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Identification Flavonoid of *Bougainvillea spectabilis* flowers in Nasiriyah by HPLC and Detection Effect of Extract as Antibacterial

Salwa Lafta [1a*] and Alla N.AL- Waheeb[1]

[1]*Department of Biology, Collage of Science, University of Thi-Qar, Thi-Qar, 64001, Iraq*

[a*]Corresponding author: Salwa.daham@utq.edu.iq

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***Abstract*- Many burn wound infections are caused by bacteria such as *Staphylococcus aureus, Staphylococcus epidermis, Klebsiella pneumonia,* and *Pseudomonas aeruginosa,* which can be difficult to treat due to antibiotic resistance. As a result, researchers are exploring complementary therapies, such as medicinal plants. In this investigation, a methanolic extract of *Bougainvillea spectabilis* flowers was prepared using the maceration method at concentrations ranging from 12,5mg/ml to 200mg/ml. Testing was done on the extract separately for antibacterial properties against each of the four bacteria on the Mueller Hinton Agar medium. Results showed that the extract was particularly effective against Gram-positive bacteria, with the greatest effect seen in S. aureus (22.3 mm). The extract was also tested at varying concentrations of 200 %, 100%, 50%, 25%, and 12.5%. HPLC was used to do a qualitative examination of the chemical composition, indicating variety and richness in the chemical constituents in flavonols and phenolic acid. Based on these findings Bougainvillea spectabilis flowers show promise as a source of compounds for clinical usage.**

***Keywords*— Bougainvillea spectabilis, Antimicrobial Activity, HPLC.**

I. INTRODUCTION

In recent years, Because of the rise in the incidence and severity of viral and chronic illnesses, scientists are looking for novel therapeutic strategies [1]. Plants are a promising natural alternative to conventional drugs due to their high levels of Comprising secondary metabolites, including molecules with flavonoids and phenolic groups, which possess potent antioxidant properties [2-3]. The World Health Organization (WHO) has stated that 80% of the developing world still benefits from the use of traditional medicines derived from medicinal plants [4]. With the advancement of phytochemistry and pharmaceutical chemistry, the ability to use active ingredients derived from plants or their synthetic equivalents in medicine has improved [5]. Antibacterial treatments' loss of effectiveness is a significant public health concern, as only a few new classes of drugs are being released. Therefore, discovering new antimicrobial agents is a top priority that demands constant attention. A hybrid species of roses.*Bougainvillea spectabilis* (Family-Nyctaginaceae) is referred to as a Paper flower, native to tropical South America, it grows around the world. It is known as Booganbel in Hindi, Booganvel in Marathi, and Kagithala puvvu in Telugu in the Arab world it is named jahanamya, growing from 1-2 meters.With the advancement of phytochemistry and pharmaceutical chemistry, the ability to use active ingredients derived from plants or their synthetic equivalents in medicine has improved. Only four species (*B. buttiana, B. glabra, B.spectabilis*, and *B. peruviana*) are commercially exploited [6]. It is a rich source of macro and micronutrients that aid in the prevention of various diseases, including phenols, tannins, alkaloids, saponins, quinones, terpenoids, bougainvinones, pinitol, quercetagetin, quercetin terpinolene, glycosides, calcium, magnesium, manganese, potassium, iron, and sodium [7]. B. spectabilis has traditionally been used in various countries to treat a range of health problems. Different parts of this plant are used as an anti-inflammatory, antiulcer, anticancer, anti-microbial, antihyperlipidemic, antifertility, thrombolytic, and hepatoprotective agent. This plant is also used to treat lung pain, flu, bronchitis, coughing, and pertussis [6].

II. MATERIALS AND METHOD

Bougainvillea spectabilis flowers were carefully picked in October 2023 from the gardens. They were then thoroughly cleaned with tap water and distilled water and left to dry for three days at 30°C in the shade. The flowers were then brought to the laboratory and thoroughly cleaned of any dirt or dust, and they were left to dry for seven to fourteen days in the shade at room temperature. Lastly, the samples were classified by Dr. Alla Nasir-ALWaib in the renowned Plant Taxonomy Laboratory, which is housed in the Department of Biology at the College of Science.2-2.

A. Preparation of Methanolic extract

To get ready for the qualitative screening of chemical compounds, 250 milliliters of methanol and 20 grams of dried powder were combined and subjected to a Soxhlet continuous extraction process. The resulting solution was filtered through Whatman No. 13 filter paper, concentrated

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at 50°C under reduced pressure using a rotary evaporator and dried at 25°C.The resulting extract was collected in sterilized glass tubes, ready for use [8].

B. Separation of Flavonoid from Plant Extract

To prepare, take 100g of dried petals, finely ground plant material, and mix with petroleum ether. After filtering, dry the solution at room temperature. Next, 300ml of 80% methanol is added and filtered through a Buchner funnel. Concentrate the resulting solution to 10ml using a rotary evaporator then add 5ml of water and ethyl acetate, shake well, and remove the lower layer containing flavonoids. Confirm the presence of flavonoids with NaOH reagent and dry the filtrate on glass plates [9].

C. HPLC Quantitive Chemical Composition Analysis

To identify and assess flavonoid compounds, we conducted HPLC analysis using the method outlined by Mradu et al. [9]. Our solvent contained distilled water, 5% formic acid, and 25% and 70% methanol. We utilized a C18-ODS (25 cm * 4.6 mm) column with a flow velocity of 1.0 and a UV detector set to 280 nm ml/min. Our injection volume was 100µL and we maintained a column temperature of 35C. Using a SYKAM HPLC model from Germany, which includes a S 2100 Quaternary Gradient Pump, a S 5200 autosampler, a S 2340 UV detector, and a S 4115 column oven, we conducted this investigation. Formic acid, methanol, and distilled water were mixed in a 70:25:5 ratio to create our mobile phase.

D. Detection of Alkaloid

Five milliliters of a plant extract were combined with a few drops of 0.1% picric acid (C6 H3 N3 O7).To utilize Draken dorff's reagent. Alkaloids are present in the test tube when a yellow color starts to develop [9].

E. Detection of Glycoside

The presence of glycosides is indicated by the formation of a brown ring on the inside surface [10] , when One milliliter of concentrated sulfuric acid (H2SO4) and a drop of ferric chloride (FeCl3) are added after two milliliters of acetic acid and five milliliters of plant extract were added.

F. Detection of Flavonoid

A yellow precipitate developed, indicating a positive reaction [11]. When one milliliter of 5% potassium hydroxide ethanol was added to one milliliter of the extract

G. Microbial Strain and Growth Condition

Four reference strain isolates from skin burns were utilized to assess the plant's antibacterial qualities. These included both Gram-positive bacteria such as *Staphylococcus* aureus and *Staphylococcus epidermis*, as well as Gram-negative bacteria such as *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. To prepare fresh cultures a loop of cells was moved from the agar slant into a test tube that had 5 mL of brain heart infusion (BHI), and the tube was left to incubate at 37 °C for the whole night.

H. Analytical Profile Index

To identify the isolated bacteria, a fully automated system called VITEK, which performs bacterial identification and antibiotic susceptibility testing, was used.

III. RESULTS

A. Quantitative phytochemical

Quantitative Plant methanol extracts from two research locations underwent phytochemical screening, which produced positive results for bioactive chemicals.

Table 1: Phytochemical test results for methanolic extracts of *Bougainvillea spectabilis* flowers

Test	Observation	MERR,
Flavonoid	yellow	+
alkaloid	Orange	+
Phenolics	yellow	+
Glycosides	yellow	+

Many researchers have authenticated that the extracts *of Bougainvillea spectabilis* flowers contain many bioactive compounds such as flavonoids, alkaloids, Phenolic, and Glycosides according to the change in color of the methanolic extract that detects the compounds.

B. Identification of Flavonoid for Bougainvillea spectabilis flowers Extract by HPLC

When we used High-Performance Liquid Chromatography (HPLC) to analyze the extract we detection flavonoids three types of flavanols according to Figure (1) The concentration of Quercetin, apigenin, and kaempferol was (92.6), (88.9),(80.1), (table- 2). This is study identify these three types of flavonols, Quercetin, apigenin, and kaempferol for *Bougainvillea spectabilis* flowers. Quercetin, apigenin, and kaempferol in Bougainvillea spectabilis flowers Extract were done by the measurement of the integrated peak area.

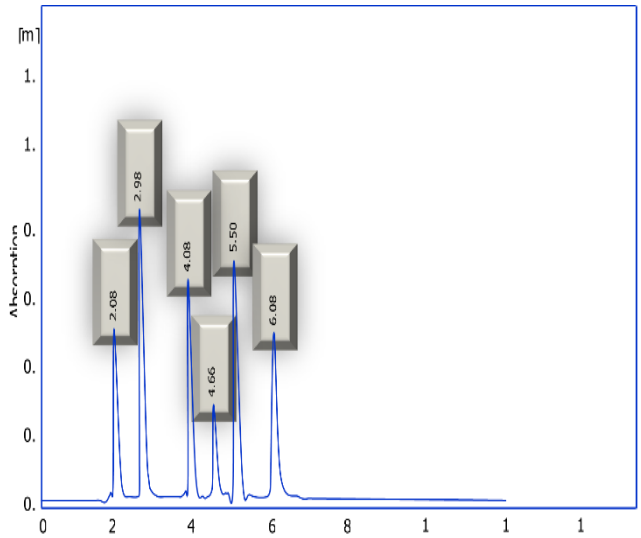


Fig.1: HPLC Chromatogram of *Bougainvillea spectabilis* flowers extract by retention Time and UV spectrum.

Table 2- The concentration of Quercetin, apigenin, and kaempferol

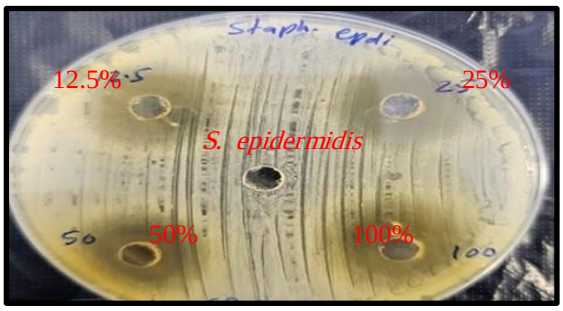
sample	peak area	Con.of sample	Formula of sample	Chemical structure
Quercetin	2541.80	92.6	$C_{15}H_{10}O_7$	
apigenin	1874.49	88.9	$C_{15}H_{10}O_5$	
kaempferol	1901.36	80.1	$C_{15}H_{10}O_6$	

C. Antibiotic Activity of Extract

In a recent study, the effects of various concentrations of methanol extract of *Bougainvillea spectabilis* flowers extract on four strains of bacteria - *S. aureus, S. epidermidis, P. aeruginosa, and Klebsiella pneumonia* - were compared with antibiotic standards (Amikacin, Vancomycin, Tetracycline, Levofloxacin, Ciprofloxacin, Trimethoprim, Cephalexin, Cefoxitin), The research revealed that the extract had a stronger inhibitory impact on Gram-positive bacteria than on Gram-negative bacteria. The methanolic extract concentration showed the most considerable effect on S. aureus. among the tested concentrations (200%, 100%, 50%, 25%, and 12.5%). These findings indicate a significant difference in the sensitivity of the *Bougainvillea spectabilis* flower extract among the tested bacteria ($P < 0.05$).

Table 3: Activity of *Trigonella foenum* against isolated bacteria

Con.	Cases No.	*S.epidermidis*	*S. aureus*	*P. aeruginosa*	*K. pneumonia*
		B. spectabilis Mean ± S. D			
12.5	9	4.00 ± 1.11	12.2 ± 0.83	12.3 ± 1.50	12.4 ± 1.74
25	9	5.11 ± 1.16	15.1 ± 1.45	12.0 ± 3.16	13.1 ± 1.29
50	9	5.88 ± 1.45	15.8 ± 1.05	14.0 ± 1.93	14.1 ± 1.96
100	9	7.44 ± 0.88	22.3 ± 1.93	15.4 ± 4.41	13.7 ± 1.30
200	9	13.7 ± 1.09	12.7 ± 1.22	14.5 ± 1.66	12.5 ± 1.66
p. value		< 0.001	< 0.001	0.045	0.042
LSD		1.10	1.28	2.62	1.53



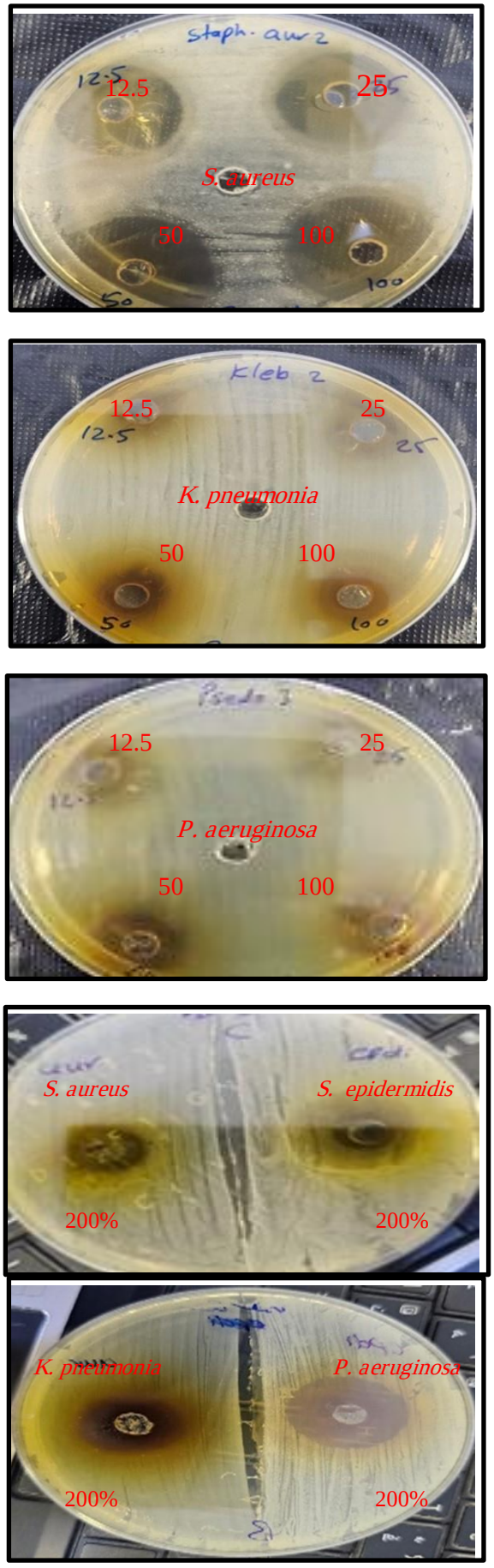


Fig.2: The antimicrobial activity of *Bougainvillea spectabilis* extract at the concentrations (200%,100%, 50%,25%,12,5%).

Table 4- Antibiotic susceptibility of *S. aureus* against different antibiotics

Antibiotics	*S. aureus* Inhibition Zone Mean ± S. D
Amikacin	22.6 ± 0.57^{b}
Gentamycin	0.00 ± 0.00^{e}
Levofloxacin	10.6 ± 1.15^{d}
Ciprofloxacin	12.6 ± 2.08^{d}
Trimethoprim	17.6 ± 3.21^{c}
Cephalexin	0.00 ± 0.00^{e}
Vancomycin	20.3 ± 1.52^{b}
Cefoxitin	22.3 ± 1.52^{b}
Tetracycline	27.3 ± 0.57^{a}
p. value < 0.001, LSD= 2.89	

IV. DISCUSSION

Plant-derived antimicrobial compounds are gaining popularity as bacterial resistance to antibiotics increases [12, 13]. The medicinal properties of herbs have been recognized and utilized for both traditional and modern medicine [14] Given the prevalence of antibiotic resistance among *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia*, and *Pseudomonas aeruginosa*, a study was conducted to investigate the potential antibacterial effects of Bougainvillea spectabilis flower extracts as an alternative to antibiotics. Our in vitro experiments revealed significant variations in the antibacterial activity of the extracts at different concentrations and when tested against different bacterial strains. The results in Table (3) A study conducted on the flavonoid extract of Bougainvillea spectabilis flowers revealed that it exhibited the highest inhibitory activity at a concentration of (100%) against Staphylococcus aureus with an inhibitory diameter of (22.3±1.93). When compared to antibiotics such as Levofloxacin, Ciprofloxacin, and Trimethoprim, the inhibitory diameters of the flavonoid extract were significantly higher at (10.6±1.15), (12.6±2.08), and (17.6±3.2), respectively. The diameter results were equivalent with Vancomycin and Cefoxitin. Furthermore, it was discovered that the extract's inhibitory efficacy was comparable to that of the medicines cephalexin and gentamycin. These Results suggest that flavonoid-rich plant extracts could be utilized instead of antibiotics. The plant extract's broad spectrum of antimicrobial properties is attributed to the presence of flavonoid ingredients, including kaempferol, quercetin, and apignin, which were found by HPLC analysis and are well-known for their biological efficacy against both Gram-positive and Gram-negative bacteria. These findings concur with those of numerous other studies, including [15].

V. CONCLUSION

The active components of the *Trigonella foenum* alkaloid seed were identified in the previous investigation. Because this research aids in the production and development of contemporary medications, GC-MS analysis is the initial stage in identifying the active components in the plant extract. The biologically active compounds in the extracts, together with some plant chemical components, are what manage diseases and make them a natural source of treatment for a variety of ailments due to their antibacterial and antioxidant qualities.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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