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Phytochemicals, Antibacterial, and Antioxidant Properties of *Blumea balsamifera* and *Graptophyllum pictum* Ethanol Extracts

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Abstract

Natural products are an important source of bioactive compounds with antioxidant and antimicrobial potential. *Blumea balsamifera* and *Graptophyllum pictum* are two medicinal plants traditionally used in Southeast Asia, yet their comparative phytochemical content and synergistic biological activities have not been fully explored. This study aimed to evaluate the phytochemical composition, antioxidant activity, and antibacterial potential of ethanol extracts of *B. balsamifera* and *G. pictum*, both individually and in combination. Phytochemical quantification was performed for flavonoids, tannins, and phenolics. Antioxidant activity was assessed using the DPPH radical scavenging assay, while antibacterial activity against *Staphylococcus aureus* was evaluated using the Kirby–Bauer disc diffusion method. Results showed that *B. balsamifera* contained higher levels of flavonoids (5315.67 mg/100 g), tannins (5299.07 mg/100 g), and phenolics (4637.04 mg/100 g) compared to *G. pictum*. Both extracts exhibited strong antioxidant activity with IC_{50} values of 73.73 ± 10.49 ppm (*B. balsamifera*) and 97.44 ± 1.68 ppm (*G. pictum*), indicating high radical scavenging capacity. *B. balsamifera* showed moderate antibacterial activity (9.83 ± 0.25 mm), while *G. pictum* showed no inhibition. Interestingly, the combination of extracts produced larger inhibition zones (up to 16.3 ± 0.23 mm at a 1:1 ratio), indicating synergistic or additive effects. These activities are likely driven by the combined mechanisms of phenolics, flavonoids, and tannins, including radical scavenging, metal chelation, protein precipitation, and membrane disruption. In conclusion, the findings highlight the potential of *B. balsamifera* and *G. pictum*, particularly in combination, as promising natural sources of multifunctional antioxidant and antibacterial agents. Further studies on compound isolation and formulation development are warranted to support their application in pharmaceutical and cosmeceutical products.

Keywords: antibacterial; antioxidant; *Blumea balsamifera*; *Graptophyllum pictum*; phytochemicals.

INTRODUCTION

Plants represent invaluable reservoirs of bioactive compounds, offering a remarkable chemical diversity that has long been crucial for drug discovery and development (Atanasov et al., 2021). Many medicinal plants traditionally used in ethnomedicine have been scientifically validated through modern pharmacological studies, confirming their therapeutic potential (Ulriksen et al., 2022). Their importance arises from evolutionary adaptations, whereby plants synthesize a wide variety of secondary metabolites—such as alkaloids, flavonoids, terpenoids, and phenolic compounds—to cope with environmental stressors, many of which exhibit significant pharmacological activities (Oliveira et al., 2023; et al 2022). Indeed, plant-derived compounds have provided the chemical basis for well-established drugs, including morphine, quinine, and paclitaxel, illustrating their central role in medicine. Importantly, these natural products often possess unique chemical scaffolds and mechanisms of action, making them valuable candidates for addressing diseases with unmet medical needs, such as cancer and infectious diseases (Dikpınar & Süzgeç-Selçuk, 2020; Gezici & Şekeroğlu, 2019). Thus, the exploration of plant biodiversity remains a cornerstone of natural product research, underscoring the enduring importance of plants as sources of novel therapeutic agents (Atanasov et al., 2021; Oliveira et al., 2023; Ulriksen et al., 2022; Yeshi et al., 2022).

Among the many medicinal plants of interest, *Blumea balsamifera* has been widely studied due to its broad spectrum of pharmacological activities. Traditionally used in Southeast Asian medicine, this plant has been reported to exhibit anti-inflammatory, antimicrobial, antioxidant, and anticancer effects (Hanafiah & Usman,

2022; Widhiantara & Jawi, 2021). Its bioactive compounds, such as sesquiterpenoids, flavonoids, and phenolic constituents, are thought to play a major role in these activities (Fan et al., 2022; Hanafiah & Usman, 2022; Widhiantara & Jawi, 2021). Modern investigations have extended its applications beyond traditional use, highlighting potential roles in infectious diseases, chronic inflammatory conditions, autoimmune disorders, and cancer therapy, which strengthens the rationale for exploring its therapeutic value in greater depth (Ulriksen et al., 2022; Widhiantara & Jawi, 2021). Given this diverse pharmacological profile, *B. balsamifera* represents a promising candidate for further studies targeting antibacterial and antioxidant properties.

Similarly, *Graptophyllum pictum*, another plant used in traditional medicine, demonstrates a rich array of pharmacological effects. Reports have documented its anti-inflammatory, antioxidant, antimicrobial, antidiabetic, and even anticancer activities (Makkiyah, et al 2021; Priyanto, et al 2024a). These activities are largely attributed to the presence of flavonoids, phenolics, and alkaloids, which contribute to its bioactive profile (Makkiyah et al., 2021; Priyanto et al., 2024a). Ethnomedicinally, *G. pictum* has been applied in treating arthritis, hemorrhoid, wound healing, menstrual problems, and fever (Diah, et al. 2023; Makkiyah et al., 2021). Its wide therapeutic potential, particularly in the context of oxidative stress and microbial infections, provides strong justification for evaluating its antibacterial and antioxidant activities alongside *B. balsamifera*.

Despite various reports on the pharmacological potential of *Blumea balsamifera* and *Graptophyllum pictum*, comprehensive studies directly comparing their antibacterial and antioxidant activities remain limited. Furthermore, the potential synergistic effects of combining extracts from both plants in antibacterial assays have not been widely explored, representing an important gap in current knowledge. Key research considerations include the need to evaluate their qualitative phytochemical profiles to understand the presence of secondary metabolites that may underlie their bioactivities, as well as the use of reliable and standardized assays for antibacterial and antioxidant testing. In this study, ethanol extracts of *B. balsamifera* and *G. pictum* will be prepared and screened for their phytochemical content, tested individually for antibacterial and antioxidant activities, and further evaluated in combination to determine possible synergistic antibacterial effects. The main objectives are therefore to (1) characterize the qualitative phytochemical composition of both plants, (2) determine the antibacterial and antioxidant activities of their ethanol extracts, and (3) assess the antibacterial potential of their combined extract. By addressing these aims, this research is expected to provide new insights into the pharmacological relevance of these two medicinal plants and to highlight the potential of their combination as a novel strategy for enhancing antibacterial activity.

MATERIALS AND METHODS

Materials

Fresh leaves of *Blumea balsamifera* and *Graptophyllum pictum* were collected from Peguyangan, North Denpasar, Denpasar, Bali. Ethanol 96% served as the extraction solvent, while streptomycin discs were used as the positive control for antibacterial assay against *Staphylococcus aureus* ATCC 25923. A DPPH kit and a UV–Vis spectrophotometer were employed to evaluate antioxidant activity. Reagents for quantitative phytochemical analysis included $AlCl_3$, Folin–Ciocalteu reagent, Folin–Denis reagent, Na_2CO_3, $NaNO_2$, NaOH, tannic acid, gallic acid, and quercetin as standards. Laboratory equipment comprised standard glassware, micropipettes with sterile tips, analytical balance, vortex mixer, rotary evaporator, autoclave, incubator, oven, Bunsen burner, inoculating loop, and centrifuge. Mueller–Hinton agar (MHA) medium was prepared as the culture medium for antibacterial testing.



Figure 1. *B.balsamifera* (left) and *G. pictum leaves* (right).

Preparation of extracts

Fresh leaves of *B. balsamifera* and *G. pictum* were sorted, washed, and oven-dried at 50°C for 48 hours. The dried leaves were then pulverized into a fine powder using a blender and sieved to obtain uniform particles. Each powdered sample was macerated with 96% ethanol at a ratio of 1:10 (w/v) for three days with daily stirring. The procedure was repeated three times (re-maceration) to maximize the extraction process. The filtrates from each maceration cycle were pooled separately for *B. balsamifera* and *G. pictum*, and subsequently concentrated using a rotary vacuum evaporator at 60°C and 100 rpm to obtain thick ethanol extracts.

Quantitative Phytochemical Analysis

The total contents of flavonoids, tannins, and phenolic compounds in *B. balsamifera* and *G. pictum* ethanol extracts were determined using spectrophotometric methods adapted from Sakanaka et al. (2005), Singh et al. (2012), and Suhardi (1997). All measurements were performed in triplicate, and concentrations were

calculated using linear regression equations from the respective standard curves.

For total phenolics, 0.01 g of extract was diluted in 5 mL citrate–phosphate buffer. A 0.1 mL aliquot was mixed with 0.3 mL of 70% ethanol and 0.4 mL of Folin–Ciocalteu reagent, then incubated for 6 minutes. Subsequently, 4.2 mL of 5% Na_2CO_3 was added, vortexed, and incubated for 90 minutes at room temperature. Absorbance was measured at 760 nm using a UV–Vis spectrophotometer, and total phenolics were expressed as mg gallic acid equivalent (GAE) per g of extract, calculated from a gallic acid calibration curve (Sakanaka et al 2005).

For total flavonoids, 0.01 g of extract was diluted in 5 mL citrate–phosphate buffer. A 1 mL aliquot was mixed with 4 mL distilled water and 0.3 mL $NaNO_2$ (10%), incubated for 5 minutes, then added with 0.3 mL $AlCl_3$ (10%) and 2 mL NaOH (1%). Absorbance was read immediately at 510 nm. Total flavonoid content was expressed as mg quercetin equivalent (QE) per g of extract using a quercetin calibration curve (Singh, Verma, & Singh, 2012).

For total tannins, 0.01 g of extract was diluted in 5 mL citrate–phosphate buffer. A 0.25 mL aliquot was mixed with 0.25 mL Folin–Denis reagent, vortexed, and added with 2 mL 5% Na_2CO_3. After vortexing and 30 minutes incubation at room temperature, absorbance was measured at 725 nm. Total tannin content was expressed as mg tannic acid equivalent (TAE) per g of extract using a tannic acid standard curve (Suhardi, 1997).

Antioxidant Assay

The antioxidant activity of the extracts was evaluated using the DPPH radical scavenging assay. Extracts were diluted in ethanol to concentrations of 20–100 ppm, and 4 mL of each dilution was mixed with 1 mL of 0.25 mM DPPH solution. The mixtures were homogenized, incubated, and absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of inhibition was calculated, and IC_{50} values (the concentration required to inhibit 50% of DPPH radicals) were determined. A lower IC_{50} value indicates stronger antioxidant activity. Each measurement was performed in duplicate.

Antibacterial Assay of Individual Extracts

The antibacterial activity of *B. balsamifera* and *G. pictum* ethanol extracts was evaluated against *S. aureus* using the Kirby–Bauer disc diffusion method. Mueller Hinton Agar (MHA) plates were prepared by pouring sterilized medium into Petri dishes and allowing it to solidify. A bacterial suspension was standardized to 0.5 McFarland turbidity and evenly spread on the agar surface with a sterile cotton swab. Sterile discs were loaded with the plant extracts and then left to dry at room temperature before being carefully placed on the inoculated agar plates. Streptomycin discs were used as the positive control. Plates were incubated at 37 °C for 24 hours, after which inhibition zones were measured using a vernier caliper. All tests were performed in triplicate, and results were expressed as mean inhibition zone diameters (mm).

Antibacterial Activity of Combination Extracts

The potential synergistic antibacterial activity of combined *B. balsamifera* and *G. pictum* extracts was also evaluated against *S. aureus* using the Kirby–Bauer disc diffusion method. MHA plates were prepared as previously described. A standardized *S. aureus* suspension equivalent to 0.5 McFarland turbidity was evenly spread on the agar surface using a sterile cotton swab. Sterile discs were loaded with mixtures of the two plant extracts at ratios of 1:3, 1:1, and 3:1, and then left to dry at room temperature to ensure proper absorption and minimize excess moisture. The dried discs were carefully placed on the inoculated MHA plates, with streptomycin discs serving as the positive control. Plates were incubated at 37 °C for 24 hours, and inhibition zones were measured using a vernier caliper. All assays were performed in triplicate, and results were expressed as mean inhibition zone diameters (mm). Possible synergistic or additive effects between the extracts were evaluated by comparing the inhibition zones of the combinations with those of the individual extracts.

RESULTS AND DISCUSSION

Phytochemical analysis of *B. balsamifera* and *G. pictum* leaf extracts

Phytochemical quantification revealed a high abundance of flavonoids, tannins, and phenolic compounds in the ethanol extracts of *B. balsamifera* and *G. pictum* (Table 1). *B. balsamifera* contained substantially higher levels of all three classes of compounds compared to *G. pictum*. These secondary metabolites are known to contribute to various biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects.

Table 1. Result of Phytochemical Analysis of *B. balsamifera* and *G. pictum* leaf extracts.

Secondary Metabolites	Concentration (mg/100g)	
	B. balsamifera	***G. pictum***
Flavonoid	5315.67 ± 52.25	1293.68 ± 23.75
Tanin	5299.07 ± 15.66	1986.61 ± 12.46
Fenol	4637.04 ± 92.94	2191.94 ± 52.43

Ethnomedical evidence supports the traditional use of *B. balsamifera* for ailments such as sinusitis, colic pain, cough, kidney stones, and as a diuretic, particularly in Southeast Asia. The plant contains both volatile (terpenoids, fatty acids, phenols, aldehydes, ethers, ketones, pyridines, furans, alkanes) and non-volatile (flavonoids, flavanones, chalcones) compounds, which have been linked to its therapeutic potential (Widhiantara & Jawi, 2021). Further chemical screening has also

shown the presence of alkaloids, steroids, and terpenoids, which may enhance its biological activity (Ismail et al., 2022).

The high levels of flavonoids, tannins, and phenolics in *B. balsamifera* provide a strong biochemical basis for its antioxidant and antibacterial activities. Tannins, as high molecular weight polyphenols, are particularly important due to their ability to bind proteins, precipitate enzymes, and disrupt microbial membranes—mechanisms that contribute to both radical scavenging and antimicrobial effects (Olchowik-Grabarek et al., 2022; Szczurek, 2021).

In contrast, *G. pictum* exhibited lower levels of flavonoids, tannins, and phenolics compared to *B. balsamifera*. Quantitative studies have reported solvent-dependent but appreciable polyphenolic levels in *G. pictum* leaves: fractionation studies report TPC 11.69–102.57 mg GAE/g and TFC 2.02–28.21 mg QE/g across hexane, ethyl acetate, butanol, and aqueous fractions, while a methanolic crude extract showed TPC 41.17 ± 2.38 mg GAE/g and TFC 26.52 ± 0.61 mg QE/g (Jiangseubchatveera et al., 2024; Makkiyah, et al 2024; Priyanto, et al 2024b). These data support the presence of moderate polyphenolic content under common extraction conditions. *G. pictum* is also known to contain flavonoids, alkaloids, and other bioactive molecules associated with wound healing, anti-inflammatory, and antimicrobial properties (Priyanto et al., 2024b). Although its phytochemical levels are lower, these compounds may still play a role in biological activity, particularly in combination with other plant extracts.

Antioxidant activity of *B. balsamifera* and *G. pictum* leaf extracts

Given the differences in total flavonoid, tannin, and phenolic contents between the two extracts, further evaluation was carried out to determine whether these phytochemical profiles translate into functional antioxidant capacity. The antioxidant activity of the ethanol extracts was assessed using the DPPH radical scavenging assay. Both *B. balsamifera* and *G. pictum* exhibited strong antioxidant activity, with IC_{50} values of 73.73 ± 10.49 ppm and 97.44 ± 1.68 ppm, respectively (Table 2). IC_{50} values between 50-100 ppm are categorized as strong antioxidant activity (Wijaya, Surya, Indraningrat, & Udiyani, 2025), confirming the potency of both extracts.

Table 2. DPPH radical scavenging activity of *B. balsamifera* and *G. pictum* leaf extracts.

Extracts	IC50 (ppm)				Category
	I	II	III	Average	
B. balsamifera	85.13	64.47	71.60	73.73 ± 10.49	Strong
G. pictum	98.16	95.52	98.64	97.44 ± 1.68	Strong

The stronger antioxidant activity of *B. balsamifera* is consistent with its higher content of flavonoids, phenolics, and tannins. These compounds exert antioxidant effects primarily through hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms. Phenolics and flavonoids can donate hydrogen atoms or electrons to neutralize free radicals, while tannins can chelate metal ions and stabilize radical intermediates, enhancing the overall radical scavenging effect (Parcheta et al., 2021; Tumilaar, et al 2024; Zhang, 2022).

Although *G. pictum* exhibited lower levels, it still demonstrated strong antioxidant capacity, possibly due to other active compounds such as alkaloids or saponins that were not quantified in this study. The relatively low standard deviation in *G. pictum* indicates consistent activity across replicates, while the slightly higher variability observed in *B. balsamifera* may reflect natural differences in extract composition.

The results obtained in this study are in line with previous reports. Putra et al. (2021) reported strong antioxidant activity for 70% ethanolic extracts of *B. balsamifera* with an IC_{50} of 32.02 ± 0.09 µg/mL, while Priyanto et al. (2024) observed an IC_{50} of 70.18 ± 3.27 µg/mL for *G. pictum* methanol extracts (Priyanto et al., 2024b; Putra, et al 2022). Differences in absolute IC_{50} values may reflect variations in extraction solvents and methodologies; however, the overall trend confirms that both species are potent sources of natural antioxidants. In addition to their antioxidant potential, flavonoids, phenolics, and tannins are also known to possess antimicrobial properties, which prompted further investigation into the antibacterial activity of both extracts, individually and in combination, against *S. aureus*.

Antibacterial activity of *B. balsamifera* and *G. pictum* leaf extracts

Building on the phytochemical and antioxidant findings, the antibacterial potential of *B. balsamifera* and *G. pictum* was further evaluated to explore the broader biological activities of these plants. Flavonoids, tannins, and phenolics have been shown to exhibit antimicrobial effects through multiple mechanisms, including disruption of bacterial cell membranes, interference with nucleic acid synthesis, inhibition of enzyme activity, and suppression of biofilm formation. Considering these properties, the extracts were tested against *Staphylococcus aureus*, a clinically relevant Gram-positive bacterium commonly associated with skin and

soft tissue infections. The antibacterial activity of the extracts was assessed individually and in combination using the Kirby–Bauer disc diffusion method to identify potential synergistic effects.

Antibacterial activity of individual extracts

The antibacterial activity of each extract was evaluated against *S. aureus*. *B. balsamifera* leaf extract demonstrated a moderate inhibition zone of 9.83 ± 0.25 mm, while *G. pictum* exhibited no detectable antibacterial activity (Table 3). The positive control (streptomycin) showed an inhibition zone of 12.7 ± 0.14 mm.

Table 3. Result of Kirby-Bauer Test of *B. balsamifera* and *G. pictum* leaves extract.

Extracts	Zone of Inhibition (mm±SD)				Category
	I	II	III	Average	
B. balsamifera	10,25	9,75	9,50	9,83 ± 0,25	Medium
G. pictum	0	0	0	0	-
Positive Control	12,5	12,75	12,75	12,7 ± 0,14	Strong

The moderate activity of *B. balsamifera* may be attributed to its high content of flavonoids, tannins, and phenolics. Flavonoids have been reported to inhibit bacterial nucleic acid synthesis, particularly RNA synthesis in *S. aureus* (Shamsudin et al., 2022). Phenolic compounds can disrupt bacterial cell membranes and interfere with virulence factors (Miklasińska-Majdanik, et al 2018), while tannins can precipitate bacterial proteins, inhibit enzymes, and destabilize membranes, leading to growth inhibition (Huang et al., 2024). In contrast, the lower phytochemical content of *G. pictum* may explain its lack of observable antibacterial effect in this assay.

Antibacterial activity of combination extracts

The combination of *B. balsamifera* and *G. pictum* extracts exhibited greater antibacterial activity against *S. aureus* compared to individual extracts (Table 4). The 1:1 ratio produced the largest inhibition zone (16.3 ± 0.23 mm), followed by 1:3 (14.2 ± 0.23 mm) and 3:1 (12.3 ± 0.47 mm). Although the activity did not surpass that of streptomycin, these results indicate a synergistic or additive effect between the two extracts.

Table 4. Antibacterial activity of *B. balsamifera* and *G. pictum* leaves combination extract.

Concentration Ratio (*B. balsamifera* : *G. pictum*)	Zone of Inhibition (mm±SD)				Category
	I	II	III	Average	
1 : 3	14,5	14	14	14,2±0,23	Strong
1 : 1	16,5	16,5	16	16,3±0,23	Strong
3 : 1	12	13	12	12,3±0,47	Strong
Negative Control (Ethanol)	0	0	0	0	-
Positive Control (Streptomycin)	12,5	12,75	12,75	12,7 ± 0,14	Strong

The observed enhancement may be explained by complementary or cumulative actions of phytochemicals from both plants. Flavonoids, phenolics, and tannins can act on multiple bacterial targets, including cell wall integrity, energy metabolism, and biofilm formation, thereby enhancing antibacterial efficacy (Kanedi, et al 2021). This broader mechanism of action could explain the greater inhibition observed in the combination compared to the individual extracts.

Interpretation and Implications

Overall, the findings demonstrate that *B. balsamifera* possesses higher levels of flavonoids, tannins, and phenolics, which correlate with stronger antioxidant and moderate antibacterial activities. *G. pictum*, although lower in phytochemical content, still exhibits strong antioxidant activity, and when combined with *B. balsamifera*, contributes to enhanced antibacterial effects. The synergistic activity observed in combination likely results from multiple interactions among phenolics, flavonoids, and tannins acting on different bacterial targets, thereby increasing overall efficacy. These results align with previous findings suggesting that combining plant extracts can enhance biological activity through multi-target mechanisms (Kanedi et al., 2021; Miklasińska-Majdanik et al., 2018; Shamsudin et al., 2022).

CONCLUSIONS

This study demonstrated that *Blumea balsamifera* and *Graptophyllum pictum* leaf extracts contain high levels of flavonoids, tannins, and phenolic compounds, which

contribute to their biological activities. Both extracts showed strong antioxidant activity, with *B. balsamifera* displaying higher potency, likely due to its higher phytochemical content. While *G. pictum* alone did not exhibit antibacterial activity against *Staphylococcus aureus*, its combination with *B. balsamifera* produced larger inhibition zones than either extract alone, indicating a synergistic or additive interaction. Tannins, together with phenolics and flavonoids, likely play a central role in both antioxidant and antibacterial mechanisms through hydrogen atom transfer, metal chelation, protein precipitation, and membrane disruption. These findings highlight the potential of *B. balsamifera* and *G. pictum*, particularly in combination, as promising natural sources of multifunctional therapeutic agents. Further studies focusing on compound isolation, mechanistic elucidation, and formulation development are warranted to support their application in pharmaceutical or cosmeceutical products.

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Competing Interests: he authors declare that there are no competing interests.

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