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Exploring *Bougainvillea glabra* flowers: a promising source of natural antimicrobial and anticancer agents

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ABSTRACT

In response to the challenge posed by antimicrobial resistance and the demand for innovative anticancer drugs, the aim of this study was to examine the potential source of natural antioxidant, antimicrobial, and anticancer compounds from purple flowers of *Bougainvillea glabra*. The dried purple flower of *B. glabra* was sequentially extracted with hexane, ethyl acetate, methanol, and water. The extracts were used for investigating antioxidants, antimicrobial activity, anticancer properties, and phytochemical composition. The methanol extraction exhibited the highest total phenolic content and in vitro antioxidant activity. The hexane and ethyl acetate extracts exhibited antimicrobial activity against a broad range of microorganisms in both American type of culture collection (ATCC) and clinical strains, while the methanol extract displayed a narrower antimicrobial profile that implied the promise of *B. glabra* as a developmental reservoir of specific antimicrobial agents. Notably, the hexane extract demonstrated the most promising anticancer activity against various cancer cell lines, including lung cancer (A549), colon cancer (HT-29), breast cancer (MCF-7), and bile duct cancer (KKU-213A). In conclusion, this study reveals the pharmaceutical potential of crude extracts from *B. glabra* flowers; thereby, paving the way for future exploration of these compounds within the drug development industry.

1. INTRODUCTION

Throughout history, medicinal plants have been used to manage infectious diseases, and their use has gained wide acceptance in many countries as an alternative approach to the treatment of specific ailments [1]. The growing use and acceptance of medicinal plants reflect their potential as a reservoir of bioactive compounds for drug discovery and development. *Bougainvillea glabra*, a hardy species of *Bougainvillea*, is distinguished by its colorful bracts. In Thailand, this species is popular as an ornamental plant and is recognized for its medicinal properties. *B. glabra* has been found to harbor phytoconstituents such as phenolic compounds, flavonoids, and alkaloids that impart a wide range of therapeutic properties [2]. *B. glabra* has generated significant interest in the diverse properties of its phytochemical composition. In addition to bioactive compounds, this plant contains various antioxidative molecules that counter oxidative activity, including the activities of free radicals that disrupt the stability of cellular molecules [3].

Certain phenolic compounds obtained through plant extraction exhibit pharmaceutical properties. Consequently, plants that produce large quantities of phenolics may potentially be sources of higher concentrations of antioxidants, antibiotics, anticancer agents, and antidiabetics [4]. The majority of natural phenolics are hydrophilic, while some are lipophilic. In the assessment of bioactive compounds, researchers frequently prioritize, as we do here, the determination of total phenolic compounds (TPC).

The surge in antibiotic overuse has raised concerns about antimicrobial resistance, as it has seriously narrowed the available healthcare choices for effective antimicrobial treatments [5]. The search for innovative antimicrobial agents has been an ongoing topic and the phytochemical composition of *B. glabra* holds possibilities for the discovery of new substances with natural antimicrobial properties. Recognized as an edible medicinal plant, *B. glabra* has emerged as a potential source

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of antimicrobial compounds, given the diverse phytochemical classes presented within its flowers [2].

Phytochemical compounds found in *Bougainvillea* have also demonstrated anticancer properties. Studies have revealed that specific compounds in the genus of *Bougainvillea* can impede the growth of various cancer cell lines [2,6]. For example, dichloromethane and methanol extracts of *B. glabra* inhibited a wide range of cancer cells, including MCF-7, MDA-MB-231, CaSki, DU-145, and SW-480 [7]. Another study showed that the new bioactive compound given the name bougainvinones, from the bark of *B. spectabilis*, displayed cytotoxic effects on KB, HeLa S-3, HT-29, MCF-7, and HepG2 cancer cells [6].

Numerous studies have explored the components and phytochemical properties of bioactive compounds in the purple flowers of *B. glabra*. However, our study contributes additional insights by providing information on antimicrobial resistance against previously unstudied stains of clinically infectious bacteria and anticancer properties against cell lines that have not been investigated before. These novel findings are based on the purple flowers of *B. glabra* collected in Songkhla Province, situated in the southern part of Thailand.

2. MATERIALS AND METHODS

2.1. Sample Collection and Preparation

Fresh purple flowers of *Bougainvillea* sp. had been collected from the Songkhla Province of Thailand and were then taxonomically identified and confirmed as *B. glabra* following the series of dichotomous keys of Flora of Thailand, Volume 5, Part 3 [8].

Fresh purple flowers from *B. glabra* were harvested and carefully rinsed with distilled water. The flowers were then dried in an oven at 60°C for 24 h. The dried flowers were ground into a fine powder that was sequentially extracted using the chosen analytical solvent grade: hexane, ethyl acetate, methanol, and water. The powdered flower was first extracted with hexane with shaking at 200 rpm for 16 h at a ratio of 1:10 w/v, with 100 g of flower in 1 L of hexane. The extract was then filtered using Whatman No.1 filter paper, and the clear extract was collected. The residue after hexane extraction then underwent subsequent extraction in the same condition using ethyl acetate, methanol, and water as solvents. The clear extracts obtained from hexane, ethyl acetate, and methanol were concentrated using a Buchi R-134 rotary evaporator and the clear extract obtained from water was dehydrated using a freezer dryer.

2.2. Total Phenolic Contents

To assess phenolic contents, a 100 mg extract sample was dissolved in 1 mL of 70% ethanol with regular shaking for 1 h at 4°C. The determination of TPC was carried out using a 96-well microplate format following a previous work [9] with some modifications. Each reaction included 20 µL of either (1) 70% ethanol as a blank, (2) various concentrations of gallic acid (Merck Co., Germany) as a standard, or (3) one of the extracts. Subsequently, 100 µL of 10% Folin–Ciocalteu reagent (Sisco Research Laboratories, India) and 80 µL of 7.5% Na_2CO_3 were added. The reaction was incubated at room temperature for 1 h. TPC was then determined by measuring absorbance at 765 nm and was reported as milligrams of gallic acid equivalent per gram of dry weight (mg GAE g^{-1} DW).

2.3. Antioxidant Activities

2.3.1. DPPH-free radical scavenging activity

To evaluate free radical scavenging activity, a 100 mg extract sample was dissolved in 1 mL of 70% ethanol with regular shaking at 4°C for 1 h. The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay was conducted using a 96-well microplate format [10]. Each well contained 190 µL of DPPH and 10 µL of either (1) 70% ethanol as a blank, (2) various concentrations of Trolox (Merck Co., Germany) as a standard, or (3) one of the extracts. The total volume of the DPPH assay (200 µL) was incubated at room temperature for 1 h. The optical density, measured at an absorbance of 517 nm, was used to determine the percentage of inhibition in the DPPH assay. The % inhibition was calculated as follows:

$$\text{Inhibition of DPPH (\%)} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

The scavenging capacity of DPPH was expressed as the Trolox equivalent antioxidant capacity (TEAC) in milligrams Trolox per gram of DW.

2.3.2. ABTS-free radical scavenging activity

To investigate 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging activity [11], a working solution of $ABTS^+$ was prepared by combining 40 mM potassium persulfate ($K_2S_2O_8$) with 7 mM ABTS. This working solution was stored at 4°C for 16 h before use. The $ABTS^+$ working solution was diluted with 50 mM phosphate buffer (pH 7.4) until the absorbance at 734 nm coincided with an optical density (OD) of 0.7. Extract samples of 100 mg were dissolved in 1 mL of 50 mM phosphate buffer (pH 7.4) with regular shaking for 1 h. The $ABTS^+$ assay was conducted using a 96-well microplate spectrophotometer. In each well, 180 µL of the working $ABTS^+$ solution was mixed with 20 µL of either (1) 50 mM phosphate buffer (pH 7.4) as a blank, (2) various concentrations of Trolox as a standard, or (3) one of the extracts. All reactions were incubated at 30°C for 6 min. OD (A) for absorbance was then measured at a wavelength of 734 nm and % inhibition was calculated as follows:

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

The scavenging capacity of ABTS was expressed as the TEAC in milligrams Trolox per gram of DW.

2.3.3. Ferric-reducing antioxidant power

Free radical scavenging activity was also investigated by using the ferric-reducing antioxidant power (FRAP) assay [12]. The FRAP reagent was prepared by combining 10 mL of 300 mM acetate buffer at pH 3.6, 1 mL of 10 mM 2,4,6-tripyridyl-*S*-triazine (TPTZ) in 40 mM HCl, and 1 mL of 20 mM $FeCl_3$. A volume of 150 µL of the FRAP reagent was added to a 96-well plate along with 20 µL of either (1) distilled water as a blank, (2) various concentrations of Trolox as a standard, or (3) one of the extracts. The reaction was incubated in darkness at 25°C for 10 min. Absorbance was then measured at 593 nm. Reducing power was calculated and reported in milligrams Trolox per gram of DW.

2.4. Antimicrobial Properties

2.4.1. Determination of antimicrobial activity

All the microbial strains in this study were obtained from the Department of Microbiology, School of Medicine, Walailak University, Thailand. The following Gram-positive bacteria were used to investigate antimicrobial screening properties of the extracts: *Bacillus cereus* ATCC11778, *B. subtilis* 7988 (clinical isolate), *B. subtilis* ATCC6051, *Listeria monocytogenes* (clinical isolates), *Staphylococcus aureus* ATCC25923, *S. aureus* ATCC29213, methicillin-resistant *S. aureus* (clinical isolate), *S. epidermidis* ATCC35984, and *Streptococcus pyogenes* ATCC19615. The following Gram-negative bacteria were used to screen the antimicrobial properties of the extracts: *Acinetobacter baumannii* MDR, *Escherichia coli* ATCC25922, *E. coli* O157:H7 (clinical isolates), *Klebsiella pneumoniae* ATCC70063, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* serotype Typhi (clinical isolates), *Shigella enteritis* (clinical isolates), and *Vibrio cholerae* (clinical isolates).

Gram-positive and -negative bacteria were maintained at 4°C on nutrient agar (Gibco; Thermo Fisher Scientific, Inc) and Luria–Bertani (LB) agar (Gibco; Thermo Fisher Scientific, Inc), respectively. The cultures were grown overnight in Mueller Hinton (MH) broth (Gibco; Thermo Fisher Scientific, Inc) at 37°C and then diluted to approximately 8×10^8 colony-forming units (CFU/mL) for all tests. For the positive control, ampicillin was employed specifically against Gram-positive bacteria, while gentamicin was utilized against Gram-negative bacteria.

2.4.2. Overlay spotted screening for antimicrobial activity

The protocol used to assess antimicrobial activity was slightly adjusted based on Wang et al. [13]. In brief, bacterial strains were cultured overnight at 37°C in MH broth, then diluted to a concentration of bacterial suspension at 10^7 CFU/mL, and swabbed on MH agar in three different directions. Extracts were individually prepared at 100 mg/mL in DMSO and sterilized through a 0.22 sterile hydrophilic PTFE filter. To screen extract sensitivity, 10 µL of each extract at 100 mg/mL were spotted on the swabbed MH agar plates and then incubated overnight at 37°C. Bacterial sensitivity to the extract was determined by observing clear spots on the bacterial lawn.

2.4.3. Microdilution technique for minimum inhibitory concentration and minimum bactericidal concentration

Microdilution technique for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in this study was adapted from Mordmuang et al. [14]. To determine the MIC, 100 µL of MH broth was transferred into each well of a 96-well plate. Extracts were subjected to twofold serial dilution. In brief, 100 µL of each sterile extract, initially diluted in MH broth at 32 mg/mL, underwent twofold dilutions in each well, resulting in final extract concentrations ranging from 16 mg/mL to 0.25 µg/mL. Additionally, 100 µL of each diluted bacterial cell suspension containing 10^6 CFU/mL was added to each well of the 96-well plate. For the growth control, 1% DMSO diluted in MHB was used instead of the extract. The entire plate was then incubated at 37°C. The MIC was determined as the lowest concentration of extract that rendered the solution in each well visibly clear.

For the investigation of MBC, 10 µL of each well, starting from the MIC concentration and onward, was plated onto MH agar and subsequently incubated at 37°C with 5% CO_2. The MBC was defined as the concentration in the well that exhibited no bacterial growth on the agar plates.

2.5. Anticancer properties

2.5.1. Cancer cell culture

Four cancer cell lines were used in this study—A549 (representing lung cancer), HT-29 (representing colon cancer), MCF-7 (representing breast cancer), and KKU-213A (representing bile duct cancer). Additionally, two normal cell lines, human dermal fibroblast (HDF) and an immortalized human cholangiocyte cell line, MMNK-1, were included for comparative analysis. All cell lines were cultivated in high-glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS) (Gibco; Thermo Fisher Scientific, Inc.) and 1% antibiotic–antimycotic (Gibco; Thermo Fisher Scientific, Inc.). Cells were cultured and incubated under standard conditions at 37°C with 5% CO_2.

2.5.2. Cytotoxicity assay

The cytotoxic effects of the studied extracts were assessed through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, 3×10^3 cells in 100 µL of medium were seeded into each well of a 96-well plate and incubated overnight. In the following day, the old media were replaced with media containing various concentrations of the extracts (62.5, 125, 250, 500, 750, and 1,000 µg/mL), and medium containing DMSO was used as a vehicle control (0 µg/mL). The cells were incubated for 72 h before being subjected to the MTT assay to assess the effect of each extract on cancer and normal cell lines. In the MTT assay, a final concentration of 0.5 mg/mL MTT solution was added to each well and incubated for 2 h. Excess MTT solution was removed, and formazan crystals were solubilized with 100 µL of DMSO. Absorbance was recorded at 540 nm using a microplate reader (Spark® multimode microplate reader, TECAN, Switzerland). Percent cell viability was calculated using the following formula:

$$\text{Cell viability (\%)} = (\text{OD test} / \text{OD control}) \times 100$$

2.6. GC-MS Analysis of Phytochemical Compounds

The four plant extracts were analyzed by gas chromatography (GC, Agilent 7890) coupled with triple quadrupole mass spectrometry (MS, Agilent 7000D) [15]. The less polar extracts, hexane and ethyl acetate, were separately chromatographed in an Agilent 19091-433 HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm; maximum temperature 340°C) while the more polar extracts, methanol and aqueous, were separated in an Agilent 19091N-113I HP-INNOWax capillary column (30 m × 0.25 mm × 0.25 µm; maximum temperature 340°C). Ultrahigh-purity helium, flowing at a constant rate of 1 mL/min, served as the carrier gas. The ionizing energy was set at 70 eV. The oven temperature was programmed from 60°C (held for 2 min) to 300°C at a rate of 3°C/min. Crude extract samples were diluted with ethanol in a 1:10 ratio (v/v). The resulting particle-free diluted crude extracts were injected via syringe using a spitless setting. Data were acquired by collecting full-scan mass spectra within the range of 35–550 amu. Constituent contents of the crude extracts were expressed as percentages calculated from peak areas. Peak identification of chemical compounds in the crude extracts relied on GC retention time. Individual peaks in the GC after mass spectral analyses were identified using the databases of Wiley10 and the National Institute Standard and Technology (NIST) version 17.

2.7. Statistical Analysis

Each experiment was conducted in five replicates unless otherwise specified. The normality of data distribution was assessed using the Shapiro–Wilk test, and the homogeneity of variance was examined using the Bartlett test. Comparative analysis was performed through an ANOVA with Tukey's post-hoc test. Statistical significance was considered when the p-value was less than 0.05. The presentation of all data includes mean values accompanied by the standard deviation (SD).

3. RESULTS

3.1. Total Phenolic Contents

An understanding of the chemical composition of *B. glabra* is crucial to the optimal retrieval of phenolic and antioxidant contents through solid–liquid extraction. To capture both hydrophilic and lipophilic phenolics, the solvents used ranged from low to high polarity. The methanol extract exhibited the highest TPC with 26.97 ± 1.70 mg GAE g^{-1} DW, followed by the aqueous extract (15.82 ± 2.58 mg GAE g^{-1} DW), ethyl acetate extract (8.69 ± 0.61 mg GAE g^{-1} DW), and hexane extract (2.19 ± 0.16 mg GAE g^{-1} DW) [Figure 1A].

3.2. In vitro Antioxidant Activities

To evaluate antioxidants present in the flower extracts of *B. glabra*, three colorimetric antioxidant assays were used: the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and FRAP assays. These in vitro assays are routinely employed to assess the antioxidant capacity of plant extracts.

3.2.1. DPPH-free radical scavenging activity

The fundamental principle of the DPPH assay is the conversion of stable DPPH radicals. When DPPH$^{\bullet}$ receives a hydrogen atom from antioxidant molecules, a color change from purple to clear yellow occurs. The color change reduces absorbance at 517 nm when 70% ethanol is used as the extraction solvent. Therefore, the antioxidant capacity of the extract is determined by comparing the plant extract concentration used to reduce the DPPH molecule to the standard concentration of an antioxidant molecules such as Trolox or L-ascorbic acid. The methanol extract (1.03 ± 0.04 mg TEAC g^{-1} DW) demonstrated a significantly higher antioxidant capacity than the water extract (0.62 ± 0.02 mg TEAC g^{-1} DW), ethyl acetate extract (0.17 ± 0.01 mg TEAC g^{-1} DW), and hexane extract (0.08 ± 0.01 mg TEAC g^{-1} DW) [Figure 1B].

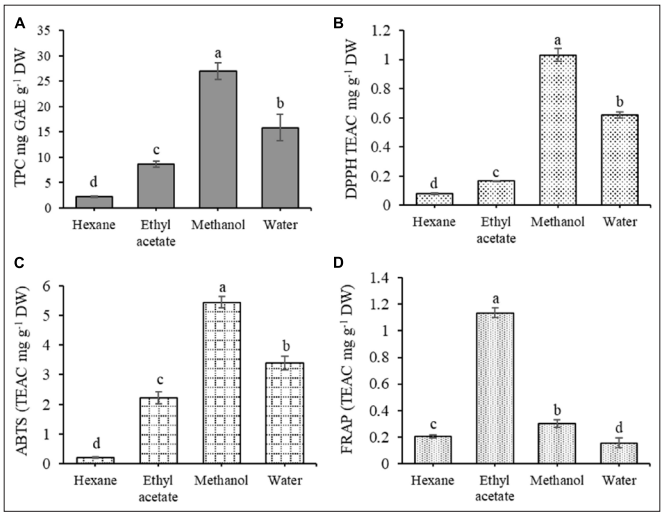

Figure 1: Total phenolic contents (TPC) **(A)** and in vitro antioxidant capacities of the dried flower of *B. glabra* extracted in different solvents were determined by DPPH **(B)**, ABTS **(C)**, and FRAP **(D)** assays and all data were reported as means ± SD from five replicate experiments. Different letters indicate a significant difference ($p < 0.05$) from the ANOVA test.

3.2.2. ABTS radical scavenging activity

The ABTS assay is based on the decolorization that occurs when the free radical molecule, ABTS$^{\bullet+}$, is reduced to ABTS by antioxidant molecules. The antioxidant capacity is determined by measuring the decolorization of ABTS$^{\bullet+}$. The advantage of the ABTS assay is that antioxidant capacity can be determined in various pH conditions. In this study, we used physiological pH (pH 7.4). The highest antioxidant capacity was found in the methanol extract (5.45 ± 0.19 mg TEAC g^{-1} DW), which demonstrated a significantly higher antioxidant capacity than the water (3.39 ± 0.23 mg TEAC g^{-1} DW), ethyl acetate (2.22 ± 0.21 mg TEAC g^{-1} DW), and hexane extracts (0.20 ± 0.02 mg TEAC g^{-1} DW) [Figure 1C]. Compared to the DPPH assay, the antioxidant capacity determined by the ABTS assay was nearly fivefold higher.

3.2.3. FRAP radical scavenging activity

Since the dried flowers of *B. glabra* are water-soluble at acidic pH (pH 3.6), the FRAP assay was also used to determine the antioxidant capacity of the flower extracts. FRAP assays are also colorimetric assays. Antioxidant activity is indicated by the blue color that appears when the ferric tripyridyltriazine complex (Fe^{3+}-TPTZ) is reduced to a ferrous complex (Fe^{2+}-TPTZ). The ethyl acetate extract (1.13 ± 0.03 mg TEAC g^{-1} DW) showed the greatest antioxidant capacity followed by methanol (0.30 ± 0.03 mg TEAC g^{-1} DW), hexane (0.20 ± 0.14 mg TEAC g^{-1} DW), and water extracts (0.15 ± 0.04 mg TEAC g^{-1} DW) [Figure 1D].

3.3. Antimicrobial properties

3.3.1. Antimicrobial screening against pathogenic

The antimicrobial screening test using 10 µL of 100 mg/mL (1 mg) of each extract revealed that the studied extracts produced a wide range of inhibitory growth effects on different bacterial lawns [Table 1]. The hexane and ethyl acetate extracts both similarly demonstrated inhibitory activity against *B. cereus* ATCC11778, *B. subtilis* ATCC6051, *B. subtilis* 7988 (clinical isolate), *L. monocytogenes* (clinical isolate), *S. aureus* ATCC29213, MRSA, *A. baumannii* MDR, *E. coli* O157:H7, and *S. enteritis* (clinical isolate). The ethyl acetate extract also inhibited *S. epidermidis* ATCC35984, *E. coli* ATCC25922, *K. pneumoniae* (clinical isolate), *S. enterica* serotype Typhi (clinical isolate), and *V. cholerae* (clinical isolate), but not *P. aeruginosa* ATCC27853, unlike the hexane extract. The methanolic extract displayed a narrower antimicrobial profile, specifically against *B. cereus* ATCC11778, *B. subtilis* ATCC6051, *B. subtilis* 7988 (clinical isolate), *S. epidermidis* ATCC35984, *S. pyogenes* ATCC19615, *A. baumannii* MDR, and *S. enteritis* (clinical isolate). The aqueous extract showed no inhibitory effects against any of the tested bacteria.

3.3.2. The MICs and MBC against pathogenic bacteria

The MIC and MBC varied across the organisms investigated [Table 2]. The hexane extract demonstrated strong inhibitory activity against Gram-positive Bacilli, specifically *B. cereus* ATCC11778, which presented relatively low concentrations to inhibit the growth

Table 1: Antimicrobial activity of four different extracts of *B. glabra* screened against various pathogenic bacteria.

Strains	Extracts				Positive Control (amp/gen)	Negative Control (1% DMSO)
	Hexane	Ethyl acetate	Methanol	Aqueous		
Gram-positive bacteria						
Bacillus cereus ATCC11778	+	+	+	–	+*	–
Bacillus subtilis 7988 (clinical isolate)	+	+	+	–	+*	–
B. subtilis ATCC6051	+	+	+	–	+*	–
Listeria monocytogenes (clinical isolates)	+	+	–	–	+*	–
Staphylococcus aureus ATCC25923	–	–	–	–	+*	–
S. aureus ATCC29213	+	+	–	–	+*	–
Methicillin-resistant *S. aureus* (clinical isolate)	+	+	–	–	–*	–
Staphylococcus epidermidis ATCC35984	–	+	+	–	+*	–
Streptococcus pyogenes ATCC19615	–	–	+	–	+*	–
Gram-negative bacteria						
Acinetobacter baumannii MDR	+	+	+	–	+**	–
Escherichia coli ATCC25922	–	+	–	–	+**	–
E. coli O157:H7 (clinical isolates)	+	+	–	–	+**	–
Klebsiella pneumoniae ATCC70063	–	+	–	–	+**	–
Pseudomonas aeruginosa ATCC27853	+	–	–	–	+**	–
Salmonella enterica serotype Typhi (clinical isolates)	–	+	–	–	+**	–
Shigella enteritis (clinical isolates)	+	+	+	–	+**	–
Vibrio cholerae (clinical isolates)	–	+	–	–	+**	–

Ampicillin (amp) was used as a positive control for Gram-positive bacteria marked as * and gentamycin (gen) was used as a positive control for Gram-negative bacteria marked as **. DMSO was used as a negative control. Symbol + and – stand for making a clear spot and not making a clear spot on bacterial lawn, respectively.

Table 2: Minimum inhibitory concentrations and minimum bactericidal concentrations of four different extracts of *B. glabra* against various pathogenic bacteria.

Strains	Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC) in µg/mL				Minimum Inhibitory Concentration (MIC) of Ampicillin/Gentamycin (µg/mL)	Negative Control (1% DMSO)
	Hexane	Ethyl acetate	Methanol	Water		
Gram-positive bacteria						
Bacillus cereus ATCC11778	256/512	512/1024	512/2048	R	0.5/0.25	R
Bacillus subtilis 7988 (clinical isolate)	4000/8000	4000/4000	4000/>8000	R	0.5/0.5	R
B. subtilis ATCC6051	2048/2048	4000/8000	4000/>4000	R	0.125/0.125	R
Listeria monocytogenes (clinical isolate)	>16000/NA	>8000/NA	R	R	0.25/2	R
Staphylococcus aureus ATCC25923	R	R	R	R	0.25/0.5	R
S. aureus ATCC29213	>16000/NA	>8000/NA	R	R	0.25/0.125	R
Methicillin-resistant *S. aureus* (clinical isolate)	>16000/NA	>8000/NA	R	R	NA/0.25	R
Staphylococcus epidermidis ATCC35984	R	>8000/NA	>16000/NA	R	0.5/0.25	R
Streptococcus pyogenes ATCC19615	R	R	>16000/NA	R	0.25/0.125	R
Gram-negative bacteria						
Acinetobacter baumannii MDR	>16000/NA	>8000/NA	R	R	NA/1	R
Escherichia coli ATCC25922	>16000/NA	>8000/NA	R	R	NA/0.031	R
E. coli O157:H7 (clinical isolates)	>16000/NA	>8000/NA	R	R	NA/0.063	R
Klebsiella pneumoniae (clinical isolate)	R	>8000/NA	R	R	NA/0.062	R
Pseudomonas aeruginosa ATCC27853	>16000/NA	R	R	R	NA/4	R
Salmonella enterica serotype Typhi (clinical isolates)	R	>8000/NA	R	R	NA/2	R
Shigella enteritis (clinical isolate)	>16000/NA	>8000/NA	>16000	R	NA/1	R
Vibrio cholerae (clinical isolate)	R	>8000/NA	R	R	NA/4	R

Ampicillin (amp) was used as a positive control for Gram-positive bacteria marked as * and gentamycin (gen) was used as a positive control for both Gram-positive and Gram-negative bacteria. DMSO (1%) was used as a negative control diluted by MHB broth. The letter R denotes resistance, a parameter that was not employed in the investigation of minimum inhibitory concentration (MIC). NA indicates that it is not applicable to MBC.

of *B. cereus* ATCC11778 at the MIC and MBC of 256 and 512 µg/mL, respectively. The MIC and MBC against *B. subtilis* ATCC6051 were at 2 mg/mL and 2 mg/mL, respectively, whereas those values against *B. subtilis* 7988 were higher in a clinical isolate at 4 mg/mL and 8 mg/mL. The inhibition effect of ethyl acetate and methanol extracts against *B. cereus* ATCC11778 showed around twofold higher at MICs and MBCs where those values revealed quite similar to both MIC and MBC against *B. subtilis* isolates. When the extracts were administered at high concentrations (measured in mg/mL), the MICs demonstrated effectiveness against the bacteria including *L. monocytogenes* (clinical isolate), *S. aureus* ATCC29213, MRSA (clinical isolate), *S. epidermidis* ATCC35984, *S. pyogenes* ATCC19615, and Gram-negative bacteria including *A. baumannii* MDR, *E. coli* ATCC25922, *E. coli* O157:H7, *K. pneumoniae* (clinical isolate), *P. aeruginosa* ATCC27853, *S. enterica* serotype Typhi (clinical isolates), *S. enteritis* (clinical isolate), and *V. cholerae* (clinical isolate).

3.4. Anticancer Activity

The studied extracts exhibited anticancer activity against various human cancer cell lines. The anticancer activity of the extracts was assessed using the MTT assay. Results indicated that the hexane extract from *B. glabra* flowers exhibited the most potent cytotoxic effects against all four cancer cell lines (colon cancer cell line [HT-29], lung cancer [A549], breast cancer [MCF-7], and bile duct cancer [KKU-213A]) followed by the ethyl acetate and methanol extracts. The water extract had a mild to moderate effect on these cell lines [Figure 2]. Among the tested cancer cell lines, the colon cancer cell line HT-29 showed the highest sensitivity to the extracts, followed by lung cancer (A549), breast cancer (MCF-7), and bile duct cancer (KKU-213A) cell lines [Figure 2].

3.5. GC-MS Analysis of Phytochemical Compounds

In the hexane extract [Table 3], the major chemical constituents were triterpene (squalene) and one natural tocotrienol, or vitamin E (1-benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-[4,8,12-trimethyltridecyl]). Several diterpenes were identified (Kaur-16-ene, phytol and phytol acetate). A group of esters was also detected, including 9,12,15-octadecatrienoic acid, methyl ester, 9,12-octadecadienoic acid, methyl ester and hexadecanoic acid, and methyl ester. The chemical components in the ethyl acetate extract

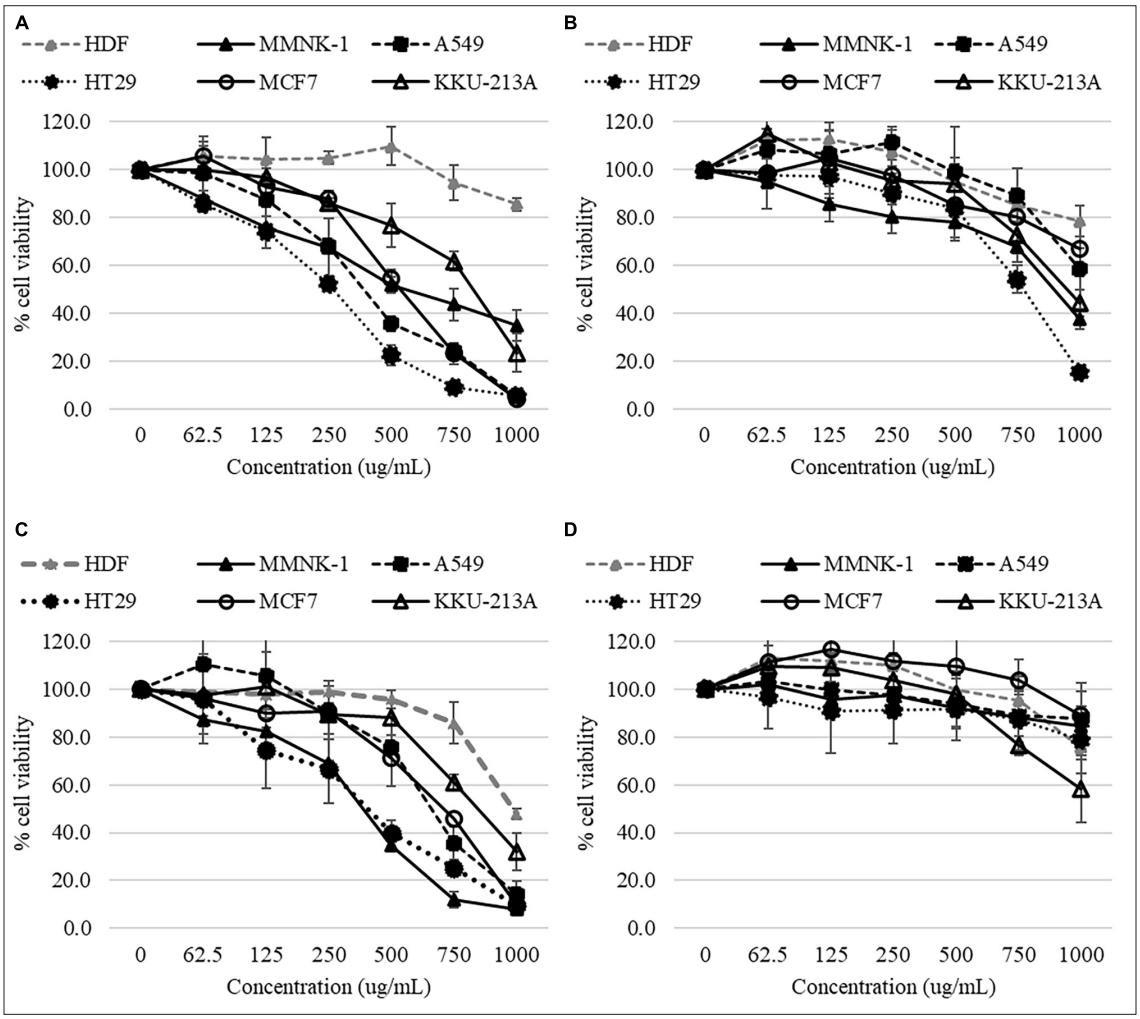

Figure 2: The anticancer activity of the dried flower of *B. glabra* extracted in different solvents was tested against four cancer cell lines: lung cancer (A549), colon cancer (HT-29), breast cancer (MCF-7), and bile duct cancer (KKU-213A) and two normal cell lines: HDF derived from human skin fibroblast cells and MMNK-1 derived from the epithelial lining of the bile duct. The charts show the results of cytotoxicity assays involving *B. glabra* flower extracted in hexane **(A)**, ethyl acetate **(B)**, methanol **(C)**, and water **(D)**. Results are means ± SD from five replicate experiments. Additional data can be found in Supplementary Data Tables 1–4.

Table 3: The major chemical components of the hexane extract of *B. glabra* flower were identified by GC-MS analysis.

No.	Identification	Formula	Rt (min)	Percentage
1	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	$C_{30}H_{50}$	67.43	14.04
2	2,5,7,8-tetramethyl-2-(4,8,12-trimethyltrideca-3,7,11-trienyl)-3,4-dihydrochromen-6-ol	$C_{29}H_{50}O_2$	73.89	9.92
3	methyl octadeca-9,12,15-trienoate	$C_{19}H_{32}O_2$	48.52	1.99
4	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	50.03	1.87
5	5,5,9-trimethyl-14-methylidenetetracyclohexadecane	$C_{20}H_{32}$	46.27	1.10
6	3,7,11,15-tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$	48.91	0.91
7	7,11,15-trimethyl-3-methylidenehexadec-1-ene	$C_{20}H_{38}$	40.33	0.90
8	methyl octadeca-9,12-dienoate	$C_{19}H_{34}O_2$	48.33	0.81
9	methyl hexadecanoate	$C_{17}H_{34}O_2$	43.18	0.26
10	3,7,11,15-tetramethylhexadec-2-enyl] acetate	$C_{22}H_{42}O_2$	41.73	0.23
11	(3,5-ditert-butylphenoxy)-trimethylsilane	$C_{14}H_{22}O$	28.43	0.15
12	Undecane	$C_{11}H_{24}$	11.31	0.15

Table 4: The major chemical components of the ethyl acetate extract of *B. glabra* flower were identified by GC-MS analysis.

No.	Identification	Formula	Rt (min)	Percentage
1	(3-acetyloxy-2-hydroxypropyl) acetate	$C_7H_{12}O_5$	17.36	5.61
2	Hexadecanoic acid	$C_{16}H_{32}O_2$	44.42	3.58
3	2,3-dihydroxypropyl acetate	$C_5H_{10}O_4$	10.93	3.45
4	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	$C_{30}H_{50}$	67.42	3.16
5	7,11,15-trimethyl-3-methylidenehexadec-1-ene	$C_{20}H_{38}$	40.32	2.67
6	3,7,11,15-tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$	48.90	2.19
7	2,3-dihydro-1-benzofuran	C_8H_8O	16.28	1.35
8	(2-acetyloxy-3-hydroxypropyl) acetate	$C_7H_{12}O_5$	17.56	1.24
9	3,7,11,15-tetramethylhexadec-2-enyl] acetate	$C_{22}H_{42}O_2$	41.72	0.62
10	6-hydroxy-4,4,7a-trimethyl-6,7-dihydro-5H-1-benzofuran-2-one	$C_{11}H_{16}O_3$	37.43	0.51
11	methyl octadeca-9,12-dienoate	$C_{19}H_{34}O_2$	48.33	0.40

Table 5: The major chemical components of the methanol extract of *B. glabra* flower were identified by GC-MS analysis.

No.	Identification	Formula	Rt (min)	Percentage
1	(2-acetyloxy-3-hydroxypropyl) acetate	$C_3H_8O_3$	21.42	6.20
2	Methyl hexadecanoate	$C_{17}H_{34}O_2$	19.99	5.34
3	Methyl octadeca-9,12,15-trienoate	$C_{19}H_{32}O_2$	26.55	4.56
4	Methyl (Z)-octadec-9-enoate	$C_{19}H_{36}O_2$	24.42	4.14
5	Hexadecanoic acid	$C_{16}H_{32}O_2$	32.82	3.63
6	Methyl octadeca-9,12-dienoate	$C_{19}H_{34}O_2$	25.29	3.28
7	4-Methoxy-4-oxobutanoic acid	$C_5H_8O_4$	22.02	1.60
8	4-Ethenyl-2-methoxyphenol	$C_9H_{10}O_2$	19.03	1.58
9	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	36.62	1.48
10	Furan-2-ylmethanol	$C_5H_6O_2$	10.36	1.15
11	5-Methylfuran-2-carbaldehyde	$C_6H_6O_2$	9.44	0.63
12	Phenylmethanol	C_7H_8O	13.39	0.32

[Table 4] were mostly 2-hydroxypropane-1,3-diyl diacetate, fatty acid (hexadecenoic acid), and triterpene (squalene). In the methanol extract [Table 5], most of the chemical constituents were classified into an ester group (hexadecanoic acid, methyl ester, 9,12,15-octadecatrienoic acid, methyl ester, and 9-octadecenoic acid, methyl ester). The aqueous extract [Table 6] contained cyclic hydrocarbons (2,3-dihydro-benzofuran, 1,2-cyclopentanedione and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl).

4. DISCUSSION

Our results demonstrated that the majority of the phenolic compounds in the dried flower of *B. glabra* were hydrophilic, as indicated by the high TPC in the methanol and aqueous extracts. This finding is consistent with previous studies of *B. glabra* [7], which also reported the highest TPC found in methanolic extracts. While one may criticize the Folin–Ciocalteu method for its indirect nature and potentially

Table 6: The major chemical components of the aqueous extract of *B. glabra* flower were identified by GC-MS analysis.

No.	Identification	Formula	Rt (min)	Percentage
1	2,3-Dihydro-1-benzofuran	C_8H_8O	22.60	6.82
2	Cyclopentane-1,2-dione	$C_5H_6O_2$	11.73	2.72
3	3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one	$C_6H_8O_4$	20.07	2.37
4	4-Hydroxy-2,5-dimethylfuran-3-one	$C_6H_8O_3$	15.90	2.18
5	4-Ethenyl-2-methoxyphenol	$C_9H_{10}O_2$	18.85	2.07
6	Methyl carbamate	$C_2H_5NO_2$	9.90	1.93
7	Furan-2-ylmethanol	$C_5H_6O_2$	10.28	1.76
8	2,3,5-Trimethylcyclohexa-2,5-diene-1,4-dione	$C_{12}H_{10}O_4$	34.10	1.66
9	2-Methoxyphenol	$C_7H_8O_2$	13.02	1.52
10	Hexadecanoic acid	$C_{16}H_{32}O_2$	32.59	1.44
11	Benzoic acid	$C_7H_6O_2$	23.41	0.98
12	Phenol	C_6H_6O	15.36	0.80

unspecific results, it has been widely used and remains an acceptable means of quantifying TPC. More direct methods, such as HPLC and GC-MS, have been employed to detect novel phenolic compounds in *B. alba* [16] and can be used to directly quantify individual phenolic compounds. Intriguingly, we found that the TPC of the purple flowers of *B. glabra* in our study (26.97 ± 1.70 mg GAE g^{-1} DW) was comparable to the TPCs reported in studies from flowers of *Bougainvillea* x buttiana Holttum and Standl (var. Rose) in Mexico (21.14 mg GAE g^{-1} DW) [17] and from the aerial parts of *B. glabra* in Pakistan (24.01 mg GAE g^{-1} DW) [7]. This similarity suggests that plants from the genus *Bougainvillea* consistently produce similar TPCs, even when cultivated in different geographical areas.

Various phenolic compounds exhibit varying degrees of free radical scavenging activity, influenced by the number of hydroxyl groups they possess. As a result, many studies have observed a positive correlation between TPC and antioxidant activities [18]. In this study, the antioxidant activities detected in the DPPH and ABTS assays were consistent with the TPC of the different extracts. Meanwhile, the ethyl acetate extract exerted the highest antioxidant activity in the FRAP assay. While antioxidant activities indicated by the FRAP assay have usually shown a positive correlation with TPC, a study of antioxidants in certain fresh fruit juices [19] found, as we did, that the results of an FRAP assay did not show a correlation with TPC. The criteria for quantifying antioxidants and the individual chemical characteristics of bioactive compounds in plant extracts are the main factors influencing the degree of antioxidant activity.

B. glabra flower exhibits notable potential as an effective antimicrobial agent against a wide array of pathogenic bacterial infections in both ATCC strains and clinical isolates. In our investigation, the purple *B. glabra* flowers displayed distinctive chemical compositions and biological properties that contribute additional information to the phytochemical profiles identified in previous studies. The hexane and ethyl acetate extracts demonstrated inhibitory effects on a broader spectrum of clinically relevant bacteria, encompassing methicillin-resistant strains of *S. aureus* (clinical isolate) and *S. epidermidis* ATCC35984, clinical isolates of *Bacillus* spp. and *L. monocytogenes* (clinical isolate), and multidrug-resistant strain of *A. baumannii*. Furthermore, both hexane and ethyl acetate extracts inhibited the growth of the pathogenic Gram-negative bacteria *E. coli* O157:H7 (clinical isolates), *S. enteritis* (clinical isolate), and *S. enterica* serotype Typhi (clinical isolate) at high concentrations of the extracts in mg/mL unit. Ethanolic and methanolic flower extracts of different *Bougainvillea* species have presented antimicrobial properties against a broad spectrum of bacteria including *S. aureus, E. coli, P. aeruginosa,* and *B. cereus* [20,21]. Swamy et al. also investigated the antimicrobial activity of the *Bougainvillea* bract against *K. pneumoniae, P. vulgaris, P. aeruginosa, B. subtilis*, as well as fungi. While the bract is highlighted for its antimicrobial potential, leaf extracts of *Bougainvillea* are also considered promising antimicrobial agents as flowers are originally adapted from leaves [22]. In addition, Ravikumar and Pratheep revealed that methanolic extract of *B. glabra* flower inhibited the growth of *S. aureus*, *B. subtilis*, *P. putida*, *E. coli*, and *Candida albicans* [20]. While the microbial properties of *Bougainvillea* flowers have been tested in previous studies, this study critically contributes essential insights into the antimicrobial properties, particularly against untested bacterial strains, *S. epidermidis* ATCC35984, clinical isolates of *Bacillus* spp., *L. monocytogenes*, pathogenic Gram-negative bacteria *E. coli* O157:H7, and multidrug-resistant strain of *A. baumannii*.

Medicinal plants have been extensively exploited in the treatment of various ailments, and *B. glabra* is recognized as a promising candidate for investigation. Despite its acknowledged potential, the understanding of its phytochemical properties, antimicrobial activity, and anticancer effects is not yet complete. In this study, we have demonstrated that the purple flowers of *B. glabra* extracts possess anticancer activity testing on the colon cancer cell line HT-29, the lung cancer (A549), the breast cancer (MCF-7), and the bile duct cancer (KKU-213A), aligning with previous research findings that indicate their consistent efficacy in suppressing various cancer cell lines. Saleem et al. illustrated that both methanol and dichloromethane *Bougainvillea* extracts effectively inhibited the growth of breast (MCF-7 and MDA-MB-231), cervical (CaSki), brain (DU-145), and colon (SW-480) cancer cell lines [7]. Moreover, an ethanol extract of *B. glabra* leaf demonstrated cytotoxic effects on HT-29, AGS (gastric adenocarcinoma), and BL-13 (bladder cancer) cell lines [23].

The belief that remedies from natural sources are safer may not always be true. Therefore, safety assessments are mandatory in the search for new drugs derived from medicinal plants. The study of Krishna and Sundararajan conducted toxicity studies on a methanol extract of B. glabra using a Wistar albino rat model [24]. The study suggested that the extract was well-tolerated and did not show any observed toxicity in both acute and sub-chronic settings. Our study [Figure 2] showed that, against the normal skin fibroblast HDF cells, high concentrations of all our extracts exhibited only mild toxicity. When our extracts were

tested on normal bile duct cells, MMNK-1, results showed that water [Figure 2D] and methanol [Figure 2C] extracts had only a mild effect. On the other hand, the ethyl acetate [Figure 2B] and hexane [Figure 2A] extracts significantly suppressed the growth of MMNK-1. The major difference between these two normal cell lines is that HDF is a normal slow-growing cell derived from human skin fibroblast cells, while MMNK-1 is derived from the epithelial lining of the bile duct, modified to be immortalized cells [25]. Therefore, they may not truly represent normal cells. Our findings add to the information regarding the safety of *Bougainvillea* extracts, suggesting that they exhibit a definite antiproliferative effect against malignant cells but show low to no effect on slow-dividing normal skin fibroblast cells.

In this study, certain chemical components detected in the purple flowers of *B. glabra* flowers in the Songkhla Province, Thailand, were consistent with those detected in previous studies [26,27]. In the present investigation, certain constituents were identified within the purple flowers of *B. glabra* for the first time. For example, 6-hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one in the ethyl acetate extract and furan (2-Furancarboxaldehyde, 5-methyl-) in the methanol extract. GC-MS analysis identified myristic acid as a primary contributor to the reduced microbial growth rate, with palmitic acid also playing a partial role. Diterpenes, including phytol, were identified as potential antimicrobial agents. Palmitic acid and phytol were also detected in the GC-MS analysis of our hexane [Table 3], ethyl acetate [Table 4], and methanol [Table 5] extracts where they might potentially be the cause of the inhibition of Gram-positive and Gram-negative bacteria [28]. The differences in the number and quantity of terpenes and phenols identified in the various extracts of *Bougainvillea* flower may be key factors in explaining its high antioxidant and antimicrobial activities, as well as the promising suppression of certain cancer cell lines. As anticipated, both plant species and geographical location can impact metabolic production [29], particularly in the synthesis of secondary metabolite pathways.

Despite the high phenolic content in the methanol extract, it may not exhibit the anticipated levels of antimicrobial and anticancer activities, as demonstrated in a previous study on *Rubus idaeus* L. [30], where various types of antioxidant activity were observed, but antimicrobial activity against different strains was not detected**.** Furthermore, we cannot discount the possibility that certain microbial and anticancer agents present in hexane and ethyl acetate extracts may be present in lesser quantities but could potentially offer higher potency in terms of antimicrobial and anticancer activities.

5. CONCLUSION

This study investigated bioactive compounds present in the purple flowers of *B. glabra* cultivated in the Songkhla Province of Thailand. A methanol extract of the flower possessed the highest total phenolic content and highest antioxidant activity, as determined through DPPH and ABTS assays. Furthermore, hexane and ethyl acetate extracts exhibited comprehensive inhibition of microbial growth, while the methanol extract selectively inhibited the growth of specific bacterial strains. Notably, the hexane extract, potentially enriched with essential oils, demonstrated pronounced cytotoxicity against four cancer cell lines. These findings underscore the abundant bioactive constituents within *B. glabra*, highlighting the need for further investigation. Future investigations should include the purification of isolated active compounds and their synthesis to substantiate pharmaceutical properties through in vitro and in vivo assessments.

6. ABBREVIATIONS

ABTS: 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid). ANOVA: Analysis of variance. ATCC: American type culture collection. C: Celsius. CaSki: Epidermoid cervical cancer cell line. CFU: Colony-forming unit. DMEM: Dulbecco's modified Eagle medium. DMSO: Dimethyl sulfoxide. DPPH: 2,2-Diphenyl-1-picrylhydrazyl. DU-145: Human prostate cancer cell line. DW: Dry weight. FBS: Fetal bovine serum. FRAP: Ferric-reducing antioxidant power. g: Gram. GAE: Gallic acid equivalent. GC-MS: Gas chromatograph–mass spectrometer. h: Hour. HDF: Human dermal fibroblasts. HeLa S-3: Human cervical epithelioid carcinoma. HepG2: Human liver cancer cell line. HT-29: Human colon adenocarcinoma. KB: Keratin-forming tumor cell line. L: Liter. MBC: Minimum bactericidal concentration. MCF-7: Human breast cancer cell line. MDA-MB-231: Breast cancer cell line. MDR: Multidrug-resistant. mg: Milligram. MH: Mueller Hinton. MIC: Minimum inhibitory concentration. min: Minute. mL: Milliliter. mM: Millimolar. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. nm: Nanometer. rpm: Round per minute. SD: Standard deviation. SW-480: Colon adenocarcinoma. TEAC: Trolox equivalent antioxidant capacity. TPC: Total phenolic compound. TPTZ: 2,4,6-Tris(2-pyridyl)-*S*-triazine. µg: Microgram.

7. AUTHOR CONTRIBUTIONS

WS and SS were responsible for research design, investigation, data analysis, interpretation of data, manuscript preparation, and manuscript revision. AM and KK were responsible for investigation, data analysis, interpretation, and manuscript preparation. All authors have read and approved the current version of the manuscript.

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9. CONFLICTS OF INTEREST

The authors declare no conflicts of interest associated with this study.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

13. PUBLISHER'S NOTE

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14. SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the journal's website: [https://jabonline.in/admin/php/uploadss/1231_pdf.pdf]

REFERENCES

1. El-Saadony MT, Zabermawi NM, Zabermawi NM, Burollus MA, Shafi ME, Alagawany M, et al. Nutritional aspects and health benefits of bioactive plant compounds against infectious diseases: a review. Food Rev Int. 2023;39(4):2138–60.
2. Saleem H, Usman A, Mahomoodally MF, Ahemad N. *Bougainvillea glabra* (choisy): a comprehensive review on botany, traditional uses, phytochemistry, pharmacology and toxicity. J Ethnopharmacol. 2021;266:113356.
3. Ravikumar P, Pratheep T. Exploring the therapeutic properties of *Bougainvillea glabra*: a study of its antimicrobial and antioxidant effects in methanol extract. Ann For Res. 2022;65(1):11285–92.
4. Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. Molecules. 2023;28(4):1845.
5. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, et al. editors. Antimicrobial resistance: a growing serious threat for global public health. Healthcare. 2023;11(13):1946.
6. Do LT, Aree T, Siripong P, Vo NT, Nguyen TT, Nguyen PK, et al. Cytotoxic flavones from the stem bark of Bougainvillea spectabilis willd. Planta Med. 2018;84(02):129–34.
7. Saleem H, Zengin G, Ahmad I, Lee JTB, Htar TT, Mahomoodally FM, et al. Multidirectional insights into the biochemical and toxicological properties of *Bougainvillea glabra* (Choisy) aerial parts: a functional approach for bioactive compounds. J Pharm Biomed Anal. 2019;170:132–8.
8. Larsen K. Nyctaginaceae. In: Smitinand T, Larsen K, editors. Floral of Thailand. Bangkok: The Chutima Press; 1991. p. 367–9.
9. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat Protocols. 2007;2(4):875–7.
10. Herald TJ, Gadgil P, Tilley M. High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. J Sci Food Agric. 2012;92(11):2326–31.
11. Nowak A, Klimowicz A, Duchnik W, Kucharski Ł, Florkowska K, Muzykiewicz A, et al. Application of green-extraction technique to evaluate of antioxidative capacity of wild population of fireweed. Herba Polon. 2019;65(4):18–30.
12. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal. 2006;19(6-7):669–75.
13. Wang S, Wang Q, Yang E, Yan L, Li T, Zhuang H. Antimicrobial compounds produced by vaginal *Lactobacillus crispatus* are able to strongly inhibit candida albicans growth, hyphal formation and regulate virulence-related gene expressions. Front Microbiol. 2017;8:564.
14. Mordmuang A, Shankar S, Chethanond U, Voravuthikunchai SP. Effects of *Rhodomyrtus tomentosa* leaf extract on staphylococcal adhesion and invasion in bovine udder epidermal tissue model. Nutrients. 2015;7(10):8503–17.
15. Agiel N, Köse YB, Gülcan Z, Saltan N, Kürkçüoğlu M, İşcan G. Antioxidant and antimicrobial activity of the endemic *Mentha longifolia* subsp. cyprica growing in Cyprus. Phytochem Lett. 2023;60:243–8.
16. AbdEl-nabi E, Abdel-Aleem IM, El-Amin SM, Refahy LA, El-Shazly MA. Active cytotoxic compounds and essential oil from *Bougainvillea alba*. J Natl Remed. 2015;2015:49–57.
17. Abarca-Vargas R, Pena Malacara CF, Petricevich VL. Characterization of chemical compounds with antioxidant and cytotoxic activities in *Bougainvillea* x *Buttiana holttum* and standl, (Var. rose) extracts. Antioxidants. 2016;5(4):45.
18. Kuhn F, de Azevedo ES, Frazzon J, Noreña CPZ. Evaluation of green extraction methods on bioactive compounds and antioxidant capacity from *Bougainvillea glabra* bracts. Sustain Chem Pharm. 2021;19:100362.
19. Wern KH, Haron H, Keng CB. Comparison of total phenolic contents (TPC) and antioxidant activities of fresh fruit juices, commercial 100% fruit juices and fruit drinks. Sains Malays. 2016;45(9):1319–27.
20. Ravikumar P, Thangaraj P. Exploring the therapeutic properties of *Bougainvillea glabra*: a study of its antimicrobial and antioxidant effects in methanol extract. Chin J Otorhinolaryngol Head Neck Surg. 2022;26:399–406.
21. Gupta V, George M, Joseph L, Singhal M, Singh HP. Evaluation of antibacterial activity of *Bougainvillea glabra* 'snow white' and *Bougainvillea glabra* 'choicy'. J Chem Pharm Res. 2009;1:233–7.
22. Sudipta K, Lokesh P, Rashmi W, Vijay R, Ssn K. Phytochemical screening and in vitro antimicrobial activity of Bougainvillea spectabilis flower extracts. Int J Phytomed. 2012;4(3):375.
23. Kaisoon O, Konczak I, Siriamornpun S. Potential health enhancing properties of edible flowers from Thailand. Food Res Int. 2012;46(2):563–71.
24. Krishna R, Sundararajan R. Toxicity studies of *Bougainvillea glabra* and *Mucuna pruriens*. Int J Pharm Sci Res. 2020;11(10):4910–7.
25. Maruyama M, Kobayashi N, Westerman KA, Sakaguchi M, Allain JE, Totsugawa T, et al. Establishment of a highly differentiated immortalized human cholangiocyte cell line with SV40T and hTERT. Transplantation. 2004;77(3):446–51.
26. Saleem H, Htar TT, Naidu R, Anwar S, Zengin G, Locatelli M, et al. HPLC–PDA polyphenolic quantification, UHPLC–MS secondary metabolite composition, and in vitro enzyme inhibition potential of *Bougainvillea glabra*. Plants. 2020;9(3):388.
27. Ornelas García IG, Guerrero Barrera AL, Avelar González FJ, Chávez Vela NA, Gutiérrez Montiel D. *Bougainvillea glabra* choisy (Nyctinaginacea): review of phytochemistry and antimicrobial potential. Front Chem. 2023;11:1276514.
28. Ivanova EP, Nguyen SH, Guo Y, Baulin VA, Webb HK, Truong VK, et al. Bactericidal activity of self-assembled palmitic and stearic fatty acid crystals on highly ordered pyrolytic graphite. Acta Biomater. 2017;59:148–57.
29. Sampaio BL, Edrada-Ebel R, Da Costa FB. Effect of the environment on the secondary metabolic profile of Tithonia diversifolia: a model for environmental metabolomics of plants. Sci Rep. 2016;6(1):29265.

30. Ispiryan A, Atkociuniene V, Makstutiene N, Sarkinas A, Salaseviciene A, Urbonaviciene D, et al. Correlation between antimicrobial activity values and total phenolic content/antioxidant activity in *Rubus idaeus* L. Plants. 2024;13(4):504.

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