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Antibacterial, Antioxidant, and Sun Protection Activities of Methanolic Extract of *Bougainvillea glabra* Flowers

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ABSTRACT

Bougainvillea glabra is a popular ornamental plant in Indonesia that contains various phytochemicals that may exhibit antibacterial, antioxidant, and photoprotective activities. This study aimed to evaluate the antibacterial activity, antioxidant capacity, and SPF value of methanolic extracts of B. glabra flowers. Antibacterial activity was evaluated using the disc diffusion method, while antioxidant activity and SPF value were determined using UV-Vis spectrophotometry. The extract showed antibacterial activity against S. aureus, strong antioxidant activity (IC50 = 62.13 µg/mL), and an SPF value of 12.74. Methanolic extract of B. glabra flowers has potential as a natural antibacterial, antioxidant, and sunscreen agent.

Keywords: *Antibacterial, antioxidant, SPF, Bougainvillea glabra*

INTRODUCTION

As interest in natural, plant-based medicines for treatment grows, this includes the use of plants as sources of antibacterial and antioxidant properties. The *Bougainvillea glabra* flower, more commonly known as the paper flower, is one of the most ornamental plants found in Indonesia. It is prized for its beauty, with brightly colored petals that adorn gardens. The paper flower plant has several medicinal properties and benefits in traditional medicine, including wound healing, anti-inflammatory, analgesic, anti-diarrheal, anti-ulcer, antibacterial, and anti-diabetic properties (1,2).

The pharmacological activity of paper flowers is largely related to the chemical compounds they produce, which are believed to offer health benefits. Phytochemical screening revealed various compounds, including alkaloids, triterpenoids, tannins, flavonoids, saponins, phenolic compounds, glycosides, tannins, beta-carotene, and glucose (1,3). Paper flowers have the potential to serve as a source of active ingredients for the production of antibacterial and

antioxidant cosmetics. These cosmetics can be produced in various forms, such as creams, gels, and ointments. Formulating them as a gel generally yields more effective skin care results because the active ingredients interact with the skin for a longer period than when taken orally (4). Antioxidants are also necessary to neutralize free radicals, which can lead to cell damage, premature aging, and various degenerative diseases.

Excessive exposure to ultraviolet (UV) radiation can cause skin damage, including erythema, premature aging, and skin cancer. Consequently, developing natural ingredients for use as sunscreen agents with sun protection factor (SPF) has become a key area of research. *Bougainvillea glabra* contains phenolic compounds and flavonoids that are capable of absorbing UV radiation and protecting the skin from the harmful effects of sunlight. Previous research (5) has shown that an extract of Bougainvillea glabra flowers can be formulated into a sunscreen gel that offers moderate protection. The extract, which contains saponins, steroids, alkaloids, and polyphenols at a concentration of 1.5%, can be used as an ingredient in gels with a pH of 7.0–7.4 and stable viscosity. Mansur's study (6) developed a spectrophotometric method for determining the SPF value in vitro, and this method has been widely used in research on plant-based materials. However, studies on the SPF value of *Bougainvillea glabra* remain limited, and further research is therefore needed to investigate its photoprotective potential.

Based on various studies, *Bougainvillea glabra* has been shown to have potential as an antibacterial and antioxidant agent. To the best of our knowledge, no previous study has simultaneously evaluated antibacterial activity, antioxidant capacity, and SPF value of methanolic extracts of *Bougainvillea glabra* flowers. Therefore, this study provides a comprehensive evaluation of its bioactivity potential.

METHODS

Sampling

The instruments used were analytical scales (Aczet CY 301C), a sonicator, a UV-Vis spectrophotometer (Agilent 8453), a water bath, and Pyrex® glassware. The samples were paper flowers that grew in the Department of Pharmaceutical and Food Analysis at Poltekkes Kemenkes Jakarta II. The flowers were identified at the Botanical Characterization Laboratory of the Badan Riset dan Inovasi Nasional (BRIN). Purposive sampling was used to collect pink paper flowers between 9:00 a.m. and 10:00 a.m.

The following materials were used: methanol (Merck), DPPH (Sigma-Aldrich), vitamin C (Sigma-Aldrich), dimethyl sulfoxide (DMSO), distilled water, filter paper, Petri dishes, *Staphylococcus aureus* and *Escherichia coli* bacteria.

Procedures

A. Extraction

Bougainvillea glabra flowers were extracted once using the maceration method. A 200-gram sample of paper flowers was placed in a 500-milliliter beaker and mixed with 100 milliliters of methanol. The sample was sonicated for 30 minutes at room temperature. The sonication product was filtered using filter paper. The filtrate was transferred to an evaporating dish and evaporated at 50 °C for 12 hours to obtain a concentrated extract. The yield of the paper flower methanol extract can be calculated using the following formula [7]:

$$\text{Yield} = \frac{\text{Weight of extract obtained}}{\text{Weight of crude drug weighed}} \times 100\%$$

B. Antibacterial test

A 20% methanol extract solution of paper flower (*Bougainvillea glabra*) was prepared by dissolving 1 g of extract in 5 mL of a 10% dimethyl sulfoxide (DMSO) solution. To prepare a 10% extract concentration, 0.5 g of the extract was dissolved in 5 mL of the solution. To prepare a 5% extract concentration, 0.25 g of the extract was dissolved in 5 mL of the solution. DMSO was used as a control. This test used the disk diffusion method, often called the Kirby-Bauer test. Antimicrobial activity was tested against *Staphylococcus aureus* and *Escherichia coli* bacteria.

Blank discs were soaked in a test solution containing methanol extract from paper flowers (*Bougainvillea glabra*) at concentrations of 5%, 10%, and 20% for 30 minutes. Then, a 100 µL aliquot of the bacterial suspension diluted to 10^{-5} was pipetted into a Petri dish and spread in a clockwise direction. Using sterile forceps, the discs were removed from the test solution and placed on the medium containing the test bacteria and incubated at 37°C for 24 hours. The inhibition zones around the discs were then examined. Each sample concentration, positive control, and negative control was tested in triplicate.

C. Antioxidant test (DPPH)

Antioxidant activity was tested using the free radical scavenging method with DPPH. The DPPH solution was prepared by mixing 5 mg of DPPH and 100 mL of methanol in a volumetric flask.

The methanol extract of paper flowers was diluted to 1,000 µg/mL (ppm) to serve as a stock solution. Test solutions were prepared from the stock solution using methanol at concentrations of 100, 80, 60, 40, 20, and 10 µg/mL (ppm). Vitamin C was used as a reference solution. Ten milligrams of vitamin C powder were weighed into a 10 mL volumetric flask, and distilled water was added to the mark (1000 µg/mL concentration). This 1000 µg/mL vitamin C solution was then diluted to 100 µg/mL. The vitamin C concentration series used were 12, 10, 8, 6, 4, 2, and 1 ppm.

1 mL of each sample at various concentrations, and the positive control (Vitamin C) was reacted with one milliliter of DPPH solution in test tubes sealed with aluminum foil. The tubes were then allowed to stand for the optimal incubation time of 30 minutes. Then, the samples were measured using a UV-Vis spectrophotometer at a wavelength of 516 nm. Three replicates were performed.

The absorption results at those wavelengths were used to calculate the percentage of inhibition. This value was then substituted into a linear equation to obtain the inhibitory capacity. Then, the inhibition values were plotted against a linear regression equation to determine the 50% inhibitory concentration (IC_{50}), which is the sample concentration required to inhibit 50% of DPPH free radicals. The inhibition percentage of the methanol extract of paper flowers was calculated using the formula:

$$\%\ \text{Inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

The IC_{50} value can be obtained using a linear regression equation to calculate the percentage of inhibition against free radicals at the 50% inhibitory concentration, or by substituting y with 50. Given y = 50 and known values of a and b, these values are substituted into the equation y = bx + a to determine the IC_{50} value. The obtained IC_{50} results are then compared with Blois's criteria, which define a compound as having the following properties: very strong antioxidant (IC_{50} value less than 50), strong antioxidant (IC_{50} value of 50–100), moderate antioxidant (IC_{50} value of 100–150), or weak antioxidant (IC_{50} value of 151–200) [8].

D. Determination of SPF Value

The methanol extract of paper flowers, prepared at a concentration of 1000 ppm, was measured for absorbance at 5-nm intervals between 290 and 320 nm using a UV-Vis spectrophotometer. The SPF (sun protection factor) value was calculated using the Mansur equation [6] based on the sample absorbance data. Absorbance was measured at 290, 295, 300, 305, 310, 315, and 320 nm. Absorbance measurements were performed in triplicate. The SPF calculation formula based on Mansur's method is as follows:

$$\text{SPF}_{\text{in vitro}} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Keterangan:

CF = Correction Factor (10)
EE (λ) = Erythemal effect spectrum
I (λ) = Solar intensity spectrum
Abs (λ) = Spectrophotometer-measured absorbance of the sample

The solar radiation spectrum yields thermal efficiency (EE), where EE × I is a constant value that has been established. These values cover the wavelength range of 290–320 nm in 5-nm increments, as specified in reference [9] and shown in Table 1.

Table 1. EE × I at wavelengths of 290–320 nm

Abs (nm)	EE × I
290	0,015
295	0,0817
300	0,2784
305	0,3278
310	0,1864
315	0,0839
320	0,0180

Data Analysis

Data were analyzed descriptively and expressed as mean ± standard deviation. *Bougainvillea glabra* flowers were extracted once using the maceration method, yielding a methanol extract that was subsequently tested for antibacterial and antioxidant activity, as well as its SPF value. The antibacterial activity of the extract was determined using the disk diffusion method. Each measurement was performed in triplicate, and the results are expressed as the mean ± standard deviation. Antioxidant activity was tested using the DPPH method and performed in triplicate, with the results expressed as the mean IC_{50}. SPF values were determined using a UV-Vis spectrophotometer in triplicate and analyzed using the Mansur method.

RESULTS

The plant identification results indicate that the sample is *Bougainvillea glabra* Choisy, a species in the *Nyctaginaceae* family. Table 2 shows the results of the paper flower extraction of *B. glabra* using methanol, including the extract weight and yield percentage.

Table 2. The Yield Methanol Extract *Bougainvillea glabra* Choisy

Weight of Raw Material	Weight of Extract	Yield
200 g	2,6 g	1,3%

Tables 3 and 4 show the inhibition zone diameter measurements of the methanol extract of *Bougainvillea glabra* flowers against *Staphylococcus aureus* and *Escherichia coli*.

Table 3: Inhibition Zone Diameters of Methanol Extracts of *Bougainvillea glabra* Choisy Against *Staphylococcus aureus*

Sample	Replication			Average (mm) ± SD	Antibacterial activity
	I	II	III		
+ Control	24	26	23	24,3 ± 1,52	Very strong
- Control	0	0	0	0	-
5%	1	1	1	1 ± 0	Weak
10%	4	6	5	5 ± 1	Moderate
20%	8	7	9	8 ± 1	Moderate

Table 4. Inhibition Zone Diameters of Methanol Extracts of *Bougainvillea glabra* Choisy Against *Escherichia coli*

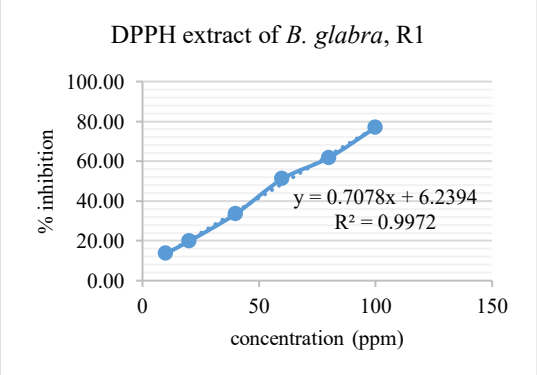
Sample	Replication			Average (mm) ± SD	Antibacterial activity
	I	II	III		
+ Control	27	29	26	27,3 ± 1,52	Very strong
- Control	0	0	0	0	-
5%	0	0	0	0	-
10%	0	0	0	0	-
20%	0	0	0	0	-

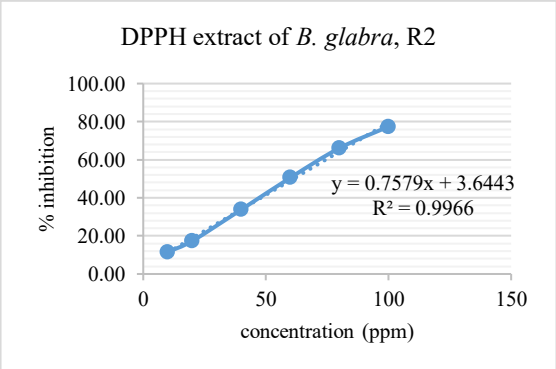
Table 5 shows the IC_{50} values from the DPPH assay of the methanol extract of *Bougainvillea glabra* flowers. Vitamin C was used as the positive control.

Table 5. Inhibition Concentration

Sample	Replication	IC_{50} (µg/mL)	Average (µg/mL)	Antioxidant activity
Methanol extract of *B. glabra*	1	61,82622	62,13	Strong
	2	61,16335		
	3	63,38649		
Vit C	1	6,3053	6,2611	Very strong
	2	6,3053		
	3	6,1727		

Figure 1 shows the IC_{50} curve for the DPPH assay of the methanol extract of *Bougainvillea glabra* flowers.





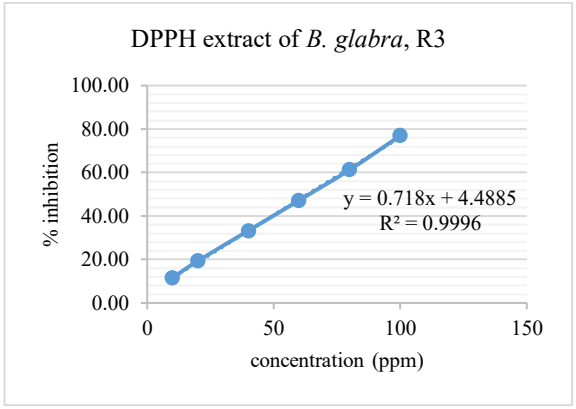


Figure 1. IC_{50} curve for the DPPH assay of the methanol extract of *B. glabra*; Replication 1, Replication 2, Replication 3

Figure 2 shows the IC_{50} curve for the DPPH assay of Vitamin C as the positive control.

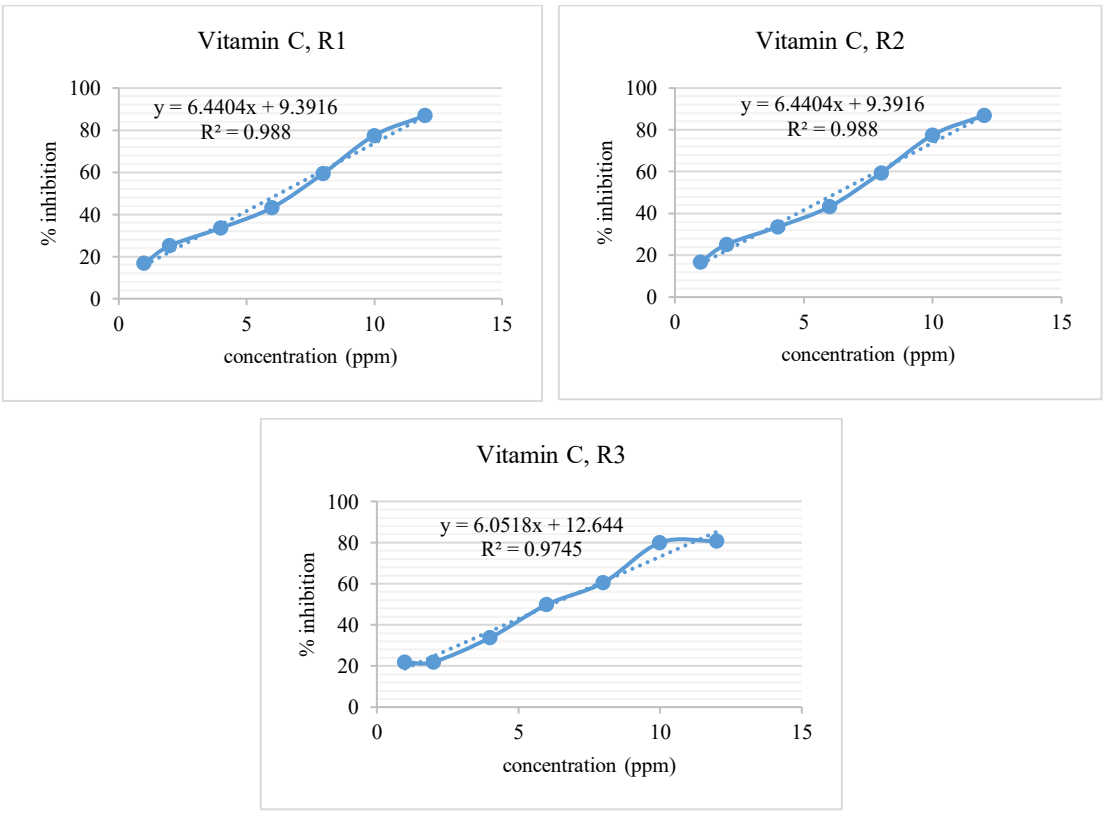


Figure 1. IC_{50} curve for the DPPH assay of Vitamin C; Replication 1, Replication 2, Replication 3

Table 6 shows the SPF values for the methanol extract of *Bougainvillea glabra* flowers.

Table 6. SPF values for the methanol extract of *B. glabra* flowers

Sample	Replication	SPF	Average	Protection Category
Methanol extract of *B. glabra*	1	12,72543	12,74032	Maximum
	2	12,77196		
	3	12,72358		

DISCUSSION

Identifying the paper flower plant confirms its identity and prevents errors in sample collection for research purposes. This identification process was conducted at the Botanical

Characterization Laboratory of Badan Riset dan Inovasi Nasional (BRIN). The results confirm that the sample is the *Bougainvillea glabra* species of the *Nyctaginaceae* family.

Paper flower samples were collected using purposive sampling. The pinkish-purple flowers used were fresh, free of defects or damage, and collected in the morning. Sampling was conducted in the morning to preserve the stability of bioactive compounds sensitive to heat and light. The temperature and humidity are generally more stable in the morning, and the concentration of secondary metabolites is highest at this time (10,11). *Bougainvillea glabra* flowers were then rinsed with water to remove dirt and dried.

The extraction process involves sonication, which breaks down cell walls, increases solvent penetration, and extracts active components, producing the extract (yield). The sonication method dissolves the sample in a solvent and subjects it to ultrasonic waves for a specific period of time. Then, the sample is filtered and concentrated. Advantages of the sonication method include faster processing, more efficient extraction, and improved solubility of compounds in the solvent, resulting in higher-quality extraction (12). *Bougainvillea* flower extract was prepared using methanol. Methanol is a universal solvent that can extract most polar and nonpolar compounds (13). Types of compounds that can be extracted by methanol include flavonoids, saponins, tannins, and terpenoids in plants. Extracting 200 g of *Bougainvillea glabra* flowers with methanol produced 2.6 g of concentrated extract, yielding 1.3%.

This study examined the antibacterial activity of *B. glabra* flower extract, using chloramphenicol as a positive control and 10% DMSO as a negative control, against the growth of *Staphylococcus aureus* and *Escherichia coli*. The concentrations used were 5%, 10%, and 20%. Chloramphenicol was used as the positive control because it is a broad-spectrum antibiotic effective against both Gram-positive and Gram-negative bacteria. It works by inhibiting bacterial protein synthesis (14). Previous studies (15) have shown that chloramphenicol is highly effective at inhibiting *E. coli*. The negative control used 10% DMSO. DMSO is a solvent capable of dissolving both polar and nonpolar compounds. Additionally, DMSO does not inhibit bacterial growth; therefore, it does not interfere with the results of antibacterial activity testing (16).

The measurements of the bacterial inhibition zones are presented in Tables 3 and 4. The results of the study indicate that the *B. glabra* flower extract exhibits antibacterial activity against *Staphylococcus aureus*, consistent with a previous study (17), but shows none against *Escherichia coli*. Antibacterial activity is classified into four categories: weak, moderate, strong, and very strong. According to Kumowal (18), antibacterial activity is classified as weak if the inhibition zone diameter is less than 5 mm, moderate if it is between 5 and 10 mm, strong if it is between 10 and 20 mm, and very strong if it is greater than 20 mm. *B. glabra* methanol extracts at 5% exhibited weak antibacterial activity, while at concentrations of 10% and 20%, they showed moderate antibacterial activity.

The selectivity of the methanol extract of *B. glabra* flowers against *S. aureus* compared to *E. coli* is due to the absence of an outer membrane in Gram-positive bacteria, allowing bioactive compounds such as flavonoids, tannins, and saponins to directly interact with the peptidoglycan cell wall and cytoplasmic membrane, causing structural and functional disruptions that ultimately lead to bacterial cell death. A phytochemical study conducted by Ornelas *et al.* (2023) (2) on methanol and dichloromethane extracts of *B. glabra* flowers revealed that of the 27 identified compounds, the majority were flavonoids and phenolic acids. Phenolic compounds are known to damage the cell membranes of Gram-positive bacteria, while gallic acid alters the hydrophobicity, charge, and permeability of the membrane. Saponins cause the release of proteins and enzymes, while alkaloids interfere with cell division.

Bougainvillea glabra flowers reportedly contain alkaloids, triterpenoids, tannins, flavonoids, saponins, and phenolic compounds (3). Antioxidant assay results indicate that B. glabra flower extract exhibits antioxidant activity with an IC50 value of 62.13 µg/mL, whereas the antioxidant activity of vitamin C, used as a reference standard, has an IC50 of 6.26 µg/mL. Antioxidant activity is classified into 4 groups based on the IC50 value: very strong antioxidant activity if the IC50 is less than 50; strong antioxidant activity for an IC50 of 50–100; moderate if the IC50 is 100–150; and weak if the IC50 is 151–200 (8). The lower the IC_{50} value, the stronger

the antioxidant activity. Based on these results, the methanol extract of B. glabra flowers exhibits strong antioxidant activity.

According to Saleem *et al.* (2019) (19), the methanol extract of *Bougainvillea glabra* flowers contains active flavonoid compounds. The antioxidant activity of the extract was tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which works on the principle that when a DPPH solution is mixed with a substance capable of donating a hydrogen atom or transferring an electron to the DPPH radical, the DPPH radical is neutralized, resulting in the reduced form of DPPH (non-radical). Free radical DPPH is neutralized, resulting in a reduced form of DPPH (non-radical). This mechanism occurs via the reaction: Fl–OH + DPPH → Fl–O + DPPH–H, where the hydroxyl group (–OH) in flavonoid compounds such as catechin, rutin, and gallic acid contained in the *B. glabra* flower extract acts as a hydrogen atom donor to the DPPH radical, converting it into a stable DPPH–H molecule. The resulting phenoxyl radical (Fl–O) is then stabilized through electron delocalization within the flavonoid's aromatic ring system, primarily via the catechol group (3',4'-dihydroxy) on ring B. As a result, the methanol extract of *B. glabra* flowers contains numerous active antioxidant compounds whose radical-scavenging capacity is comparable to the hydrogen-donating ability of the –OH groups of the flavonoids present.

Determining the SPF value indicates how long a material or product can protect against or block UV rays that cause sunburn. The SPF value is determined by measuring the absorbance of the methanol extract of *B. glabra* flowers using UV-visible spectrophotometry at wavelengths between 290 and 320 nanometers (nm), with measurements taken at 5 nm intervals. This wavelength range corresponds to the UV-B spectrum, consisting of rays capable of causing erythema. Based on Mansur's calculations, sunscreen protection levels are classified into five categories: minimal for values of 2–4; moderate for values of 4–6; higher moderate for values of 6–8; maximum for values of 8–15; and ultra for values greater than 15 [20]. Based on the data from this study, the SPF value of the methanol extract of *B. glabra* flowers is 12.74, which is in the maximum category.

Sunscreen is classified as protective if it contains an SPF of at least 2. It is considered high protection if the SPF is above 15, which categorizes it as ultra protection. An SPF above 15 provides better protection against long-term skin damage, such as skin cancer. Moreover, an SPF of 15 can protect the skin from sun exposure for about 4–5 hours, whereas an SPF of 10 only protects for approximately 1.5 hours (21) Based on these findings, the methanol extract of *B. glabra* flowers has potential as a sunscreen, due to its significant activity and sufficiently high SPF value.

Flavonoids and phenolic compounds in *B. glabra* flower extract can increase the SPF value because both possess chemical structures with conjugated double bonds and aromatic chromophore groups capable of directly absorbing UV radiation (22). Flavonoids demonstrate significant absorption capabilities in both the ultraviolet A (UVA) and ultraviolet B (UVB) regions due to the chemical structure of their conjugated double bonds, so they have the potential to be used as ingredients in cosmetic formulations for skin protection, and one of the primary mechanisms of action in their photoprotective potential is the absorption of UV light, which is directly related to the conjugated double bonds within the flavonoid molecule. Phenolic compounds, particularly flavonoids, are known for their ability to absorb UV radiation due to their conjugated double bond structure and aromatic chromophores, which help reduce UV penetration into the skin, thus protecting the skin from oxidative damage (23). Specifically in the UV-B region (290–320 nm), the energy of UV-B photons is absorbed by the delocalized π-electron system in the flavonoid's aromatic ring; this energy is then re-emitted as harmless heat or fluorescence, preventing the radiation from reaching deeper skin layers (24). Natural compounds are increasingly being studied as potential sources of sunscreen due to their UV-absorbing properties and antioxidant activity; consequently, high levels of phenolic compounds and flavonoids are positively correlated with higher SPF values (22). Thus, the higher the content of flavonoids and phenolic compounds in an extract, the more UV-B photons are absorbed before penetrating the skin, which directly increases the extract's SPF value.

CONCLUSION

Methanolic extract of *Bougainvillea glabra* flowers exhibited antibacterial activity against *Staphylococcus aureus*, strong antioxidant activity (IC50 = 62.13 µg/mL), and maximum sunscreen protection (SPF = 12.74). These findings indicate its potential as a natural pharmaceutical and cosmetic ingredient.

Further studies should investigate phytochemical profiling and formulation development of *B. glabra* extracts for pharmaceutical and cosmetic applications.

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