

Phytochemical Screening and Antibacterial Activity of Bougainvillea Glabra Plant Extract as Potential Sources of Antibacterial and Resistance-modifying Agents

Yolwin Jed Perales⁺, Merlyn Leysa

Sultan Kudarat State University, Philippines

Abstract. The crude methanol extract of different parts of *Bougainvillea glabra* were experimented and examined for its Phytochemical Constituents and Antibacterial Activity Index with four Commercial Antibiotics against 4 bacterial strains. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, reducing sugar and cardiac glycosides in the leaves of *Bougainvillea glabra*. Anthraquinones, flavonoids and terpenoids were absent in the leaves of *Bougainvillea glabra*. Different parts of *Bougainvillea glabra* have shown antibacterial potentials when used on two gram-positive bacteria, the *Bacillus Subtilis* and *Staphylococcus aureus*; and two gram-negative bacteria, the *Escherichia coli* and *Pseudomonas aeruginosa* except for flowers against two gram-positive bacteria, the *Bacillus Subtilis* and *Staphylococcus aureus*. Results show that this plant is an interesting source of antimicrobial agents, potential source of nutraceuticals, anti-inflammatory, antiallergenic, antithrombic, antiviral, analgesic, antimycotic, virostatic and other pharmacological importance.

Keywords: Bougainvillea glabra, Antibacterial Activity Index, Phytochemical Screening

1. Introduction

1.1. Background of the Study

Man in solving its numerous medical challenges for ages depends on his immediate environment taking advantages of nature's provisions of its beauty for life and survival. They learned to depend on plants and in some cases animals in providing solutions to the myriad of their health problems. Aside from these, there has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like medicinal plants [2] (Antessa 2008).

According to a research, there are 422 127 plant species growing on earth; among them about 35 000 to 70 000 plants are used as medicinal plants [4] (Fabricant, 2001). *Bougainvillea glabra* is a common flower planted everywhere in the Philippines, it is used in landscaping and beautification of Filipino houses. Since the ancient times, it is not known for any medicinal uses in the country. The people are left clueless about the wonders of healing this flower could bring to them.

In this study, the phytochemical screening and antibacterial activity index of the methanolic extract of Bougainvillea leaves, stem, flowers and roots were investigated to prove the potential medicinal use of the plant.

1.2. Objectives of the Study

⁺ Corresponding author. Tel.: 0642005816; fax: 0642004261.
E-mail address: yolwin_yjp@yahoo.com

- To identify the phytochemical constituents (alkaloids, flavonoids, tannins, saponins, reducing sugar, terpenoids, cardiac glycosides and anthraquinones) of *Bougainvillea glabra* ethanolic leaf extract.
- To determine the antibacterial activity index of *Bougainvillea glabra* leaves, stem, flowers and roots ethanolic extracts on the two gram-positive bacteria, the *Bacillus subtilis* and *Staphylococcus aureus*, and two gram-negative bacteria the *Escherichia coli* and *Pseudomonas aeruginosa*.
- To determine which part of *Bougainvillea glabra* is most effective against two gram-positive and two gram-negative bacteria.

1.3. Significance of the Study

Plants are important to lives of people throughout the world. We depend upon plants to satisfy some of our basic human needs as food, clothing, shelter, and health care. Increasing attention has also been given to the role of plants in disease management and alternative to synthetic medicines.

Different pathogens and bacteria are now immune to a wide range of synthetic medicines, thus alternative medicines from plants are in the greatest concern to replace it. The investigation stresses the possibility of extracting a new, cheaper and readily available herbal medicines form *Bougainvillea glabra* which is not ever known for its any pharmacological importance in the country, therefore provide great savings on the part of Filipinos mostly in poverty line. Similarly, it could provide a potential source of income when large scale production is feasible.

1.4. Scope and Limitations

This investigation was done to identify the phytochemical constituents namely alkaloids, flavonoids, tannins, saponins, reducing sugar, terpenoids, cardiac glycosides and anthraquinones of the methanolic leaf extract of *Bougainvillea glabra*. Methanolic leaves, stems, flowers and roots extracts were used to determine the antibacterial activity index on four bacterial isolates obtained from Notre Dame of Marbel University Biology Laboratory Culture Collection, Koronadal City, Philippines. These include to gram-positive bacteria, the *Bacillus subtilis* and *Staphylococcus aureus*, and two gram-negative bacteria the *Escherichia coli* and *Pseudomonas aeruginosa*.

This study was conducted at Notre Dame of Marbel University Biology Laboratory and at Sultan Kudarat State University, Chemistry Laboratory on the Month of September 2010 to December 2012.

1.5. Hypothesis

Alternative: Different parts of *Bougainvillea glabra* methanolic extract are effective against 4 strains of bacteria.

2. Methodology

2.1. Procedure

2.1.1. Collection of Plant Materials

Bougainvillea glabra plants were collected at Barangay Poblacion, along National Highway, Esperanza, Province of Sultan Kudarat. Identification of the plant species was confirmed by Prof. Merlyn Leysa of Sultan Kudarat State University Scientific and Review Committee.

Healthy and mature plants were gathered and were separately placed in plastic bags. These were washed with running water to remove soil and other dirt that were present in the plants. The plants were then surface sterilized with one percent (1%) hypochlorite to kill pathogenic organisms.

2.1.2. Ethanolic Extraction

Mature and intact leaves, stems, flowers and roots of the plant were cleaned and one hundred grams (100g) of each part were weighed separately using the digital balance. These were then separately chopped into small pieces and were placed in conical flasks. Ninety-five percent (95%) ethanol was added to each bottle until the plant parts were completely submerged. The plant materials were soaked for 24 hours

Extraction was done by filtering the mixture through a Buchner funnel with gentle suction. Plant residue was then discarded properly. The filtrate was concentrated in a rotary evaporator at a temperature below 50°C. The concentrates of each plant part were stored in properly labeled vials, and refrigerated until use.

2.1.3. Methodology for the Determination of Phytochemical Constituents.

Phytochemical Screening was performed using standard procedure.

2.1.4. Antimicrobial Assay Media Preparation

Muller Hinton Agar (MHA) was used for the antimicrobial activity test. MHA was prepared by weighing 39 grams of the powdered agar into 1000 ml of distilled water in a clean conical flask. It was cooked in low flame and 20 ml of the medium was pour in test tubes and were autoclaved at 121⁰c, 15 psi pressure for 15 minutes.

2.1.4.1. Antimicrobial Assay

The antimicrobial activities of the extracts were determined by the Kirby-Bauer agar diffusion method according to NCCLS standards. Under aseptic technique, the surfaces of solidified MHA were inoculated with the bacterial culture.

Amikacin 30mcg, Cephalexin 30mcg, Oxacillin 1mcg, and Penicillin 10 U were used as positive controls and distilled water was used as negative control. The discs were spaced far enough to avoid reflection wave from the edges of the petridishes and overlapping rings of inhibition. Finally, the petridishes were incubated for 18 to 24 hours at 37⁰C. The diameter of zone of inhibition (mean of triplicates+SD) as indicated by clear area devoid of microbial growth was measured.

2.1.4.2. Determination of Activity Index

The activity index of the crude plant extract was calculated as:

Activity Index (A.I) = Mean zone of inhibition of the extract/

Zone of inhibition obtained by standard antibiotic drug

2.1.4.3. Statistical Evaluation

The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates+SD of three replicates.

3. Results and Discussions

3.1. Phytochemical Constituents

The phytochemical screening revealed the presence of alkaloids, saponins, tannins, reducing sugar and cardiac glycosides in the leaves of *Bougainvillea glabra*. Anthraquinones, flavonoids and terpenoids were absent in the leaves of *Bougainvillea glabra*.

Table 1: Phytochemical constituents of *Bougainvillea glabra* ethanolic leaf extract.

Phytochemical	Test	<i>Bougainvillea glabra</i>
Reducing Sugar	Fehling's test	Positive
Anthraquinones	Borntrager's test	Negative
Saponins	Froth test	Positive
Tannins	Ferric Chloride test	Positive
Flavonoids	Aluminum Test	Negative
Alkaloids	Mayer's reagent	Positive
	Dragendorff's reagent	Positive
Cardiac Glycosides	(Keller-Killiani test)	Positive

3.2. Antibacterial Assay

The ethanolic extracts of *Bougainvillea glabra* were subjected to antibacterial assay on two gram-positive bacteria, the *Bacillus Subtilis* and *Staphylococcus aureus*; and two gram-negative bacteria, the

Escherichia coli and *Pseudomonas aeruginosa*. Amikacin 30mcg, Cephalexin 30mcg, Oxacillin 1mcg, and Penicillin 10 U were used as positive controls and distilled water was used as negative control.

The results of antibacterial assay and antibacterial activity index are shown in table 2 and table 3.

Table 2: Zone of inhibition of the *Bougainvillea glabra* leaf, stem, flower, root extracts, antibiotic and distilled water against four strains of bacteria.

Test Discs	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Leaves	7.50±57	7.75±49	7.50±57	7.0±0
Stems	7.25±49	7.0±0	7.75±49	7.25±49
Flowers	6.0±0	6.0±0	7.0±0	7.0±0
Roots	7.25±49	7.25±49	7.0±0	7.25±49
Amikacin 30mcg	17.00	35.00	17.00	20.00
Cephalexin 30mcg	18.00	8.00	8.00	8.00
Oxacillin 1mcg	12.00	12.00	10.00	16.00
Penicillin 10U	8.00	16.00	9.00	8.00
Distilled Water	6.00	6.00	6.00	6.00

Different parts of *Bougainvillea glabra* was found to be effective against two gram-positive bacteria, the *Bacillus Subtilis* and *Staphylococcus aureus*; and two gram-negative bacteria, the *Escherichia coli* and *Pseudomonas aeruginos* except for flowers against two gram-positive bacteria, the *Bacillus Subtilis* and *Staphylococcus aureus*.

4. Conclusions

After a thorough analysis of the results of the investigation, the following conclusions are made:

- *Bougainvillea glabra* contains alkaloids, saponins, tannins, reducing sugar and cardiac glycosides of methanolic leaf extract. Anthraquinones, flavonoids and terpenoids were absent in the leaves of *Bougainvillea glabra*.
- Different parts of *Bougainvillea glabra* have antibacterial potential when used on two gram-positive bacteria, the *Bacillus Subtilis* and *Staphylococcus aureus*; and two gram-negative bacteria, the *Escherichia coli* and *Pseudomonas aeruginosa* except for flowers against two gram-positive bacteria, the *Bacillus Subtilis* and *Staphylococcus aureus*.
- The Antibacterial Activity Index of stems ranged from .20 (in *S. aurues* with Amikacin 30mcg) to .97 (*E. coli* with Cephalexin 30mcg). The Antibacterial Activity Index of flowers ranged from .17 (in *S. aurues* with Amikacin 30mcg) to .88 (*P.Aeruginosa* with Penicillin 10U). The Antibacterial Activity Index of flowers ranged from .17 (in *S. aurues* with Amikacin 30mcg) to .88 (*P.Aeruginosa* with Penicillin 10U). The Antibacterial Activity Index of roots ranged from .21 (in *S. aurues* with Amikacin 30mcg) to .91 (*B. subtilis* with Penicillin 10U, *S. aureus* with Cephalexin 30mcg and *P.Aeruginosa* with Cephalexin 30mcg).
- Leaves extract was found to be the most effective against *B. subtilis* and *S. aureus*, meanwhile, stem extract was the most effective against *E. coli* then Stems and Roots extracts were the most effective against *P. aeruginosa*.

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6. References

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