

Antimicrobial and Antioxidant Activities of *Phanera aureifolia* (K.Larsen & S.S.Larsen) Bandyop., P.P.Ghoshal & M.K.Pathak Leaf Ethanolic Extracts

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History

- Submission Date: 06-05-2025;
- Review completed: 11-06-2025;
- Accepted Date: 03-06-2025.

DOI : 10.5530/pj.2025.17.45

Article Available online

<http://www.phcogj.com/v17/i3>

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ABSTRACT

Introduction: Natural antioxidants and antimicrobial agents are abundantly found in medicinal plants.

Objective: This study evaluated the antimicrobial and antioxidant activities of two colors from *Phanera aureifolia* (K.Larsen & S.S.Larsen) Bandyop., P.P.Ghoshal & M.K.Pathak leaf ethanolic extracts including gold and green leaves. **Method:** To assess antimicrobial efficacy, the agar well diffusion technique was analyzed. Scanning electron microscopy (SEM) was utilized to investigate the ultrastructural alterations in MRSA caused by leaf extracts. DPPH assay was used to assess antioxidant activity. The total amounts of phenolic compounds and flavonoids were evaluated through the Folin-Ciocalteu and aluminum chloride colorimetric techniques, respectively. The statistical analysis using an independent t-test. **Results:** Both extracts demonstrated activity against *Pseudomonas aeruginosa* TISTR146, *Micrococcus luteus* TISTR884, *Staphylococcus aureus* TISTR517 and Methicillin-resistant *S. aureus* 142 (MRSA142). Their activity against *M. luteus* TISTR884 was the highest observed. The MIC of both extracts against MRSA were 10 mg/ml. SEM analysis revealed that the cells exhibited noticeable enlargement and swelling compared to untreated MRSA cells. Extract from the green leaves of Gold leaf Bauhinia showed greater radical scavenging activity, higher total phenolic and flavonoid contents, compared to those from the golden leaves. **Conclusions:** The findings show that the extract from the green leaves of Gold leaf Bauhinia possess higher antioxidant potential compared to those from the golden leaves. In addition, both extracts exhibited antimicrobial activity, especially against MRSA.

Key words: *Phanera aureifolia* (K.Larsen & S.S.Larsen) Bandyop., P.P.Ghoshal & M.K.Pathak, Antioxidant, Antimicrobial Activity, Phenolic compound, Flavonoid compound.

INTRODUCTION

Microbial infections are a significant contributor to illness and mortality worldwide. While antibiotics were initially developed to reduce infection rates and associated mortality¹, their excessive use without prescriptions and incorrect prescribing have facilitated the development of antibiotic-resistant strains.² Antimicrobial resistance is not only a problem in developing countries but is a global problem resulting in increasing the expense of healthcare, hospital stay time, morbidity, and mortality.³⁻⁴ The inappropriate use of antibiotics in medicine and agriculture is the main cause of this.⁵ Therefore, medicinal plant extracts are an alternative treatment for infectious diseases to reduce the problem of antimicrobial resistance⁶ and reduce the adverse effects of antimicrobial agents.

Medicinal plants are a source of phytochemicals that are important and have many biological activities for example flavonoids and various phenolic compounds have been demonstrated significant, anticancer agents, antibacterial agents, cardioprotective agents, anti-inflammatories, immune health promoting, protective skin care against UV, and intriguing candidates for medical use.⁷⁻⁸ Therefore, it is interesting that extracts from medicinal plants and herbs have been researched for use in the treatment of diseases or the creation of dietary supplements.

Gold leaf Bauhinia (*Phanera aureifolia* (K.Larsen & S.S.Larsen) Bandyop., P.P.Ghoshal & M.K.Pathak), also known as Yan Da Oh is an ivy plant that is part of the Fabaceae family. It is often found growing in moist evergreen forests in the open areas along streams of Pattani, Yala, and Narathiwat provinces, Thailand. It was first discovered at Pajo Waterfall, Bacho district, Narathiwat province. It is a type of vine plant. The leaves are single, alternately arranged leaves. It appears to be quite round. The leaf tip is deeply concave into two lobes, while the base is concave and heart-shaped. The surface of the leaves is soft and has a velvety texture with golden hairs. There are two types of leaves, the green leaves group that performs photosynthesis to create food, and a group of gold leaves. The fruit looks like a sheath like a sword. The seeds appear to be flat. According to research, Gold leaf Bauhinia contain phenol compounds that crude extract with golden leaf has antioxidant properties 70.310% of DPPH free radicals. It was also discovered that the crude extract from golden leaf could against gram-negative organisms, including *E. coli*.⁹

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid, gallic acid, rutin and dimethyl sulfoxide (DMSO) were

Cite this article: Piboonpol G, Somsap O, Panthong W, Sujiwattanasat P, Kamnate A. Antimicrobial and Antioxidant Activities of *Phanera aureifolia* (K.Larsen & S.S.Larsen) Bandyop., P.P.Ghoshal & M.K.Pathak Leaf Ethanolic Extracts. Pharmacogn J. 2025;17(3): 365-369.

purchased from Sigma-Aldrich (Germany). Methanol and ethanol were purchased from Merck (Germany). Luria-Bertani agar was purchased from Difco (USA). All chemicals and reagents were of analytical grade.

Sample preparation and extraction

Two colors of Gold leaf Bauhinia—gold and green leaves—were obtained from Muang Narathiwat District, Narathiwat Province, Thailand (PSU herbarium number 0020232). The leaves were thoroughly washed, separated, air-dried for a day, and then subjected to three days of drying in an oven at 40 °C. The leaves were chopped into pieces and extracted with 95% ethanol over three days, with the maceration procedure repeated twice. The extracts were then filtered through cheesecloth and Whatman No. 1 paper. The filtered solution was evaporated using a rotary evaporator and allowed to cool for three days at room temperature. The crude extracts of golden and green leaves were brown and kept at room temperature for further analysis. The filtrate was concentrated by evaporation with a rotary evaporator.

Antibacterial activities test

The antibacterial activity of golden and green leaves of Gold leaf Bauhinia ethanolic extracts was assessed using the agar well diffusion method, as described by On-Anong *et al.* 2024 and On-Anong *et al.* 2025.¹⁰⁻¹¹ The test involved seven indicator strains of bacteria, categorized into four strains of gram-positive bacteria: *Bacillus cereus* ATCC 11778, MRSA142, *Micrococcus luteus* TISTR884, *Staphylococcus aureus* TISTR517 and three strains of gram-negative bacteria: *Escherichia coli* ESB182, *Pseudomonas aeruginosa* TISTR1467, *Salmonella typhimurium* TISTR292. All bacterial strains tested were cultured on Luria-Bertani agar (LB agar) and incubated at 37 °C for 18 hours. A single isolated pure colony of each bacterial strain under investigation was inoculated into LB broth and subsequently incubated at 37 °C for 18 hours. Then, each inoculated broth strain was prepared to McFarland No.5. in 0.85% normal saline. After that, each indicator strain was swabbed on an LB agar plate. Subsequently, the wells are made using a 6 mm diameter sterile tip. Next, 100 µl of crude extract from golden leaves at a concentration of 300 mg/ml was added to the well. Gentamicin (10 µg/ml) was used as the positive control, with DMSO as the negative. After 5 hours at room temperature, the plates were placed in an incubator at 35 °C for 18 to 24 hours. Vernier caliper was used to measure the zone of inhibition in millimeters. The experiment was done three times.

Minimum inhibitory concentration (MIC) test

The inhibition zone was assessed using the agar well diffusion technique, as previously described in the antibacterial activity test. The concentration of the crude extract ranged from 10 mg/ml to 0.3125 mg/ml, prepared by the two-fold dilution method. One hundred microliters of each concentration were added to the wells, and the plates were left at room temperature for 5 hours. Afterward, the plates were placed in an incubator at 35 °C for 18 to 24 hours. The diameter of the inhibition zones was then measured using a vernier caliper. The experiment was conducted in triplicate. The MIC value was defined as the minimum concentration of extracts that displayed an inhibition zone.

Scanning electron microscope (SEM) analysis

The experiment was carried out following the method described by On-Anong *et al.* 2024; On-Anong *et al.* 2025¹⁰⁻¹¹, the treated cells of indicator strains were prepared. The cells were scraped and rinsed two times using 0.15M phosphate buffer adjusted to pH 7.2, then fixed with 2.5% glutaraldehyde for 1-2 h. After washing twice with distilled water and phosphate buffer, the cells were dehydrated using acetone concentrations from 5% to 100%. Critical Point Drying was used to dry the cells, which were then mounted on stubs with carbon tape and paint, coated with gold using a sputter coater, and monitored with SEM.

Total phenolic content

Total phenolic contents (TPC) in the crude extracts were measured using the Folin–Ciocalteu reagent with some alterations according to woisky *et al.*¹² All extracts were formulated to a concentration of 100 mg/mL by mixing 20 µL of each extract with 50 µL of Folin–Ciocalteu reagent, and then 100 µL of 20% sodium carbonate solution was added and mixed. The absorbance of the reaction mixture was recorded at 730 nm after an hour at room temperature. The total phenolic content was presented as milligrams of Gallic acid equivalents (GAE) per gram of dry weight.

Total flavonoid content

The total flavonoid content (TFC) was assessed following the methodology of Woisky *et al.*¹² with some modifications. Each extract was diluted to a concentration of 10 mg/mL by mixing 250 µL of extract with 1.5 mL of methanol, 2.8 ml of distill water, 100 ul of 10% AlCl₃ and 100 ul of 1M potassium acetate. Following a 30-minute incubation period at room temperature, The absorbance of the solution was recorded at a wavelength of 415 nm. The total flavonoid content was determined using rutin as the standard. The total flavonoid content value was presented as milligrams of rutin equivalents per gram of extract.

Antioxidant activity

The antioxidant activity of Gold leaf Bauhinia extracts was evaluated using DPPH free radical scavenging assays according to Murugan, Mishra & Paul¹³ with a slight modification. Each extract and L-ascorbic acid standard were dissolved in appropriate solvents to prepare stock solutions, which were then diluted with methanol to various concentrations. Each extract was prepared at a defined concentration, and 1 mL of the extract was mixed with 1 mL of DPPH solvent in methanol. After being kept in the dark for 30 minutes at room temperature, the mixture's absorbance was recorded at 517 nm. The percentage of DPPH inhibition was evaluated relative to the control sample, according to the equation: % DPPH inhibition = (Ac - As) / As × 100, that Ac was the absorbance of the control while As was the absorbance of the samples. The results for the inhibition of the free radical DPPH are shown as IC₅₀ values, which indicate the concentration required to decrease free radical levels by 50%. IC₅₀ was calculated by graphing the inhibition percentages versus sample concentrations. All tests were carried out in triplicate.

Statistical analysis

The statistical analysis was determined using IBM SPSS Statistics Standard Version 29.0.1.0. Data are presented as mean ± SD. To evaluate antioxidant activities, independent T-test (P < 0.05) was used to compare the differences in IC₅₀, TPC, and TFC for each extract.

RESULTS AND DISCUSSION

Antibacterial activities test

The results of the antibacterial activity assessment of the green and golden leaves of Gold leaf Bauhinia crude extracts by the agar well diffusion method are presented in Table 1. It was observed that the green and golden leaves of Gold leaf Bauhinia ethanolic crude extracts at a concentration of 300 mg/ml can inhibit gram-positive bacteria, including MRSA142, *M. luteus* TISTR884, *S. aureus* TISTR517 and gram-negative bacteria, such as *P. aeruginosa* TISTR1467. The ethanolic crude extracts from green and golden leaves were able to inhibit *M. luteus* TISTR884 with the highest inhibitory zone 23.7 ± 0.060 and 23.0 ± 0.001 mm, respectively. For the inhibition zone of green leaves extract against MRSA142, *S. aureus* TISTR517 and *P. aeruginosa*

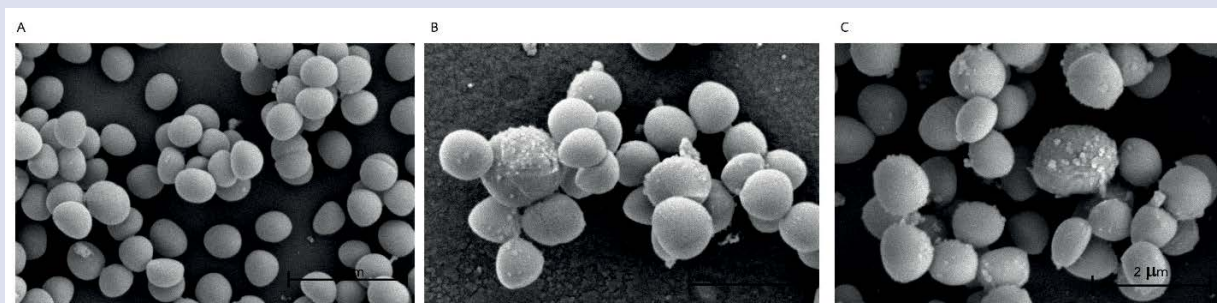


Figure 1: Scanning electron micrographs of untreated MRSA142 (A) and treated MRSA142 with green leaves (B) and golden leaves of Gold leaf Bauhinia (C). Magnification of x 30000.

Table 1: Antimicrobial activities of ethanolic extract from green and golden leaves of Gold leaf Bauhinia.

Bacteria	Inhibition zone (mm)			
	green leaves	golden leaves	gentamicin (10 µg/ml)	10 % DMSO
<i>B. cereus</i> ATCC 11778	NZ	NZ	16.0±0.001	NZ
<i>E. coli</i> ESBL182	NZ	NZ	16.0±0.001	NZ
<i>M.luteus</i> TISTR884	23.7±0.060	23.0±0.001	29.0±0.001	NZ
MRSA142	21.0±0.001	19.3±0.060	NZ	NZ
<i>S. aureus</i> TISTR517	20.0±0.001	19.3±0.060	23.0±0.001	NZ
<i>S. typhimurium</i> TISTR292	NZ	NZ	25.0±0.001	NZ
<i>P. aeruginosa</i> TISTR1467	19.0±0.001	17.7±0.060	23.0±0.001	NZ

Zone of growth inhibition values are presented as mean ± SD (n = 3), NZ: no inhibition zone

Table 2: Minimum inhibitory concentration (MIC) of the ethanolic extract from green and golden leaves of Gold leaf Bauhinia.

Bacteria	MIC (mg/ml)	
	green leaves	golden leaves
<i>M.luteus</i> TISTR884	2.5	5
MRSA142	10	10
<i>S. aureus</i> TISTR517	5	5
<i>P. aeruginosa</i> TISTR1467	2.5	2.5

Table 3: Antioxidant activities, TPC and TFC of Gold leaf Bauhinia.

Extract	IC ₅₀ (µg/mL)	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
green leaves	10.51±0.2 ^a	469.99 ± 26.08 ^a	204.84 ± 15.82 ^a
golden leaves	14.77±0.19 ^b	272.90 ± 24.74 ^b	157.29 ± 3.46 ^b

TISTR1467 were 21.0 ± 0.001 mm. and 20.0 ± 0.001 mm. and 19.0 ± 0.001 mm., respectively. The golden leaves extract exhibited inhibition zones of 19.3 ± 0.060 mm against MRSA142 and *S. aureus* TISTR517, and 17.7 ± 0.060 mm against *P. aeruginosa* TISTR1467. The green and golden leaves extracts of Gold leaf Bauhinia showed no inhibitory activity against the growth of *B. cereus* ATCC 11778, *E. coli* ESBL182 and *S. typhimurium* TISTR292 bacteria. From these results, Minimum inhibitory concentration (MIC) values were assessed through the agar well diffusion method. The data demonstrated that the lowest MIC for *M.luteus* TISTR884 and *P. aeruginosa* TISTR1467 was 2.5 mg/mL. The MIC for *S. aureus* TISTR517 and MRSA142 were 5 mg/mL. and 10 mg/mL., as presented in Table 2. This result is similar to the findings reported on the biological properties of an aqueous crude extract of gold leaf Bauhinia, which inhibits gram-positive bacteria, such as *S. aureus*, and gram-negative bacteria, such as *E. coli*.⁹ Additionally, several research studies on the *Phanera* genus have demonstrated notable antimicrobial properties, such as *Phanera vahlii*, which demonstrated bacterial

inhibition activity against pathogens, such as *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *K. pneumoniae*, and *B. subtilis*.¹⁴⁻¹⁵

Scanning electron microscope (SEM) analysis

The ethanolic extract of green and golden leaves of Gold leaf Bauhinia on MRSA, Gram-positive bacterial strains were investigated using scanning electron microscopy (SEM) to examine morphological changes of the bacterial cells with swelling. The study's findings, depicted in figure 1, reveal the untreated MRSA normal cell shape in figure 1 A. When subjected to green leaves and golden leaves extract in figure 1 B and figure C, it was observed that the cell structure appeared morphology changing in comparison to untreated cells. The cells manifested signs of enlargement and swelling. In contrast to the control, which bore a uniform surface, the cell surface exhibited a coarse texture. This result revealed that the bacterial cell walls of MRSA142 were damaged by the green and golden leaves extracts. According to Zeng *et al.*, the aqueous extract of *Polygonum chinense* L. showed antimicrobial effects by causing strong damage to the bacterial cell walls and extracellular solutes existed¹⁶. Moreover, the autolysis of bacterial cells was demonstrated in treated cells.

Antioxidant activities

The antioxidant activities of the crude extract of Gold leaf Bauhinia was presented in table 3. DPPH free radicals and vitamin C were used to evaluate the antioxidant capacity of Gold leaf Bauhinia, owing to their notable ability to scavenge free radicals. Antioxidant activity in the DPPH radical scavenging test was evaluated by monitoring the reduction in absorbance resulting from the DPPH radical's acceptance of an electron or hydrogen radical from the antioxidant resulting in the formation of a stable diamagnetic molecule.¹⁷ The antioxidant activity was quantified as IC₅₀, represents the concentration of the antioxidant needed to decrease 50% of the initial DPPH radicals. The findings indicated that the ethanolic extract from the green leaves of Gold leaf

Bauhinia exhibited greater radical scavenging activity, with an IC_{50} of $10.51 \pm 0.2 \mu\text{g/mL}$, compared to $14.76 \pm \mu\text{g/mL}$ for golden leaves. The IC_{50} value for ascorbic acid, used as a positive control, was $5.57 \pm 0.17 \mu\text{g/mL}$.

Total phenolic content (TPC), and total flavonoid content (TFC)

The extracts from the green and gold leaves of Gold leaf Bauhinia exhibited total phenolic contents of 469.99 ± 26.08 and 272.90 ± 24.74 mg gallic acid equivalent (GAE) per gram of fresh weight, respectively. Similarly, the total flavonoid contents were 204.84 ± 15.82 and 157.29 ± 3.46 mg quercetin per gram of fresh weight, respectively, as shown in table 3. This was consistent with previous reports that the green leaves of Gold leaf Bauhinia exhibited greater radical scavenging activity and higher total phenolic content compared to the golden leaves.⁹ In addition, several studies on species within the *Phanera* genus and relate species have revealed significant pharmacological properties, including antioxidant activity.¹⁸⁻²¹ The antioxidant potential of a substance is significantly influenced by its content of polyphenols, including flavonoids and other phenolic compounds.²² According to the results of this study, the green leaves extracts contained greater amounts of total phenolics and flavonoids compared to the golden leaves extracts, which could contribute to its enhanced antioxidant properties.

CONCLUSION

This study indicated that ethanolic crude extracts from the green and golden leaves of Gold leaf Bauhinia exhibit significant antimicrobial and antioxidant properties. Both extracts show antibacterial effects against various gram-negative and gram-positive bacteria, including antibiotic-resistant strains. These findings highlight the potential for developing Gold leaf Bauhinia-derived value-added products as raw materials for use in medical treatments. Future studies will aim to isolate the bioactive compounds in this plant responsible for its therapeutic properties.

ACKNOWLEDGEMENT

The authors wish to acknowledge the Faculty of Medicine at Princess of Naradhiwas University for providing research funding. The authors also extend their thanks to Assoc. Prof. Dr. Monthon Lertcanawanichakul from the School of Allied Health Sciences at Walailak University for providing bacterial test strains.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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Cite this article: Piboonpol G, Somsap O, Panthong W, Sujiwattanasat P, Kamnate A. Antimicrobial and Antioxidant Activities of *Phanera aureifolia* (K.Larsen & S.S.Larsen) Bandyop., P.P.Ghoshal & M.K.Pathak Leaf Ethanolic Extracts. *Pharmacogn J.* 2025;17(3): 365-369.