst areas originating from limestone rocks. This soil has 7–9% clay, 14–20% silt and 2–5% sand (Setiaki, 2002), with distinct characteristics, ranging from a brown-yellow to red-brown shallow solum and a red-brown to dark-red subsoil, contributing to high infertility (Arifin, 2012). Mediterranean or calcareous soil has a high pH and high Ca, and Mg contents, which disrupt the availability of nutrients for plants (Isnatin et al., 2019), resulting in low bioavailability of micronutrients and macronutrients, particularly iron (Fe) and phosphorus (P), causing stunted root growth and a decrease in the biomass of several fruit species grown in Mediterranean soil, due to reduced photosynthetic activity (Ipek et al., 2021).

The latosol soil type belongs to the ultisol order (Dharma et al., 2021), characterized by a low cation exchange capacity and a low nutrient content, with an acidity level of approximately 4.5–6.0 (Saptiningsih and Haryanti, 2015). The general morphological characteristics include a clay-to-loamy texture, crumbly-to-weakly lumpy structure and loose consistency, with 20–45% sand, 15–53% dust and 27–40% clay (Hanafiah, 2009).

Regosol soil is included in the entisol order (Fajrina et al., 2019), characterized by a sandy texture and loose-leaf consistency. Other features include a neutral pH, moderate fertility, gray-to-yellow-colored soil and a clay content less than 40%. Due to its sandy texture, a regosol soil has a low ability to hold water and nutrients, resulting in the expansion of root growth to the absorption area (Prasetyo et al., 2018). A study on *Tanacetum vulgare* L. (from the *Asteraceae*) grown in sandy soil reported a reduction in the thickness of the leaf mesophyll, palisade parenchyma and the adaxial and abaxial epidermis (Stevovic et al., 2010).

Plants generally respond to characteristics in their environments by producing secondary metabolites for protection against predators, insects and infection by harmful microorganisms (Pavela, 2016). Although identification studies have been conducted, there is no published information on the anatomical characters and distribution of secondary metabolites at the tissue level for Senggani grown in Mediterranean, regosol and latosol soil types. Therefore, this study aimed to determine the effect of soil type on Senggani anatomical structures and the distribution of secondary metabolites, specifically phenolics, terpenoids and alkaloids. The results should provide valuable information to improve and support the effective and efficient development of Senggani.

Materials and Methods

Preparation and planting procedure

Senggani (*M. malabathricum*) plants (height and stem diameter approximately 15 cm and 3 mm, respectively) were obtained from Pugeran Forest, Semoyo, Patuk, Gunungkidul, Yogyakarta, Indonesia, with identification Letter No. 0123/S.Tb/VII/2022, 4 July 2022, by Plant Systematic Laboratory, Faculty of Biology, Universitas Gadjah Mada. Each plant was replanted in a separate polybag filled with 2.5 kg growing media, comprising Mediterranean, latosol or regosol soil type from Gunungkidul, Bantul and Sleman Regencies, respectively. The experiment was carried out in the greenhouse of the Biodiversity Research and Development Station II, Faculty of Biology, Universitas Gadjah Mada (7°45’35”S 110°23’03”E). Each treatment was replicated five times and plants were watered twice daily to field capacity

(700 mL, 400 mL and 500 mL for the Mediterranean, latosol and regosol soil types, respectively. Before being used as planting media, all soil samples were analyzed in the Soil Laboratory Department of Soil Science, Faculty of Agriculture, Universitas Gadjah Mada. The properties of soil analysis applied in this study are presented in Table 1.

Observations and anatomical data collection

Observations and data collection for anatomical analysis were conducted after 60 d of plant growth Anatomical slides of roots and leaves were prepared using the paraffin embedding method (Ruzin, 1999). Stomatal size and stomatal and trichome density were measured and counted on tissue prints of the leaf paradermal prepared using nail polish. Samples were observed using an Optilab-connected microscope equipped with an Optilab Viewer and Image Raster software (Miconos, Indonesia) The stomatal index (SI) was calculated using the formula described by Salisbury (1928), namely SI = (S / (S + E)) × 100, where S is the number of stomata per unit area and E is the number of epidermal cells in the same unit area. The trichome density was calculated by counting the number of trichomes on each microscope field of view (10 × 40 magnification or 0.173 mm^2), according to Maryani et al. (2009).

Cellular localization of secondary metabolites

The secondary metabolites of phenolic, terpenoid and alkaloid compounds were observed using a histochemical method on free-hand sections of the root, stem and leaf. Phenolic observations used a 5% potassium dichromate (K_2CrO4) reagent, with a positive reaction by the color change of cells or tissues to a dark brownish-yellow color (Badria and Aboelmaaty, 2019). Subsequently, terpenoids were detected using a 5% CuSO4 reagent with a yellow-to-brownish color, where the yellow color indicated a positive reaction (Wiryo et al., 2015). While observations of alkaloids were carried out using Dragendorf reagent, with a positive reaction indicated as a change to a reddish-brown color (Santhi and Sengottuvel, 2016).

Statistical analyses

Data on the anatomical parameters of roots, stems, and leaves were analyzed using one-way analysis of variance, followed by Duncan's new multiple range test for mean comparisons. Statistical significance was set at $p < 0.05$.

Results and Discussion

Anatomical structure of roots

Fig. 1 shows that soil type affected the arrangement of the cortical cells of Senggani roots. In the cortex of Mediterranean (Figs. 1A, 1B) and regosol soils (Figs. 1C, 1F) ± 4 layers of parenchymatous tissues were identified, while ± 7 cell layers of parenchyma cells were observed in latosol soil roots (Figs. 1B, 1E). However, the size of the cortex cells in the Mediterranean soil was larger and more densely arranged than in the latosol and regosol samples (Figs. 1A, 1D).

According to Schneider et al. (2020), the cortical cell size increased in soil with a low water concentration to reduce the bending of root cells. Chimungu et al. (2014) stated that the adaptation of root cells facilitated the absorption of water by plants in dry environments. Fig. 1B shows that the roots grown in the latosol had more regular xylem constituent cells. In latosol soil roots, the xylem vessels, and pith ray cells were laterally arranged compared to roots in the Mediterranean and regosol soil types.

Table 1 Physico-chemical properties of soil types used in the present study

No.	Parameter		Soil type		
			Mediterranean	Latosol	Regosol
1.	Moisture content (%)	0.5 mm	0.96	8.84	2.21
		2 mm	1.65	9.76	1.69
2.	Cation exchange capacity (%)		10.57*	47.42***	10.17*
3.	Soil pH		5.7	5.8	6.2
4.	Organic carbon (%)		0.96*	0.83*	0.88*
5.	Available N (ppm)		3.09*	13.85**	1.48*
6.	Available P (ppm)		6.63*	1.04*	6.82*
7.	Available K (%)		0.35**	0.63***	0.61***

ppm = parts per million; * = low content; ** = medium content; *** = high content.

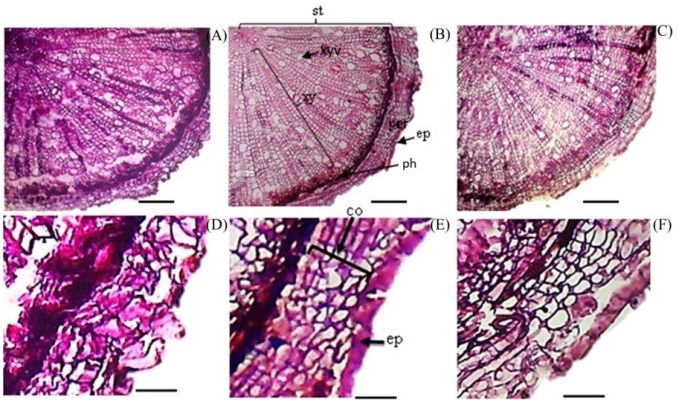

Fig. 1 Transverse section of *Melastoma malabathricum* roots (upper) and root cortex (lower) grown in three different soil types: Mediterranean (A, D); latosol (B, E); regosol (C, F), where ep = epidermis, co = cortex, ph = phloem, xy = xylem, xyv = xylem vessel, and per = periderm; scale bars in (A–C) = 100 µm and in (D–F) = 50 µm

Soil type did not significantly ($p \geq 0.05$) affect epidermal thickness, but affected cortex thickness and stele diameter (Table 2). According to Suharti et al. (2017), cortex thickening is related to water storage capacity in the roots. In the Mediterranean and regosol soils, the Senggani primary roots had lower cortex thickness compared to the latosol soil (Table 2). This could have been due to the low moisture content levels (Table 1), as was also reported in Mediterranean soil by Nurilmi et al. (2017). The thin cortex can act as a plant's tolerance mechanism to shorten the distance of water transport into the xylem, thereby enhancing efficient water vascularization (Roswanti et al., 2015).

The lowest root stele diameter was in the Mediterranean soil treatment, followed by xylem vessel diameter and number, as shown in Table 2. Generally, a low root xylem diameter is an adaptation mechanism to dry environments by preventing embolism to facilitate the absorption of water from the soil (Kisman et al., 2002). Embolism is the formation of air bubbles in the xylem that can interfere with the conductivity of water which is adapted for the reduction in xylem diameter (Akmalia, 2021). According to Prabowo and Rachmawati (2020), an increase in stele diameter is supported by the high number and diameter of xylem elements. As shown in Table 1, cation exchange capacity value of the latosol soil (47.42%) was higher than the others, indicating increased absorption of nutrients in this soil (Peniwiratri and Afany, 2022). A thinner stele with smaller xylem vessels on the roots in the Mediterranean soil was assumed to support water transport efficiency, considering that the presence of water in this soil was lower than in the ragosol and latosol soils. This was consistent with the lowest soil moisture content being in the Mediterranean soil (Table 1).

Anatomical structure of stems

Fig. 2 shows the transverse section of Senggani stems grown in the Mediterranean, latosol and regosol soils. Anatomically, the stem is composed of the periderm which

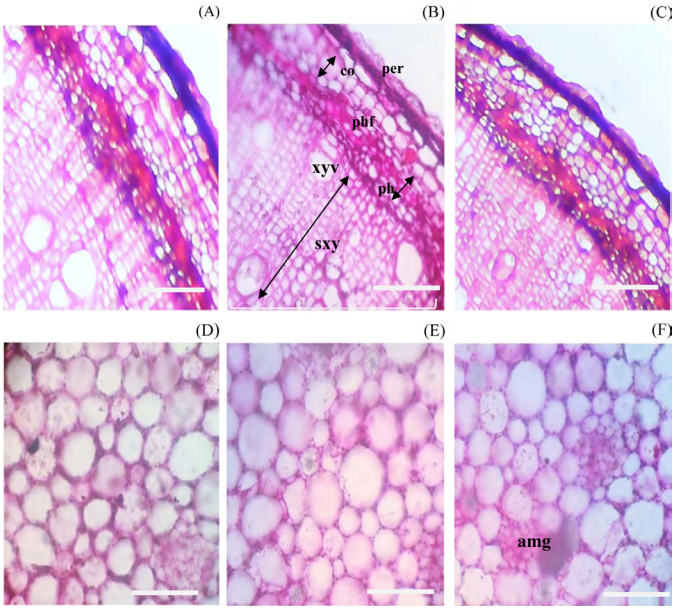

Fig. 2 Transverse section of *Melastoma malabathricum* stem grown in different soils: (A, D) Mediterranean; (B, E) latosol; (C, F) regosol, where top row photos show periderm and cortex and vascular tissues, while lower photos show pith tissue with amylum grains; per = periderm, co = cortex, phf = phloem fibers, ph = phloem, sxy = secondary xylem, xyv = xylem vessel, amg = amylum grains; scale bar = 100 µm

Table 2 Anatomical parameters of *Melastoma malabathricum* roots grown in different soil types

Parameter	Soil types		
	Mediterranean	Latosol	Regosol
Epidermal thickness (µm)	24.86±10.88[a]	14.86±2.91[a]	18.43±1.50[a]
Cortex thickness (µm)	54.56±7.72[a]	105.96±13.29[c]	84.67±1.88[b]
Stele diameter (µm)	1,151.50±52.89[a]	1,536.76±28.65[b]	1,730.26±28.38[c]
Xylem vessel diameter (µm)	27.86±5.38[a]	41.16±2.15[b]	33.46±2.66[a]
Number of xylem vessels/ transverse section	93.00±8.00[a]	120.00±9.29[b]	118.00±16.65[b]

Values (mean ± SD) in each row, superscripted with different lowercase letters are significantly different ($p < 0.05$).

provides replacement of epidermal tissue, the cortex with various shapes of parenchymal cells and the stele occupying the inner part. The stele is composed of vascular tissue and hexagonal-shaped pith parenchymal tissue and phloem fibers with thickened cell walls. Primary xylem tissue has thickened cell walls, including xylem vessels with a smaller size near the pith. Furthermore, the pith parenchyma is characterized by the presence of amylum grains, as shown in Fig. 2F. The periderm cell wall layer in the Mediterranean soil sample was thicker than the two other soil types, with the cortex being composed of 4–5 layers of parenchymal cells of smaller sizes closely arranged, followed by 2–3 layers of parenchymal cells of a larger size in the latosol, as shown in Figs. 2A–2C. The xylem tissues from plants grown in the three different soil types were irregularly arranged, with various sizes in radial lines. As presented in Figs. 2D–2F, the pith parenchyma in the regosol sample contained more amylum grains than in the Mediterranean and latosol soils.

Table 3 presents the quantitative results for the stem anatomical parameters of Senggani grown in the different soil types. Senggani stems grown in the Mediterranean soil had a higher periderm thickness than in the other two soil types. Based on the results of soil analysis, the moisture content of the Mediterranean soil was the lowest, was also reported by Nurilmi et al. (2017). This thickening periderm aimed to prevent water loss as an adaptation mechanism to the environment (El-Sherbeny et al., 2021). There were no significant ($p \geq 0.05$) differences in cortex thickness for all soil types; however, the lowest thickness and moisture content were in the Mediterranean soil root. Similarly, Mangena (2018) reported that the stem cortex in the soybean plant under water shortage conditions was thinner compared to the control. The parenchymatous cells of the cortex can easily shrink in dry conditions, leading to a decrease in the thickness of the cortex (Hidayati et al., 2017). However, treatment of the different soil types did not affect the diameters of stele and xylem vessels. Based on the current results, the Mediterranean soil had fewer xylem vessels, serving as a strategy for efficient water transport.

Anatomical structure of leaves

Anatomically, the leaf blade of Senggani has a uniseriate epidermis and a thick cuticle on both surfaces, as presented in Figs. 3A, 3C and 3E. In transverse section, the adaxial epidermal cells tend to be rectangular, as are the abaxial epidermal cells. Below the adaxial epidermis, there is a layer of hypodermal cells with large, polyhedral-isodiametric or rectangular-shaped cells. Furthermore, the mesophyll is composed of one layer of palisade cells, closely adjacent to the hypodermis and made up of about 5 layers of spongy cells.

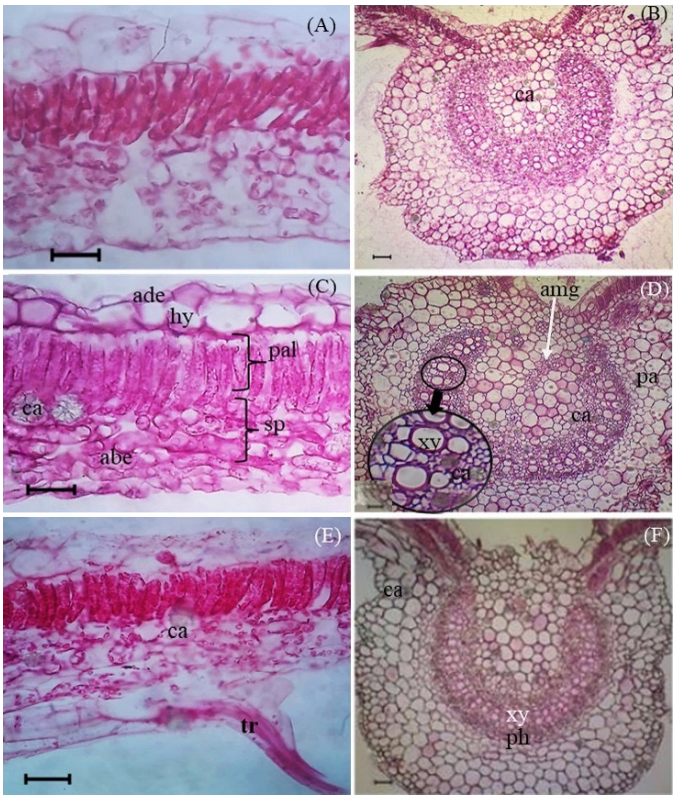

Fig. 3 Transverse section of lamina (left) and leaf midrib (right) from plants grown in various soil types: (A, B) Mediterranean; (C, D) latosol; (E, F) regosol; ade = adaxial epidermis, hy = hypodermis, ca = Ca-oxalate crystal, pal = palisade, sp = sponge, abe = abaxial epidermis, pa = parenchyma, ph = phloem, xy = xylem, tr = trichomes, amg = amylum grains; scale bar = 50 µm

Table 3 Stem anatomical parameters of *Melastoma malabathricum* grown in different soil types

Parameter	Soil type		
	Mediterranean	Latosol	Regosol
Periderm thickness (µm)	44.73±5.36^{b}	31.63±0.92^{a}	28.16±2.34^{a}
Cortex thickness (µm)	45.20±1.35^{a}	46.67±4.22^{a}	49.63±6.97^{a}
Stele diameter (µm)	2,426.56±544.27^{a}	2,773.83±75.00^{a}	2,774.06±79.71^{a}
Xylem vessel diameter (µm)	34.30±1.11^{a}	40.33±6.18^{a}	40.93±2.57^{a}
Number of xylem vessels/transverse section	304.00±12.16^{a}	397.00±52.30^{b}	416.00±37.87^{b}

Values (mean ± SD) in each row superscripted with different lowercase letters are significantly different ($p < 0.05$).

The transverse section of the midrib is approximately circular in shape and is composed of a layer of epidermal cells, 2–3 layers of cholenchymal cells and several parenchymatous cells. Other morphological characteristics include vascular tissues arranged in a circular shape, consisting of phloem and xylem rays and filled with parenchymal cells in the center of the costae, as shown in Figs. 3B, 3D and 3F. The druse form of Ca-oxalate crystals ($Ca\text{-}O_x$) was evident in leaf blades, predominantly observed in midribs, as presented in Fig. 3. Additionally, druses were found in the sponge tissue of the leaf blade and in epidermal and parenchymal cells in the peripheral area, as well as in the phloem, xylem and central parenchymal cells of the leaf midrib.

The current study showed that in Mediterranean soil leaf had thinner hypodermis layer compared to others. Palisade tissue arrangement in Mediterranean and regosol leaf are more tightly packed (Fig. 3A, Table 4). The spongy tissue on plant leaf grown in the latosol soil was also observed to be denser (Fig. 3C), while regosol produced continuous spongy cells with wider intercellular spaces (Fig. 3E). Excessive calcium (Ca) absorbed by Mediterranean soil is stored as Ca-oxalate due to the presence of carbonate content. As shown in Fig. 3B, 3D, and 3F, Ca-oxalate crystal usually appear scattered in parenchymatic tissue, specifically in leaves and stems, close phloem from veins (Paiva and Machado, 2005).

Palisade and sponge tissues from leaves grown in the Mediterranean soil were significantly ($p < 0.05$) thicker compared to those grown in the regosol soil (Table 4). This was influenced by an increase in the photosynthetic rate of the plant because palisades and sponges contain chloroplasts where photosynthetic reactions occur and generally, plant species with a thick mesophyll have better photosynthetic capacity (Liu et al., 2018), because the thicker palisade mesophyll cells contribute to maintaining a higher internal temperature and water status within the leaf, thereby facilitating adaptation (Liu et al., 2020). Thickening cells in the leaf facilitate water flow and reinforce the organ (Lamalakshmi et al., 2017).

The environment has a significant effect on xylem vessel diameter and number, which contributes to vascularization in the plant (Qaderi et al., 2019). The current results indicated that the xylem vessels in the leaf of Senggani plants grown in the Mediterranean soil had the smallest diameter (Table 4). The Mediterranean soil had a low moisture content, which facilitated the development of a smaller xylem vessel diameter by the plant to avoid cavitation. According to Irawan and Putra (2020), cavitation in the vessel network can reduce water vascularization and hydraulic conductance, resulting in plant death.

The stomatal index percentages for the Senggani leaf grown in the Mediterranean, latosol, and regosol soils were not significantly ($p \geq 0.05$) different, as shown in Table 4. Based on the current results, the trichome density in the adaxial and abaxial paradermal leaves of Senggani was higher in the Mediterranean and regosol soils due to their low water content compared to the latosol soil type, as well as the reduced availability of elemental C, N and P. Cahyono et al. (2022) suggested that a high trichome density reduced evaporation, causing a lack of water in the plant. Non-glandular trichomes were reported to play a significant role in regulating temperature, reducing water loss and absorbing water and nutrients (Wagner et al., 2004).

Table 4 Leaf anatomical parameters of *Melastoma malabathricum* grown in three different soil types

Parameter		Soil type		
		Mediterranean	Latosol	Regosol
Adaxial epidermal thickness (µm)		10.65±0.24^a	14.16±1.6^b	14.13±0.32^b
Hypodermis thickness (µm)		18.05±0.76^a	27.47±1.05^c	24.46±0.47^b
Abaxial epidermal thickness (µm)		8.25±0.18^a	11.20±1.90^b	11.54±1.62^b
Palisade thickness (µm)		64.95±2.72^b	63.05±1.72^b	51.72±3.56^a
Sponge thickness (µm)		75.51±6.18^b	83.3±1.41^b	59.90±4.39^a
Xylem vessel diameter (µm)		23.24±1.90^a	32.80±2.55^b	25.33±2.30^a
Stomatal index (%)	Adaxial	44.69±3.71^a	45.62±1.73^a	42.39±0.55^a
	Abaxial	36.13±2.57^a	41.99±2.57^b	41.45±0.48^b
Trichome density/mm^2	Adaxial	4.13±0.30^b	2.60±0.52^b	4.13±1.10^b
	Abaxial	10.20±0.64^b	7.26±0.52^a	9.73±0.17^b

Values (mean ± SD) in each row superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Distribution of secondary metabolites

The distribution of phenolic compounds in root tissue includes the epidermis, idioblast cells in the cortex, phloem and xylem parenchyma. Positive reactions to terpenoids were found in the epidermis, cortex parenchyma and vascular tissues, while alkaloid compounds were distributed in the epidermis, phloem and xylem parenchyma, as shown in Fig. 4 and Table 5. According to Pratiwi et al. (2020), the main cinchona alkaloid storage is in the periderm, distributed in the secondary phloem.

According to Kumar et al. (2021), Senggani roots are used for rheumatic therapy by local people in Dibrugarh, India. In Indonesia and China, Senggani fresh roots are used as traditional medicine to treat excessive vaginal discharge and irregular menstruation (Liu et al., 2018). Based on previous studies, phenolics, such as flavonoids, and melastomic acid, have been identified from Senggani (Giri and Rajbhandari, 2018). Phenolic localization was marked in the root epidermis. However, this root area is fragile, leading to the recommendation of maintaining the root epidermal area intact. Through this process, as the root gets older, there is a replacement of the function of the epidermis by secondary tissue, namely the periderm.

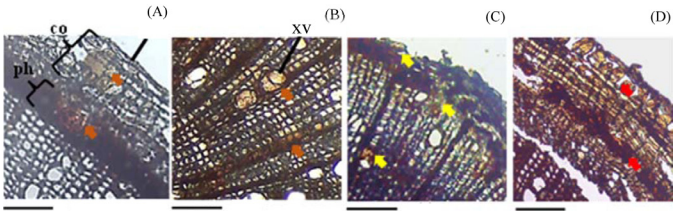

Fig. 4 Distribution of secondary metabolites in *Melastoma malabathricum* root tissues: (A) phenolics (brown arrow) in epidermis, cortex and phloem; (B) xylem vessel; (C) Senggani roots grown in Mediterranean soil, where terpenoid (yellow arrow) is distributed in cortex parenchyma, phloem, and xylem vessels of root grown in latosol soil; (D) alkaloid (red arrow) on epidermis and phloem area of Senggani root grown in regosol; co = cortex, ph = phloem, xv = xylem vessel; scale bar = 100 µm

Table 5 Distribution of secondary metabolites in *Melastoma malabathricum* grown in different soil types

Type of secondary metabolite	Organ	Tissue	Soil type		
			Mediterranean	Latosol	Regosol
Phenolic	Root	Epidermis	++	+	+++
		Cortex	++	+	+++
		Xylem vessel	+++	+	+++
	Stem	Epidermis	++	+	+++
		Cortex	++	+	++
		Vascular tissue	+	+	+
	Leaf	Epidermis	++	+	++
		Mesophyll	++	++	++
		Midrib parenchyma	+++	++	++
Terpenoid	Root	Epidermis	++	++	++
		Cortex	+	++	++
		Vascular tissue	++	++	++
	Stem	Epidermis	++	+	++
		Cortex	++	++	+++
		Vascular tissue	+++	+++	+++
	Leaf	Epidermis	++	++	+
		Mesophyll	+	+	+
		Vascular tissue	+++	++	++
Alkaloid	Root	Epidermis	++	+	++
		Cortex	+	+	+
		Vascular tissue	+++	+	+++
	Stem	Epidermis	+++	++	+++
		Cortex	++	+	++
		Vascular tissue	++	+	++
	Leaf	Epidermis	+++	+++	+++
		Mesophyll	-	-	-
		Vascular tissue	++	++	++

+ = less distributed, ++ = medium distributed, +++ = more distributed.

In the stem, phenolic compounds were detected in the periderm, cortex parenchyma, vascular bundles and stele parenchyma, while terpenoids were observed in the cortical and pith parenchyma (Fig. 5, Table 5). Based on the results, alkaloid detection was positive in the periderm and parenchyma of vascular bundles. Furthermore, the regosol soil had a greater distribution of idioblast cells with a deeper color compared to the other two soils, due to its lowest N content. Secondary metabolite compounds, particularly alkaloids that protect plant from insects and herbivores, neutralize toxins and store N as an essential element (Kuntorini et al., 2022). Joffry et al. (2012) showed that the stem could be used to treat puerperal infections, high blood pressure and diabetes, while the shoot juice was effective as a mouthwash to relieve toothache or treat leukorrhea. Phenolic compounds in the leaf were identified in the epidermis, trichomes and mesophyll in the lamina, midribs parenchyma cells and vascular bundle area (Fig. 6, Table 5). The terpenoid compounds were located in the spongy mesophyll, midribs parenchyma and vascular tissue. Alkaloid compounds showed a positive reaction in the periderm, transport bundle and stele.

The Mediterranean and regosol soils had a higher accumulation of secondary metabolites compared to the latosol soil sample. Specifically, the Mediterranean soil had relatively low levels of nutrients (Arifin, 2012). Tannins are beneficial for plant growth in less fertile ecosystems, while flavonoids can protect from free radicals and inhibit lipid oxidation (Arnoldi et al., 2020), while Haberstroh et al. (2018) reported that the plant of *Cistus ladanifer* in a Mediterranean soil with dry and hot conditions could adapt biochemically with increasing terpenoid levels. Based on the current results, alkaloid distribution in Mediterranean and regosol soils is higher compared to latosol. A low water moisture content in the Mediterranean and regosol soils may trigger the synthesis of alkaloid as a self-defense mechanism. This finding was reported for *Stellaria dichotoma* L. var. *lanceolata* Bge where water stress increased secondary metabolites, especially the total flavonoid content (Zhang et al., 2017).

Sari et al. (2018) found flavonoids and tannins in Senggani leaves with potential as antioxidants, playing a role in repairing damage to body cells. In addition, Kartina et al. (2019) quantified the leaf extract of Senggani and produced contents phenol (36.32%), terpenoids (9.13%) and alkaloids (4.8%). Senggani leaves store phenolics in the epidermis and mesophyll, supporting their use in treating external wounds. Therefore, mature leaves that accumulate higher levels of secondary metabolites, such as phenolics, can be used for such treatment.

The Mediterranean soil produced the highest stem periderm thickness, as well as greatest number of leaf trichomes, with the lowest number of stem tracheids and the lowest leaf epidermal thickness. The latosol soil produced the highest root cortex thickness, root xylem vessel diameter and sponge thickness. The regosol produced the largest root stele diameter and stomatal size, the greatest number of stem xylem vessels and the greatest leaf trichome density. Histochemically, the Mediterranean and regosol soils had higher cellular contents of phenolics, terpenoids and alkaloids compared to the latosol soil, indicating that both the Mediterranean and regosol soil types were effective in producing anatomical adaptation of the plant.

Conflict of Interest

The authors declare that there are no conflicts of interest.

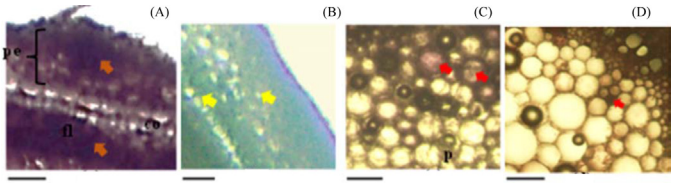

Fig. 5 Distribution of secondary metabolites in *Melastoma malabathricum* stem tissues: phenolics (brown arrow) in periderm and phloem of Senggani stem grown in regosol; (B) terpenoid (yellow arrow) distributed in cortex parenchyma, phloem area of Senggani stem grown in latosol soil; (C) alkaloid (red arrow) shown on stele parenchyma in regosol; (D) alkaloid (red arrow) shown on stele parenchyma in Mediterranean soil; co = cortex, fl = phloem, p = parenchyma, pe = periderm; scale bar = 50 µm

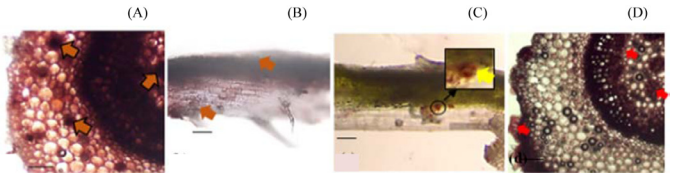

Fig. 6 Distribution of secondary metabolites in *Melastoma malabathricum* leaf tissues: (A) phenolic (brown arrow) in parenchyma of midribs from leaf grown in Mediterranean soil; (B) epidermis from leaf grown in Mediterranean soil; (C) terpenoid (yellow arrow) distributed in mesophyll area of leaf grown in latosol soil; (D) alkaloid (red arrow) on stele parenchyma area of Senggani grown in regosol; scale bar = 100 µm

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