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Anthocyanins, Betalains, and Antioxidant Activity in Bougainvillea Petal Extracts: A Novel Source of Natural Colorant

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Abstract

The objective of this research was to analyze the pigment composition and antioxidant capacities present in water extracts from four Bougainvillea varieties: Jawa White (White), Tequila Sunrise (Orange), Sanderiana (Purple), and Texas King (Lilac). The petals of Bougainvillea were extracted using water, with a petal-to-water ratio of 1:1(w/v). The extracted compounds were evaluated for their antioxidant activity and bioactive compound content, including total flavonoids, phenolic compounds, anthocyanins, and betalains. The results indicated significant differences in CIELAB, Hue, and Chroma values ($p<0.05$) among the aqueous extracts, corresponding to the petal colors. All examined varieties demonstrated notable levels of bioactive compounds. Texas King exhibited the highest content of betalains, anthocyanins, and antioxidant activity (FRAP assay: 110.67 mM $Fe2+$/mg), while Jawa White displayed the highest antioxidant activity (DPPH assay: with 88.99% radical scavenging activity). Therefore, Bougainvillea varieties show promising potential as valuable natural sources of colorants and antioxidants..

Keywords: *Anthocyanin; Betalain; Bougainvilleas Varieties; Natural Colorant*

Introduction

The current market exhibits a heightened demand for natural colorants, primarily for the restoration of color in processed foods and food decoration purposes. This demand stems from a growing awareness of health implications associated with synthetic colorants, particularly red and yellow azo-dyes, which have been documented to potentially form carcinogenic aromatic amines upon metabolism in the human gut. Consequently, consumers are increasingly seeking products that utilize natural colors. Various researchers have highlighted the significance of anthocyanins and betalains derived from natural sources, emphasizing their potential as safe colorants and antioxidants beneficial to human health. Antioxidants are acknowledged for their potential to mitigate the risk of numerous diseases linked to oxidative stress. Anthocyanins and betalains, commonly found in vibrantly colored plants, have demonstrated diverse biological effects, including antibacterial, anti-inflammatory, and antioxidant activities. Numerous studies have established a correlation between anthocyanins, betalains, and their antioxidant properties. Plants, particularly vegetables containing anthocyanins and betalains, exhibit antioxidant properties. Bougainvillea, known locally as 'Faung-Fa' or 'paper flower,' is prevalent across all regions of Thailand. Bougainvillea belongs to a genus of thorny ornamental vines, bushes, and trees characterized by flower-like spring leaves near its flowers. Thriving in the Thai climate, Bougainvillea has given rise to numerous new mutations and is a staple in Thai gardens, celebrated for its evergreen nature and vibrant hues ranging from pink, magenta, purple, red, orange, white, to yellow. Despite its popularity, there has been limited research on the pigments present in Bougainvilleas, particularly as a novel source of natural colorants. Consequently, this study was undertaken to explore the color variations, colorant compound contents, and their associated antioxidant activities.

Materials and methods

Sample preparation

Preparation of Bougainvilleas

Freshly cut flowers from various selected Bougainvillea varieties, namely Jawa White (White), Tequila Sunrise (Orange), Sanderiana (Purple), and Texas King (Lilac), were carefully collected from a local garden in Mahasarakham Province, Thailand. The flowers were subjected to a thorough rinse with tap water, and subsequently, the flower leaves were meticulously trimmed. The preparation of samples was carried out in triplicate for each variety to ensure reliability and consistency in the analysis.

Preparation of colorant extract

Each color variant of Bougainvillea petals, amounting to a total of 100 g per shade, underwent extraction through blending with water at a precise 1:1(w/v) ratio for a duration of 3 minutes. The blended samples were subjected to filtration using cheesecloth, a process that was repeated three times to ensure thorough extraction. Subsequently, the residual material was subjected to three additional extractions using the same solvent.

Determination of color

CIELAB measurements were conducted on the color extracts using a Hunter Lab Color Flex EZ colorimeter to assess the color properties after preparation. The color properties were expressed in terms of L* (representing lightness), a* (indicating magenta hue, with positive values), and b* (indicating blue hue, with negative values). The hue, or color angle, was calculated based on the a* and b* values.

First quadrant [+a, +b] Hue = [Arc tan (b/a)]
Second quadrant [-a, +b] Hue = 180-Arc tan(b/a)
Third quadrant [−a, −b] Hue = 180+Arc tan(b/a)
Fourth quadrant [+a, −b] Hue = 360- Arc tan(b/a)

The chroma, was calculated using the following formula:

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

Determination of total betalain content

An appropriate volume of the filtered extract was selected and diluted with the extracting solvent to ensure an optical density falling within the optimal range of the measuring instrument. The absorbance of the solution was then quantified at 538 nm. The determination of total betalain content utilized the equation.

Betalain content (mg/100g) = A×DF×MW×1000)/(ε×1)

Whereas

A = Absorbance (538 nm)
DF = Dilution factor
MW = Molecular weight of betalain (550 g /mol)
ε = Molar extinction coefficients (60,000 L/mol cm)
l = Path length of cuvette (1 cm)

Determination of total anthocyanin content

A portion of the filtered extract was diluted with the extracting solvent to achieve an optical density within the optimal range for the measuring instrument. The appropriately diluted extracts were then stored in darkness for a duration of 2 hours, after which their absorbance was measured at 520 nm. The obtained results were expressed in equivalents of cyanidin-3-glucoside, the prevalent anthocyanin pigment in nature. The determination of total anthocyanin content followed the equation outlined.

Total anthocyanin content (mg/100g) = (OD×DV×TEV×100)/(SV×SW×51.56)

Whereas

OD = optical density
DV = diluted volume for the OD measurement
TEV = total extract volume (mL)
SV = sample volume (mL)
SW = sample weigh (g)
51.56 = E. value for which the major constituent (Cyanidin)

Determination of total phenolic content

Total phenolic content was determined employing a modified Folin-Ciocalteu method, as described. The absorbance was measured at a wavelength of 765 nm. The analysis was conducted in triplicate for each extract. Standard solutions of gallic acid ranging from 60 to 150 mg/mL were prepared to establish a standard curve. The results were expressed as a percentage of total gallic acid equivalents per 100 g (g GAE/100 g).

Determination of total flavonoid content

The determination of total flavonoid content was based on a slightly modified method derived from Pourmorad et al., 2006. The absorbance was measured at a wavelength of 415 nm. The evaluation was performed in triplicate for each extract. Standard solutions of quercetin (QE) ranging from 40 to 160 mg/mL were prepared to generate a standard curve. The total flavonoid content was expressed as a percentage of total quercetin equivalents per 100 g (g QE/100 g).

Determination of DPPH scavenging capacity

The preparation of fresh samples involved dissolving 5 g of the sample in 20 ml of methanol, followed by sonication for 1 hour. The solution was then filtered using Millipore filter paper. Each filtered sample was further diluted with methanol at a 1:4 ratio. The DPPH assay, based on the method presented with certain modifications, was utilized for evaluating the samples. In this assay, 0.4 ml of each sample was vigorously mixed with 2 ml of 1.2 mM DPPH and allowed to react for 1 hour in the dark. Subsequently, the absorbance at 517 nm was measured using a UV-Vis spectrophotometer (Hewlett Packard 8435). Methanol was employed as a blank, and a DPPH solution without added sample served as the control. The evaluation was conducted in triplicate for each sample. The scavenging activity was calculated using the following formula:

DPPH radical−scavenging activity (%) = [1-(A sample/ A control)] x 100

Whereas

A is the absorbance at 517 nm.

Determination of FRAP capacity

The preparation of the FRAP solution followed the procedure outlined by Miraliakbari H. et al., 2008. The FRAP solution was prepared in acetate buffer at pH 3.6. For each extract, 0.3 mL was mixed with 2.7 mL of the FRAP solution and allowed to react for 30 minutes in darkness. Subsequently, the absorbance was measured at 595 nm using a UV-Vis spectrophotometer (Hewlett Packard 843). Acetate buffer was utilized as a blank. The evaluation was conducted in triplicate for both the standard and each extract. The FRAP capacity of each extract was determined based on the increase in Fe (II)-TPTZ absorbance, measured in mM Fe2+/mg.

Statistical Analysis

All experiments were executed in triplicate, and the results from each analysis were expressed as means ± standard deviation obtained from a minimum of three independent experiments. Statistical analysis involved employing ANOVA, with a set statistical significance level of $p<0.05$. Post-hoc analysis using the least significant difference procedure was conducted using statistic program.

Result and Dicussion

The analysis of color shades in the aqueous extracts is presented in **Table 1.** Various color shades were observed in the fresh petals of the four Bougainvillea varieties. Significant differences were noted in all CIELAB, Hue, and Chroma values. The CIELAB values revealed that Jawa White had the highest L* value at 72.53±0.11, whereas Sanderiana exhibited the highest a* value at 55.32±0.04. Tequila Sunrise displayed the highest b* value at 31.84±0.16. In terms of the Hue angle, Sanderiana recorded the highest value at 350.26±0.06, while Tequila Sunrise had the highest Chroma value at 33.91±0.22.

Variety of Bougainvillea	L^*	a^*	b^*	Hue	Chroma
Jawa White (White)	72.53±0.11[a]	-5.59±0.02[d]	15.55±0.08[b]	109.11±0.15[b]	16.46±0.07[c]
Tequila Sunrise (Orange)	50.24±0.38[d]	11.67±0.26[b]	31.84±0.16[a]	69.87±0.23[c]	33.91±0.22[a]
Sanderiana (Purple)	55.32±0.04[c]	26.69±0.05[a]	-4.58±0.04[d]	350.26±0.06[a]	27.08±0.05[b]
Texas King (Lilac)	66.44±0.07[b]	6.98±1.38[c]	6.57±0.04[c]	43.63±5.47[d]	9.62±1.03[d]
*Values are shown in mean ± SD of triplicate measurement **means having different superscript letters within a same column are significantly different (p≤ 0.05).					

Table 1: CIELAB, Hue and Chroma values of the extracts from different Bougainvillea variety.

The experimental results presented in **Table 1** demonstrate that the aqueous extracts obtained from Bougainvillea flowers can serve as natural colorants. This conclusion is based on two primary reasons: Suitability for Aqueous Extraction: The aqueous extraction method is feasible, simple, and safe, eliminating the need for additional chemical agents. Vibrant and True-to-Nature Colors: The resulting colors exhibit a vibrant and natural hue, resembling the shades present in the flower petals. This characteristic enhances the convenience and versatility of their utilization. Simultaneously, the range of colors obtained offers diverse possibilities for various applications.

Table 2 displays the content of betalains and total anthocyanins in each Bougainvillea variety. The findings underscored the substantial presence of both betalains and anthocyanins in all color extracts. Specifically, the Texas King variety exhibited the highest content of betalains and total anthocyanins at 6.79±0.21 g/100g and 6.52±0.10 g/100g, respectively.

Variety of *Bougainvillea*	Petal color	Betalain mg/100 g	Anthocyanin mg/100 g
Jawa White	white	0.92±0.10[d]	1.05±0.06[d]
Tequila Sunrise	orange	5.19±0.10[b]	4.85±0.06[b]
Sanderiana	purple	3.97±0.26[c]	4.28±0.10[c]
Texas King	lilac	6.79±0.21[a]	6.52±0.10[a]
*Values are shown in mean ± SD of triplicate measurement **means having different superscript letters within a same column are significantly different ($p \leq 0.05$).			

Table 2: Betalain and Total Anthocyanin content of the extracts from various Bougainvillea varieties.

Numerous studies have underscored the notable stability of betalains within the pH range of 4-5, presenting a broad pH stability profile. Betalains find valuable applications in the food industry, particularly in products with a short shelf-life, requiring minimal heat treatment, and packaged and marketed in a dry state under reduced levels of light, oxygen, and humidity. Potential applications of betalains in foods encompass gelatin desserts, confectioneries, dry mixes, poultry, dairy, and meat products. Therefore, based on the experimental results, it is indicative that the aqueous extracts from Bougainvillea flowers possess natural colorant properties.

Variety of Bougainvillea	Petal color	Flavonoid (g QE/100g)	total phenolic compound (g GAE/100g)
Jawa White	white	0.43±0.01[d]	0.02±0.04[c]
Tequila Sunrise	orange	1.03±0.04[b]	0.36±0.03[b]
Sanderiana	purple	0.63±0.01[c]	0.03±0.01[c]
Texas King	lilac	1.15±0.07[a]	0.40±0.01[a]
*Values are shown in mean ± SD of triplicate measurement **means having different superscript letters within a same column are significantly different ($p \leq 0.05$).			

Table 3: Flavonoids and Total phenolic compounds in extracts from various Bougainvillea varieties.

Table 3 presents the flavonoid and total phenolic compound content in Bougainvillea varieties. The highest content of flavonoids and total phenolic compounds was observed in the Texas King variety, measuring 1.15±0.07 g QE/100g and 0.40±0.01 g GAE/100g, respectively. However, the results indicate that Bougainvillea varieties may not be a significant source of flavonoids and total phenolic compounds**.**

Variety of Bougainvillea	Petal color	FRAP mM Fe^{2+}/mg	DPPH % radical scavenging activity
Jawa White	white	20.94±2.4[d]	88.99±017[a]
Tequila Sunrise	orange	49.11±1.8[c]	70.28±1020[b]
Sanderiana	purple	71.44±1.43[b]	61.57±0.98[c]
Texas King	lilac	110.67±3.17[a]	43.25±0.64[b]
*Values are shown in mean ± SD of triplicate measurement **means having different superscript letters within a same column are significantly different ($p \leq 0.05$).			

Table 4: Antioxidant activity of the extracts in Bougainvillea variety.

Table 4 illustrates the antioxidant activity of each Bougainvillea variety. The results obtained from the FRAP assay demonstrated that the aqueous extract of Bougainvillea variety 'Texas King' exhibited the highest antioxidant capacity at 110.67±3.17 mM Fe2+/mg. Conversely, in the DPPH radical scavenging assay, 'Jawa White' demonstrated the highest activity at 88.99±0.17%, although this result was not directly correlated to the flower pigments. The experiments revealed that the aqueous extracts from Bougainvillea flowers of the 'Texas King' variety displayed significant antioxidant activity, indicated by the substantial formation of complex iron compounds, showcasing potent free radical scavenging properties. This variety exhibited an effective mechanism for electron donation. On the other hand, the aqueous extracts from Bougainvillea flowers of the 'Jawa White' variety demonstrated notable antioxidant capabilities when compared to the standard, with a high percentage of radical scavenging activity, reaching 88.99%. Despite the petals of this variety being white, the antioxidant potential of the extract was remarkable. Moreover, considering the aqueous extracts from Bougainvillea flowers, particularly the 'Tequila Sunrise' variety, which imparts an orange hue, it was evident that the extract exhibited substantial antioxidant potential. The percentage of radical scavenging activity was notably high at 60.28%. This observation underscores the potential of aqueous extracts from Bougainvillea flowers to serve as a source of new natural colorants.

Conclusion

The study findings strongly support the potential of Bougainvillea flowers as a promising natural colorant source. The aqueous extracts were notably abundant in both betalains and total anthocyanins. Betalains, being water-soluble pigments, can manifest as red or yellow hues. Anthocyanins, on the other hand, represent a major category of water-soluble pigments highly valued in the food industry for their excellent coloring properties, offering a range of hues from red and violet to yellow. In particular, the flowers of Bougainvillea, specifically the 'Texas King' variety, are rich reservoirs of both anthocyanins and betalains. Consequently, they find commercial application as food coloring agents and natural constituents in cosmetics.

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