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Environmental Factors and Soil Conditions Affect Brazilin Content in *Caesalpinia sappan*

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ABSTRACT

Sappanwood (*Caesalpinia sappan* L.) is a valuable plant used in natural medicine, beverages, and dyes due to its bioactive compounds, particularly brazilin. This study examined the environmental and metabolite characteristics of *C. sappan* collected from four regions in Indonesia: Sumedang (SMD), Majalengka (MJK), Garut (GRT), and Purwodadi (PWD). The results indicated that environmental factors, such as light intensity and altitude, varied among locations and influenced metabolite synthesis, along with differences in soil chemical properties. GRT exhibited the highest nitrogen (0.22%) and organic carbon content (2.18%), while MJK and PWD showed lower levels. Secondary metabolite analysis revealed that GRT had the highest phenolic content (33.12 mg g^{-1} GAE), flavonoid content (13.76 mg g^{-1} QE), and antioxidant activity (80.32%). Brazilin content was also highest in GRT (0.0186 mg g^{-1}) and showed strong positive correlations with total phenolics, flavonoids, and antioxidant activity, underscoring its role in oxidative stress defense. Overall, this study highlights the environmental and biochemical determinants of brazilin biosynthesis in *C. sappan*, offering insights for optimizing its production through targeted agronomic management and breeding strategies. The findings are valuable for advancing value-added applications of sappanwood in natural medicine and the antioxidant industry.

Abbreviations: Brazilin Content (BC), Gallic Acid Equivalent (GAE), Quercetin Equivalent (QE), Soil Plant Analysis Development (chlorophyll meter reading) (SPAD), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Cation Exchange Capacity (CEC), Base Saturation (BS), Aluminum (Al), Hydrogen (H), Nitrogen (N), Phosphorus (P), Potassium (K), Sodium (Na), Calcium (Ca), Magnesium (Mg), Meters Above Sea Level (MASL), Principal Component (PC), Principal Component Analysis (PCA), Carbon-Nutrient Balance Hypothesis (CNBH), Sumedang (SMD), Majalengka (MJK), Garut (GRT), Purwodadi (PWD), Chlorophyll Leaves (CL), Compound Leaves Number (CLN), Nitrogen Total (NT), Carbon Organic (CO), Light Intensity In Heading (LIIH), Light Intensity Outside Heading (LIOH), Percentage Inhibition (%I)

Introduction

The sappan tree (*Caesalpinia sappan* L.), a member of the Fabaceae family, is a versatile species of notable economic and medicinal importance. Native to Southeast Asia, it has long been valued for its diverse applications in traditional medicine, natural dye production, and as a functional ingredient in beverages. The dried stems of sappanwood have historically been used in traditional remedies and exhibit a wide range of pharmacological properties, including antioxidant, anti-inflammatory, hypoglycemic, hepatoprotective, and vasorelaxant effects (Nirmal et al., 2015).

These therapeutic effects are largely attributed to its rich bioactive composition, particularly flavonoid compounds such as brazilin, 3'-O-methylbrazilin, sappanin, chalcone, and sappancalchone. These compounds act as primary or secondary antioxidants and play vital roles in biological processes (Bukke et al., 2015; Septiyani et al., 2024). Among them, brazilin stands out as the principal active metabolite of *C. sappan*. This homoisoflavonoid not only imparts the plant's distinctive red pigment but also exhibits potent antioxidant, antibacterial, and anticarcinogenic activities, among other health-promoting effects (Djaeni et al., 2021; Wongsooksin et al., 2007). The catechol groups within its chemical structure enhance its antioxidant potential, making brazilin highly valuable for medicinal, cosmetic, and industrial applications (Djaeni et al., 2021).

In addition to its pharmacological uses, *C. sappan* wood produces a vivid red dye when boiled, traditionally used to color textiles, foods, and inks (Taveepanich et al., 2024). Beyond brazilin, the plant contains a range of other secondary metabolites—tannins, alkaloids, phenolics, gallic acid, saponins, and essential oils—that contribute to its antifungal, antiviral, antibacterial, and anti-inflammatory properties (Cahyaningtyas et al., 2019).

The production of these secondary metabolites, including brazilin, is strongly influenced by environmental factors such as geography, soil composition, pH, and climate. These parameters affect the plant's metabolic processes and can either stimulate or inhibit the biosynthesis of key compounds (Jain et al., 2021; Pant et al., 2021). Environmental stresses, including extreme temperature fluctuations or poor soil fertility, often trigger secondary metabolite accumulation as part of the plant's defense mechanism against oxidative stress and reactive oxygen species (Muthusamy and Lee, 2024).

Understanding the relationship between environmental variables and brazilin content is therefore crucial for optimizing *C. sappan* cultivation and enhancing its pharmacological and industrial potential. Despite extensive studies on its bioactivity, relatively few investigations have examined the influence of environmental factors on the biosynthesis of key metabolites in *C. sappan*. Research comparing the effects of different regional conditions on brazilin accumulation remains particularly limited.

This study aims to assess the morphological traits, environmental parameters, and metabolite profiles of *C. sappan* cultivated in four regions of Indonesia. By exploring the correlations between environmental factors and brazilin content, the research seeks to provide insights into optimal cultivation strategies and contribute to the sustainable and efficient utilization of *C. sappan* in medicinal and industrial applications.

Materials and Methods

Plant and soil materials

Heartwood samples from four to five-year-old *Caesalpinia sappan* (sappanwood) trees and corresponding soil samples from their growing sites were collected from four regions in Indonesia: Sumedang (SMD), Majalengka (MJK), Garut (GRT), and Purwodadi (PWD) (Fig. 1). Each location provided five replicate samples. All collected materials were transported and stored at the Laboratory of Plant Analysis, Faculty of Agriculture, Universitas Padjadjaran.

The heartwood samples were sun-dried to complete dryness and subsequently ground into a fine powder for analysis. Soil samples from each planting site were air-dried and then analyzed for their physical, chemical, and biological properties to characterize the environmental conditions influencing plant growth.

Fig. 1. Sappanwood habitat and sample from four locations in Indonesia (Sumedang, Majalengka, Garut, and Purwodadi).

Leaf profile and environment analysis

Leaf profile assessment included measuring leaf nitrogen content, determining chlorophyll levels with a SPAD device, and counting mature compound leaves. Nitrogen content analysis followed the method described by Corte et al. (2009). Environmental conditions were evaluated by measuring light intensity both within and outside the plant canopy using a lux meter, recording air temperature and humidity, and documenting the elevation of the planting area.

Soil analysis

Soil samples were collected from a depth of at least 20 cm at eight points surrounding the root zone of each *Caesalpinia sappan* plant. Samples were air-dried, passed through a 2 mm sieve, and analyzed for multiple physicochemical parameters. Total nitrogen (N) content was determined using the alkali-hydrolyzable diffusion method (Mulvaney and Khan, 2001). Available phosphorus (P) was measured via the molybdenum–antimony colorimetric method, while rapidly available potassium (K) was determined using the ammonium acetate leaching method followed by flame photometry. Soil organic carbon was analyzed using the hydration heat dichromate oxidation–colorimetric method (Combs and Nathan, 1998).

Exchangeable cations in this context were potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), aluminum (Al), and hydrogen (H). These cations were extracted using the Mehlich-3 reagent (Mehlich, 1984) and quantified by inductively coupled plasma atomic emission spectrometry (ICP-AES). Cation exchange capacity (CEC) and base saturation were subsequently calculated from the exchangeable cation data. Soil pH was measured with a glass electrode in a 1:1 soil-to-water slurry. These analyses provided comprehensive information on nutrient availability, organic matter content, and the overall chemical properties of the soils.

Total flavonoid analysis

Total flavonoid content was determined using the aluminum chloride ($AlCl_3$) colorimetric method described by Chang et al. (2002), with slight modifications. In brief, 0.5 g of dried sappanwood powder was extracted with 10 mL of 96% ethanol, homogenized, and centrifuged at 6000 rpm for 5 min. A 0.1 mL aliquot of the supernatant was mixed with 0.2 mL of 10% $AlCl_3$ and 0.2 mL of 1 M potassium acetate solution, then incubated at room temperature for 30 min. Absorbance was measured at 415 nm using an Orion AquaMate 8000 UV–Vis spectrophotometer (Thermo Scientific, USA).

Total phenolic analysis

Total phenolic content was quantified using a modified Folin–Ciocalteu method based on Yan et al. (2006). Approximately 0.5 g of dried sappanwood powder was extracted with 10 mL of 96% ethanol, homogenized by vortexing, and centrifuged at 6000 rpm for 5 min. A 0.05 mL aliquot of the supernatant was transferred into a 25 mL volumetric flask, followed by the addition of 0.5 mL of diluted Folin–Ciocalteu reagent (1:1) and 2.5 mL of 20% Na_2CO_3 solution. The mixture was gently stirred, diluted to volume with distilled water, and incubated in the dark for 40 min. Absorbance was then measured at 725 nm using an Orion AquaMate 8000 UV–Vis spectrophotometer (Thermo Scientific, USA).

Percentage of inhibition by antioxidant activity

The antioxidant activity, expressed as the percentage of inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, was determined using a modified method described by Rosales et al. (2006). Briefly, 0.5 g of dried sappanwood extract powder was dissolved in 10 mL of 96% ethanol. A 0.01 mL aliquot of this ethanolic extract was mixed with 1.99 mL of methanol and 2 mL of a 160 ppm DPPH methanolic solution. The blank solution was prepared by mixing 0.5 mL of methanol with 2 mL of 160 ppm DPPH methanolic solution. All samples were incubated in the dark at room temperature for 30 min before measurement. Absorbance was recorded at 516 nm using an Orion AquaMate 8000 UV–Vis spectrophotometer (Thermo Scientific, USA). The percentage of inhibition was calculated to assess the radical scavenging activity of the extracts.

Brazilin analysis

Brazilin content was determined following the method described by Warinhomhaun et al. (2018), with modifications. One milligram of sappanwood extract from each of the four locations was dissolved in 20 mL of methanol and then diluted with 2.5% acetic acid/methanol (90:10, v/v) to obtain a final concentration of 0.2 mg mL^{-1}. The resulting solution was filtered through a 0.45 μm membrane filter before high-performance liquid chromatography (HPLC) analysis. Chromatographic separation was performed on an Eclipse Plus® C18 column (250 × 4.6 mm, 5 μm) maintained at 40 °C, with a flow rate of 1.0 mL min^{-1}.

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA), and treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% significance level. Pearson correlation analysis was performed to evaluate the relationships among parameters, and the results were visualized using a heatmap to highlight significant correlations. Statistical analyses were carried out using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA).

Results

Chemical soil properties of growth media

The chemical properties of the soils varied significantly among the sampling locations, as indicated by the 5% Duncan test (Table 1). The highest total nitrogen (N) content was recorded in Garut (GRT) at 0.22%, while the lowest was observed in Purwodadi (PWD) at 0.14%. Phosphorus content (P_2O_5 HCl 25%) was greatest in Majalengka (MJK) and Sumedang (SMD), with values of 79.65 and 78.34 mg 100 g^{-1}, respectively, whereas PWD showed the lowest value (14.69 mg 100 g^{-1}). For potassium content (K_2O HCl 25%), SMD exhibited the highest level (34.12 mg 100 g^{-1}), while GRT showed the lowest (9.71 mg 100 g^{-1}). The organic carbon content was also variable among sites, with GRT showing the highest value (2.18%), while PWD and MJK recorded the lowest, ranging from 1.73 to 1.74%. The C/N ratio reached its maximum in PWD (13.33) and its minimum in GRT (9.67). Regarding exchangeable cations, calcium (Ca) concentration was highest in SMD (33.72 cmol kg^{-1}) and lowest in PWD (8.97 cmol kg^{-1}). Magnesium (Mg) followed a similar trend, with the highest value in SMD (6.34 cmol kg^{-1}) and the lowest in PWD (2.64 cmol kg^{-1}). Aluminum (Al) content, in contrast, was highest in GRT (2.81 cmol kg^{-1}) and lowest in MJK (0.25 cmol kg^{-1}). The cation exchange capacity (CEC) exhibited substantial variation across locations, with MJK showing the highest value (134.48 cmol kg^{-1}) and PWD the lowest (26.71 cmol kg^{-1}). Correspondingly, base saturation was also highest in MJK (134.48%) and lowest in GRT (40.01%). These findings indicate that soil chemical properties differ markedly across regions, reflecting distinct fertility levels and nutrient dynamics. Such variability likely influences the physiological performance and secondary metabolite production of *Caesalpinia sappan* under different environmental and management conditions.

Table 1. Comparative analysis of soil chemical properties across four Indonesian regions cultivating *Caesalpinia sappan* L.

Properties	Originated			
	SMD	MJK	GRT	PWD
N Total (%)	0.18^{ab}	0.15^{a}	0.22^{b}	0.14^{a}
P_2O_5 HCl 25% (mg $100g^{-1}$)	78.34^{c}	79.65^{c}	43.20^{b}	14.69^{a}
K_2O HCl 25% (mg $100g^{-1}$)	34.12^{c}	25.22^{bc}	9.71^{a}	16.99^{b}
C Organic (%)	2.06^{ab}	1.74^{a}	2.18^{c}	1.73^{a}
C/N Ratio	11.67^{ab}	12.00^{ab}	9.67^{a}	13.33^{b}
K (cmol kg^{-1})	0.48^{b}	0.41^{b}	0.28^{a}	0.39^{b}
Na (cmol kg^{-1})	0.19^{a}	0.15^{a}	0.21^{b}	0.20^{ab}
Ca (cmol kg^{-1})	33.72^{b}	26.13^{b}	8.97^{a}	24.76^{b}
Mg (cmol kg^{-1})	6.34^{b}	4.79^{ab}	5.10^{ab}	2.64^{a}
Al (cmol kg^{-1})	0.27^{a}	0.25^{a}	0.42^{b}	0.29^{a}
H (cmol kg^{-1})	0.04^{ab}	0.13^{bc}	0.22^{c}	0.03^{a}
Al Saturation	0.73^{a}	0.93^{a}	2.81^{b}	1.15^{a}
Cation Exchange Capacity (cmol kg^{-1})	48.23^{b}	22.44^{a}	39.03^{b}	26.71^{a}
Base Saturation (%)	93.00^{b}	134.48^{c}	40.01^{a}	109.52^{bc}

Note: Mean values ± SE (standard error) followed by different lowercase letters in the same columns are significantly different based on Duncan's Multiple Range Test ($P < 0.05$).

Leaf profile characteristics

All leaf profile parameters showed significant variation according to the 5% Duncan test (Table 2). The highest leaf nitrogen (N) content was recorded in Majalengka (MJK) at 20.46 mg g^{-1}, whereas the lowest value was found in Purwodadi (PWD) at 16.09 mg g^{-1}. Chlorophyll content (SPAD value) followed a similar pattern, with MJK showing the highest reading (56.93) and PWD the lowest (48.97). Regarding leaf morphology, the number of compound leaf pairs was highest in PWD (11.11 pairs), which was significantly greater than in Garut (GRT) with 10.00 pairs. Sumedang (SMD) and MJK displayed intermediate values of 10.67 and 11.00 pairs, respectively. These results indicate notable differences in leaf characteristics among the studied populations, suggesting that variations in leaf nitrogen and chlorophyll content may contribute to differences in photosynthetic efficiency and overall plant growth performance.

Characteristics of the environmental profile for plant growth

Environmental parameters also varied significantly among the four locations, as shown by the 5% Duncan test (Table 3). Light intensity within the canopy ("heading") was highest in PWD (6039.33 lux), significantly exceeding that of SMD (1135.67 lux). Intermediate light intensities were recorded in GRT (4087.67 lux) and MJK (4364.33 lux), with no significant difference between them, though both were higher than SMD. For light intensity outside the canopy, PWD again had the highest value (363,766.67 lux), which was significantly greater than the other regions. MJK (32,790.33 lux), GRT (30,033.33 lux), and SMD (38,836.67 lux) had lower values that were statistically similar. Temperature also differed across locations: MJK recorded the highest temperature (33.67 °C), significantly higher than GRT (29.00 °C) and SMD (31.33 °C). GRT and SMD did not differ significantly, while PWD (32.00 °C) had an intermediate temperature, comparable to SMD.

Table 2. Leaf profile characteristics of various Sappanwood varieties.

Origin	**Leaves profile**		
	Leaf N content (mg g^{-1})	**Leaf chlorophyll (SPAD)**	**Compound leaf count (pair)**
SMD	19.22^{b}	52.21^{ab}	10.67^{ab}
MJK	20.46^{c}	56.93^{c}	11.00^{ab}
GRT	19.94^{bc}	54.59^{bc}	10.00^{a}
PWD	16.09^{a}	48.97^{a}	11.11^{b}

Note: Mean values ± SE (standard error) followed by different lowercase letters in the same columns are significantly different based on Duncan's Multiple Range Test ($P < 0.05$).

Table 3. Characteristics of the environmental profile for plant growth in sappanwood varieties.

Origin	**Environment profile**				
	Light Intensity in Heading (lux)	**Light Intensity outside the Heading (lux)**	**Temperature (°C)**	**Humidity (%)**	**Altitude (MASL)**
SMD	1,135.67^{a}	38,836.67^{a}	31.33^{b}	64.00^{c}	462.75^{c}
MJK	4,364.33^{ab}	32,790.33^{a}	33.67^{c}	43.00^{a}	130.79^{b}
GRT	4,087.67^{ab}	30,033.33^{a}	29.00^{a}	69.00^{c}	50.00^{a}
PWD	6,039.33^{b}	363,766.67^{b}	32.00^{b}	46.67^{b}	45.00^{a}

Note: Mean values ± SE (standard error) followed by different lowercase letters in the same columns are significantly different based on Duncan's Multiple Range Test ($P < 0.05$).

Relative humidity (%) varied markedly, with GRT showing the highest value (69.00%), significantly greater than MJK (43.00%) and PWD (46.67%). SMD (64.00%) presented an intermediate humidity level that did not differ significantly from PWD. Overall, these results highlight distinct environmental conditions among the four regions, particularly in light intensity, temperature, and humidity, i.e., factors that likely play crucial roles in influencing *Caesalpinia sappan* growth, photosynthetic activity, and metabolite accumulation.

Regarding altitude (MASL), the highest value was recorded in SMD (462.75 m), which was significantly higher than in PWD (45.00 m), the lowest-altitude location. GRT (50.00 m) and MJK (130.79 m) had intermediate altitudes, showing no significant difference between them. These findings indicate substantial variation in environmental factors among the studied habitats. PWD exhibited the highest light intensity both within and outside the canopy but was characterized by the lowest altitude and moderate temperature. MJK and GRT had moderate values for both light intensity and temperature but differed significantly in humidity, with GRT showing the highest humidity. Overall, the variations in altitude, temperature, and humidity suggest that these environmental conditions may distinctly influence the growth and physiological performance of the studied plants.

Characteristics of secondary metabolite profiles

Brazilin content was analyzed using the HPLC. Figure 2 shows the overlapping chromatograms of samples collected from the four locations and the brazilin standard. The chromatograms showed peaks between the 2nd and 15th min, with several prominent peaks clearly visible. The major peak in each sample was detected around 7.1–7.3 min, which closely matched the retention time of the brazilin standard (7.152 min). This confirms that the dominant peak corresponds to brazilin, the primary metabolite in the samples.

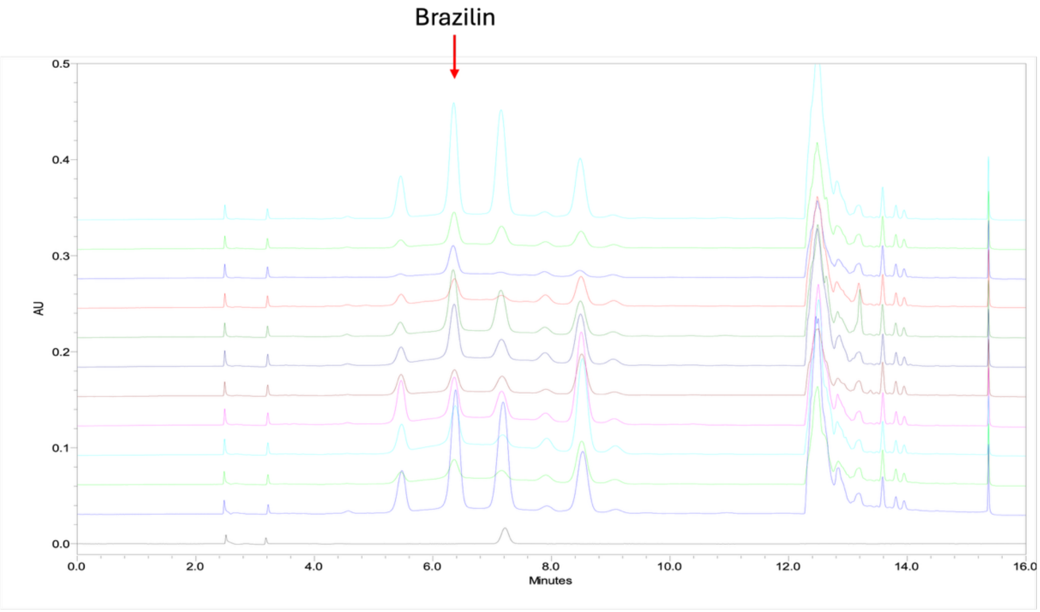

Fig. 2. Overlapping chromatograms of samples and brazilin standard.

Based on Duncan's multiple range test at the 5% significance level, phenolic content varied significantly among the locations (Fig. 3). GRT exhibited the highest phenolic content (33.1219 mg g^{-1} GAE), significantly higher than all other sites. PWD had the second-highest value (30.0699 mg g^{-1} GAE), which was significantly higher than SMD (24.8594 mg g^{-1} GAE) and MJK (15.4587 mg g^{-1} GAE). SMD was also significantly higher than MJK, which showed the lowest phenolic concentration. Similarly, for flavonoid content, GRT again showed the highest value (13.7626 mg g^{-1} QE), which was significantly higher than at all other locations. PWD followed with 8.4551 mg g^{-1} QE, which was significantly higher than both SMD (4.9555 mg g^{-1} QE) and MJK (5.0182 mg g^{-1} QE). No significant difference was found between SMD and MJK.

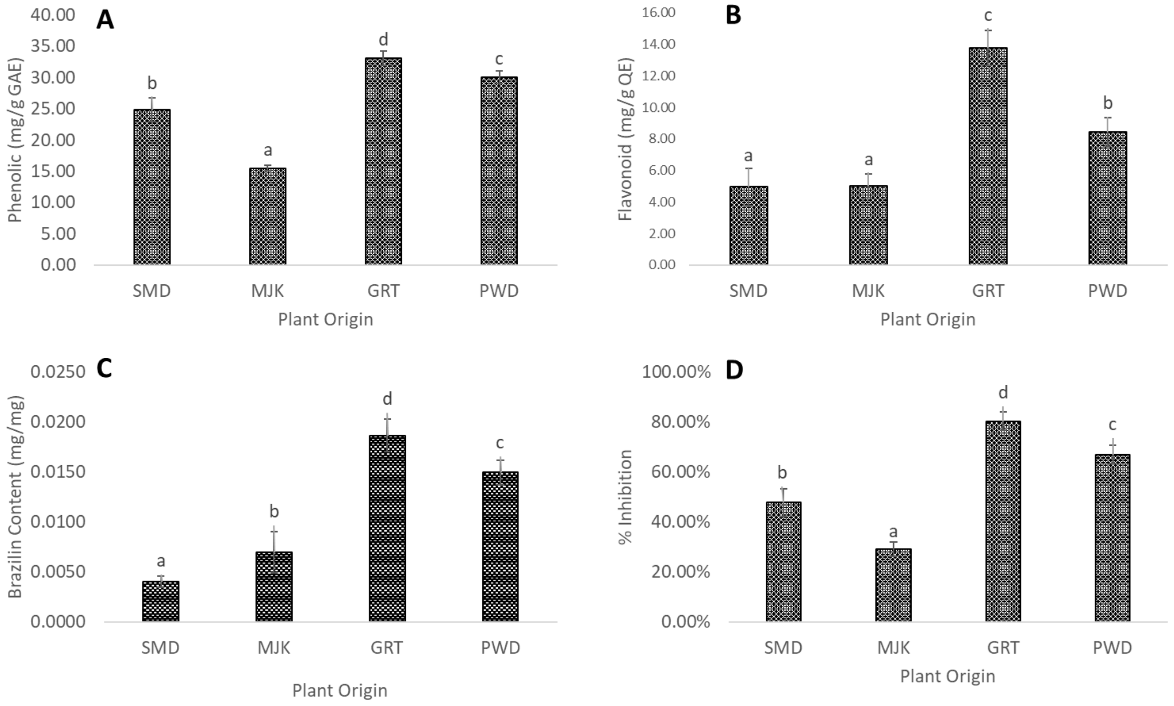

Fig. 3. Characteristics of secondary metabolite profiles of the sappanwood varieties. (**A**) phenolic content, (**B**) total flavonoid, (**C**) brazilin content, (**D**) percentage of inhibition of antioxidant. Mean values ± SE (standard error) followed by different lowercase letters in the same row are significantly different based on Duncan's Multiple Range Test ($P < 0.05$).

In terms of antioxidant activity, as indicated by the percentage of inhibition, GRT showed the highest value (80.32%), which was significantly higher than those of all other varieties. PWD showed the second-highest inhibition percentage (66.98%), significantly higher than SMD (47.71%) and MJK (28.99%). SMD was also significantly higher than MJK, which demonstrated the lowest inhibition percentage. Regarding brazilin content, GRT again recorded the highest value (0.0186 mg g^{-1}), significantly different from all other varieties. PWD recorded 0.0149 mg g^{-1}, which was significantly higher than MJK (0.007 mg g^{-1}) and SMD (0.004 mg g^{-1}). SMD exhibited the lowest brazilin concentration, differing significantly from all other varieties.

These findings indicate that GRT consistently displayed higher levels of phenolic and flavonoid content, antioxidant activity, and brazilin concentration, suggesting that it is the most promising variety for maximizing secondary metabolite production and antioxidant potential. The significant variations observed among the varieties highlight the influence of both genetic and environmental factors on the accumulation of bioactive compounds and their associated functional properties.

Correlation of environmental conditions, soil characteristics, leaf profile content, and secondary metabolites with brazilin content in sappanwood varieties

The analysis revealed that brazilin content is significantly influenced by a combination of soil properties, environmental conditions, and other secondary metabolites, establishing it as a key indicator of plant physiological status and biochemical synthesis (Fig. 4). Brazilin content showed a strong positive correlation with phenolic and flavonoid contents, indicating that higher concentrations of these metabolites are closely associated with increased brazilin production. This relationship suggests a shared biosynthetic pathway or a mutual enhancement within the plant’s secondary metabolite network. In addition, brazilin content was strongly correlated with soil C-organic and total nitrogen (N total) contents, emphasizing the importance of soil organic matter and nitrogen availability for brazilin biosynthesis. These elements likely act as crucial precursors or regulatory factors in the metabolic pathways responsible for brazilin formation.

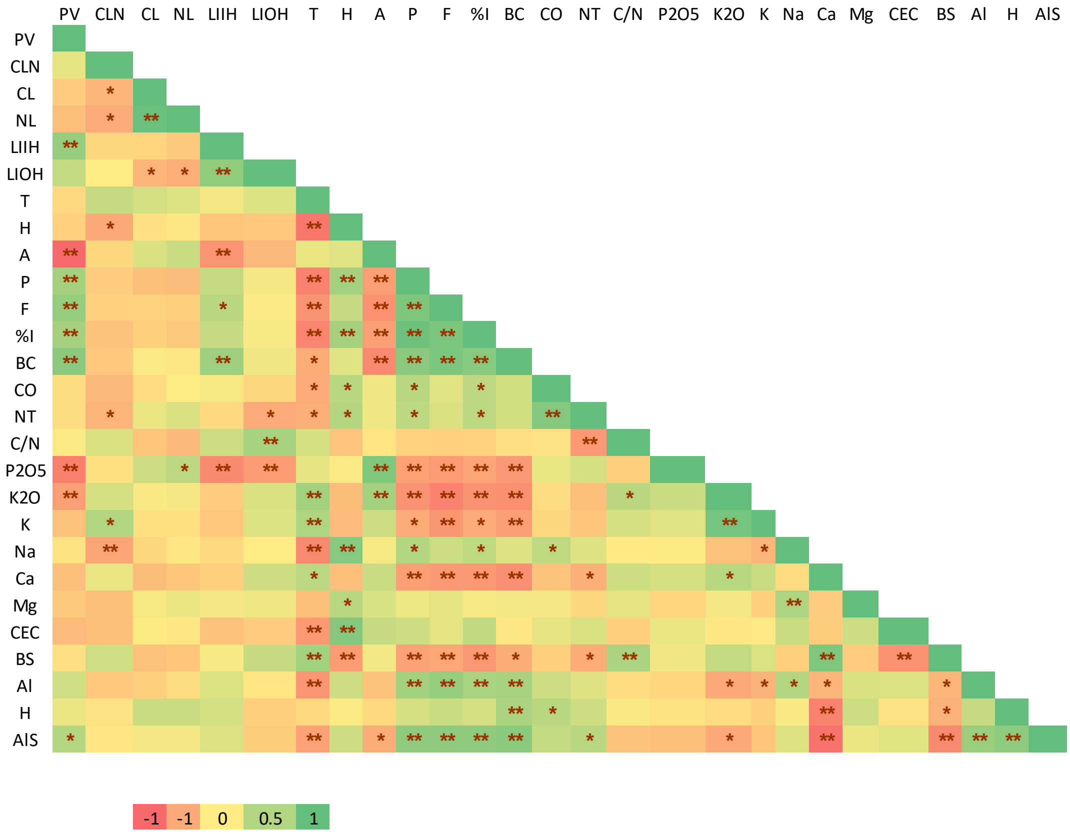

Fig. 4. Correlation plot between environmental conditions, soil characteristics, leaf profile content, and secondary metabolites in sappanwood varieties. PV: Plant variety, CLN: Compound leaf number, CL: Chlorophyll leaves, N: Leaf N content, LIIH: Light intensity in heading, LIOH: Light intensity outside the heading, T: Temperature, H: Humidity, A: Altitude, P: Phenolic content, F: Flavonoids, %I: Percentage of inhibition, BC: Brazilin content, CO: C Organic material content, NT: N Total, C/N: C/N Ratio, CEC: Cation exchange capacity, BS: Base saturation, AIS: Al saturation.

Soil nutrients, particularly P_2O_5 and K_2O, showed a strong positive correlation with BC, underscoring the crucial role of phosphorus and potassium in the metabolic and physiological processes that enhance Brazilin production. In addition, BC was significantly correlated with Cation Exchange Capacity (CEC) and Base Saturation (BS), emphasizing the importance of soil fertility and nutrient exchange efficiency in promoting its synthesis. Environmental factors also influenced BC levels. A moderate positive correlation was observed between BC and both temperature and altitude, suggesting that higher temperatures and altitudes favor Brazilin biosynthesis. These conditions may slow plant metabolic activity, allowing greater accumulation of secondary metabolites, including Brazilin. Conversely, BC exhibited a negative correlation with aluminium saturation and hydrogen ion concentration (H), indicating that acidic soil conditions and aluminium toxicity can suppress Brazilin content. This reduction may stem from impaired nutrient uptake or stress-induced disruptions in plant metabolism under such conditions.

Principal Component Analysis (PCA) was performed to examine the relationships among multiple traits, including Brazilin content, by reducing the dataset's dimensionality while retaining the most significant sources of variability (Fig. 5). A total of 27 principal components (PCs) were identified, with the first five PCs exhibiting eigenvalues greater than one and collectively explaining approximately 82.89% of the total variance. The first principal component (PC1) accounted for the largest portion of the variability (36.27%), followed by PC2 (20.36%), PC3 (11.79%), PC4 (7.81%), PC5 (6.66%), PC6 (5.72%), and PC7 (4.20%) (Table 4). The distribution of variance across the components is illustrated in the scree plot (Fig. 4). Together, these seven components explained the majority of the variability in the dataset (Table 5).

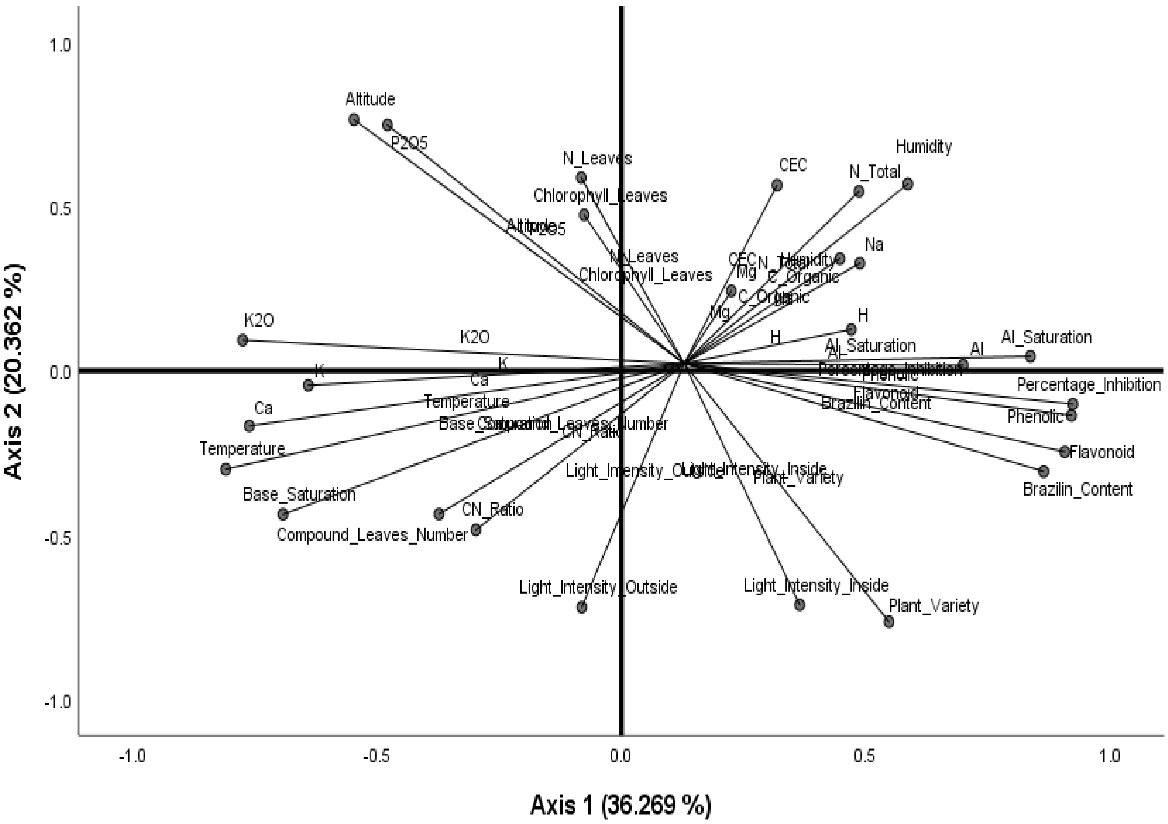

Fig. 5. Principal component biplot for first two principal components between environmental conditions, soil characteristics, leaf profile content, and secondary metabolites in sappanwood varieties.

Table 4. Eigen values, percentage of variance, and cumulative variance of the various principal components.

Principal Components	**Eigen Value**	**Variance Percentage**	**Cumulative Variance Percentage**
PC1	9.7925816013406100	36.2688207457060000	36.27
PC2	5.4978550907916900	20.3624262621915000	56.63
PC3	3.1843669191558300	11.7939515524290000	68.43
PC4	2.1078469861563100	7.8068406894678100	76.23
PC5	1.7977950682037100	6.6585002526063200	82.89
PC6	1.5435810363396800	5.7169668012580600	88.61
PC7	1.1344097675591800	4.2015176576265800	92.81
PC8	0.6126595377903260	2.2691093992234300	95.08
PC9	0.5222820536052520	1.9343779763157500	97.01
PC10	0.4702055905693130	1.7415021872937500	98.75
PC11	0.1458622325251580	0.5402304908339170	99.29
PC12	0.0841974626181562	0.3118424541413190	99.61
PC13	0.0655496757793661	0.2427765769606150	99.85
PC14	0.0258311793040948	0.0956710344596103	99.94
PC15	0.0149757982613335	0.0554659194864203	100.00
PC16	0.0000000000000008	0.0000000000000029	100.00
PC17	0.0000000000000003	0.0000000000000012	100.00
PC18	0.0000000000000003	0.0000000000000011	100.00
PC19	0.0000000000000002	0.0000000000000006	100.00
PC20	0.0000000000000000	0.0000000000000001	100.00
PC21	0.0000000000000000	0.0000000000000000	100.00
PC22	-0.0000000000000001	-0.0000000000000003	100.00
PC23	-0.0000000000000002	-0.0000000000000007	100.00
PC24	-0.0000000000000004	-0.0000000000000016	100.00
PC25	-0.0000000000000006	-0.0000000000000021	100.00
PC26	-0.0000000000000009	-0.0000000000000034	100.00
PC27	-0.0000000000000026	-0.0000000000000095	100.00

Table 5. Environmental, physiological, and biochemical traits affecting principal components in PCA of *Caesalpinia sappan* L.

Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**
Plant Variety	0.5476	-0.7647	-0.2074	-0.1614	-0.0780	-0.0495	0.0374
Compound Leaf Number	-0.3734	-0.4366	-0.0634	-0.3811	0.5723	-0.1967	-0.3507
Chlorophyll Leaves	-0.0764	0.4744	-0.7222	0.2875	-0.2499	-0.1783	0.1482
N Leaves	-0.0824	0.5883	-0.6540	0.2991	-0.2777	-0.0406	0.0741
Light Intensity in Heading	0.3649	-0.7128	-0.1448	0.3869	-0.1017	0.1909	0.2402
Light Intensity outside the Heading	-0.0812	-0.7201	0.3266	0.4166	-0.0280	0.0681	0.3485
Temperature	-0.8099	-0.3002	-0.4704	0.1063	-0.0168	0.0465	0.1063
Humidity	0.5863	0.5688	0.5121	0.0247	-0.0209	-0.1237	0.1069

Altitude	-0.5476	0.7647	0.2074	0.1614	0.0780	0.0495	-0.0374
Phenolic	0.9213	-0.1362	0.2283	-0.1085	0.1077	0.0503	0.1048
Flavonoid	0.9076	-0.2469	0.0003	-0.0572	-0.0735	-0.1211	-0.1170
% Inhibition	0.9241	-0.1015	0.1393	-0.1006	0.0765	-0.0138	0.2226
Brazilin Content	0.8642	-0.3069	-0.2705	0.1160	-0.0595	0.0728	0.0235
C Organic	0.4471	0.3412	0.1320	0.0824	0.2610	0.7536	0.1048
N Total	0.4863	0.5458	-0.1523	-0.2936	0.0816	0.5064	0.2520
C/N Ratio	-0.2979	-0.4846	0.2963	0.5360	0.2106	0.1274	-0.1604
P2O5	-0.4791	0.7483	-0.0568	-0.1630	0.0070	0.2346	-0.2066
K2O	-0.7752	0.0929	0.0947	0.3450	0.4505	0.0356	0.1909
K	-0.6411	-0.0445	0.0464	0.0114	0.6231	-0.0691	0.2837
Na	0.4881	0.3272	0.5592	0.2661	-0.2317	0.0983	-0.1969
Ca	-0.7616	-0.1678	0.4772	-0.1284	-0.3065	0.1570	0.0259
Mg	0.2248	0.2428	0.2806	0.7396	-0.0104	-0.2726	-0.2253
Cation exchange capacity	0.3185	0.5648	0.4474	0.0160	0.2001	-0.4072	0.2883
Base Saturation	-0.6928	-0.4376	0.1935	0.0173	-0.2445	0.3985	-0.2317
Al	0.6994	0.0165	0.1328	0.0788	0.0329	0.0783	-0.3710
H	0.4706	0.1257	-0.5244	0.4445	0.4039	0.2053	-0.2573
Al Saturation	0.8371	0.0437	-0.3115	0.0010	0.3977	-0.0465	-0.1178

The biplot analysis revealed that Brazilin content exhibited a strong positive loading on PC1, indicating its significant contribution to the variability captured by this component. In contrast, PC2 and subsequent components showed mixed positive and negative loadings across different traits, reflecting their distinct contributions to the overall variation. Traits such as total phenolic content, flavonoids, and percentage of inhibition also displayed strong associations with PC1, highlighting their close relationship with Brazilin content. Overall, this analysis emphasizes the significance of Brazilin content as a key determinant of variability within the dataset, consistent with its central role in metabolite-related studies.

The results provided significant insights into the correlations between Brazilin content and other measured traits. Brazilin content emerged as a key variable contributing to the overall variability within the dataset, particularly in the first two principal components, which together accounted for more than 56% of the total variance (Table 4). A strong positive association was observed between Brazilin content and secondary metabolites such as total phenolic content, flavonoid content, and percentage inhibition. This relationship highlights the pivotal role of Brazilin in antioxidant activity and its close linkage with phenolic biosynthesis pathways. Overall, these findings highlight the importance of biochemical traits in explaining the variability associated with Brazilin content. Environmental factors such as light intensity within the canopy, humidity, and cation exchange capacity (CEC) exhibited moderate correlations with Brazilin content, suggesting their potential influence on the metabolic processes underlying its synthesis. In contrast, variables including temperature, altitude, and nutrient levels (e.g., K_2O and Ca) showed weaker or negative correlations, indicating a limited or inverse impact on Brazilin accumulation. These patterns suggest that biochemical traits govern Brazilin content more strongly than environmental or agronomic factors. Overall, the PCA highlights the complex interplay among biochemical, environmental, and physiological factors affecting Brazilin content. The findings provide a valuable framework for identifying key traits that can be targeted to optimize Brazilin production, particularly through the enhancement of phenolic biosynthesis and antioxidant-related pathways. Such insights are essential for guiding breeding strategies, agronomic management, and in vitro culture approaches aimed at improving Brazilin yields in relevant plant species.

Discussion

Environmental conditions, including climate and soil fertility, play a significant role in shaping the secondary metabolite composition of *Caesalpinia sappan* (sappanwood), particularly its Brazilin content. This observation aligns with the findings of Bi et al. (2024), who reported that both aboveground and belowground environmental factors contribute to variations in the secondary metabolite profiles of plants such as *Reynoutria japonica*. Secondary metabolites, including phenolic compounds and flavonoids, are essential for plant survival and function primarily as defense mechanisms against biotic stressors such as herbivory and microbial attack (Xiao et al., 2019). The relationship between Brazilin content and environmental factors reflects a complex, multivariate response, as illustrated in Figure 4. Brazilin exhibits a negative correlation with high base saturation, which was determined by cationic nutrients such as potassium, sodium, and calcium, as well as with elevated phosphorus levels. Similarly, higher temperatures and elevations are associated with reduced Brazilin accumulation. In contrast, positive correlations were observed between Brazilin content and aluminium concentration, acidic soil conditions, and secondary metabolites such as phenolic compounds, flavonoids, and antioxidant activity. Notably, aluminium saturation showed an opposite response pattern to base saturation, suggesting it may be a more reliable indicator of plant physiological responses than soil pH (Schoenholtz et al., 2000).

Base saturation is closely linked to cation exchange capacity, a key determinant of nutrient availability and retention in soils. The carbon can further explain this relationship–nutrient balance hypothesis (CNBH), which posits that increased nutrient availability reduces the production of carbon-based secondary metabolites, such as phenolics and terpenoids, in woody plant species (Bryant et al., 1983; Bustamante et al., 2020; Ormeño and Fernandez, 2012). Under nutrient-limited conditions, woody plants tend to allocate more carbon resources to defensive secondary metabolites, as growth is constrained both above- and belowground (Bryant et al., 1983).

Brazilin, a flavonoid belonging to the chalcone class, is initially pale or colorless but undergoes oxidation to form brazilein, a red pigment (Ngamwonglumlert and Devahastin, 2023; Vij et al., 2023). This chemical transformation explains the positive correlation observed between Brazilin and other phenolic and flavonoid compounds. Both Brazilin and brazilein contribute to oxidative stress defense mechanisms in plants due to their potent antioxidant properties (Han et al., 2023). Although Brazilin enhances antioxidant activity in sappanwood (Fig. 1), other phenolic compounds also play substantial roles, indicating that Brazilin levels do not solely determine antioxidant capacity.

Altitude likewise influences Brazilin content. Sappanwood grown at higher elevations, such as Majalengka (130.79 MASL) and Sumedang (462.75 MASL), contains lower Brazilin levels than plants from lower elevations, such as Garut (50 MASL) and Purwodadi (45 MASL). According to Topaloğlu et al. (2016), altitude affects the structural characteristics of woody plants, with higher elevations promoting fibrous wood formation and lower elevations favoring denser, thicker wood. Metabolite distribution also varies with altitude: low-elevation environments tend to promote secondary metabolite accumulation in woody organs, such as roots, whereas high-elevation environments favor accumulation in leaves (Pan et al., 2023).

Furthermore, soils in high-altitude areas such as Sumedang and Majalengka generally contain higher levels of phosphorus and potassium than those in low-altitude regions such as Garut and Purwodadi. Elevated phosphorus and potassium concentrations enhance nitrogen metabolism efficiency, photosynthetic activity, and leaf growth, even under constant light conditions (Liu et al., 2024). Although data on sappanwood growth and metabolism remain limited, comparisons with related species provide valuable insights.

The positive influence of soil aluminium on Brazilin content suggests that sappanwood may function as an aluminium hyperaccumulator capable of thriving in acidic soils. Hyperaccumulation may stimulate the biosynthesis of secondary metabolites, including Brazilin, in woody tissues (Ofoe et al., 2023). For example, *Cunila galioides* exposed to high aluminium concentrations exhibited increased flavonoid production despite reduced biomass and dry weight (Mossi et al., 2011). Aluminium exposure can be beneficial for higher plants, enhancing biomass production and promoting metabolic processes such as chlorophyll synthesis, sugar accumulation, amino acid and hormone production, and secondary metabolite biosynthesis via the shikimic acid pathway (Moriyama et al., 2016; Xu et al., 2016).

Aluminium may also affect pigment production in plant organs, as evidenced by enhanced anthocyanin and quercetin formation in flowers exposed to specific aluminium concentrations (Bojórquez-Quintal et al., 2017). However, its influence on wood pigments, such as those found in sappanwood, remains unverified. Environmental stress, particularly under challenging growth conditions, can trigger defensive responses in plants, often resulting in increased synthesis of secondary metabolites (Srivastava et al., 2020). The elevated Brazilin levels observed in plants from Garut and Purwodadi may therefore represent adaptive responses to acidic soils and other local environmental factors. Temperature also plays a crucial role in secondary metabolite accumulation. The relatively moderate average temperature in Garut, around 29 °C, falls within the optimal range for secondary metabolite and pigment production reported by Verma and Shukla (2015). Similarly, Singh and Sharma (2020) identified 17–29 °C as the ideal temperature range for metabolite and pigment accumulation, noting significant declines above 30

°C. This pattern is further supported by Sobuj et al. (2018), who reported a 55% reduction in bark phenolic content in *Populus tremula* under high-temperature conditions. Collectively, these findings highlight the intricate interplay between environmental factors, such as temperature, soil chemistry, and stress conditions, and secondary metabolite biosynthesis in sappanwood, emphasizing the plant's adaptive metabolic responses to its environment.

Conclusion

This study elucidated the complex interactions between environmental factors and nutrient dynamics in regulating Brazilin production in *Caesalpinia sappan*, highlighting distinct mechanisms that governed secondary metabolite biosynthesis. The negative correlations between Brazilin content, base saturation, and phosphorus availability were consistent with the carbon–nutrient balance hypothesis, suggesting a trade-off between primary and secondary metabolic pathways. In contrast, acidic soils with elevated aluminium and hydrogen saturations were positively associated with Brazilin accumulation, indicating a stress-induced enhancement of flavonoid and phenolic synthesis as part of the plant's defensive response. The positive association between Brazilin content and antioxidant activity further underscored its functional role in mitigating oxidative stress. Environmental parameters such as elevation and temperature also modulated Brazilin levels. Lower altitudes and moderate temperatures favoured Brazilin synthesis, whereas higher altitudes with increased phosphorus and potassium concentrations appeared to promote primary metabolic processes over secondary metabolite accumulation. Moreover, the potential of *C. sappan* to function as an aluminium hyperaccumulator highlighted its adaptability to acidic soils, which simultaneously supported Brazilin and flavonoid production. Overall, these findings revealed the intricate balance among genetic, environmental, and nutritional factors that influenced Brazilin biosynthesis, providing valuable insights into its ecological functions and adaptive significance under varying environmental conditions.

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Author Contributions

Conceptualization, Formal Analysis, Funding Acquisition, Investigation Methodology, Supervision, Validation, Visualization, Writing – Original Draft, Writing – Review and Editing, SR; Formal Analysis, Investigation, Supervision, Writing – Original Draft, MA and FF; Data Curation, Formal Analysis, Investigation, Writing – Original Draft, ALR; Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – Original Draft, Writing – Review and Editing, SM. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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