

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan



Research Article

Phytochemical Profiling, Antioxidant Potential and Antibacterial Activity of *Fagraea fragrans* Roxb. Flower Extract Against Antibiotic-Resistant Bacteria

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Abstract

Background and Objective: *Fagraea fragrans* Roxb. is a medicinal plant traditionally used in herbal medicine; however, information regarding the phytochemical composition and antibacterial activity of its flower extract against antibiotic-resistant bacteria remains limited. This study aimed to investigate the phytochemical constituents, antioxidant capacity and antibacterial activity of *F. fragrans* Roxb. flower ethanol extract against selected antibiotic-resistant bacterial strains. **Materials and Methods:** Qualitative phytochemical screening was performed to identify major bioactive compounds in the ethanol extract. Antioxidant activity was assessed using the DPPH radical scavenging assay, while antibacterial activity against clinically important antibiotic-resistant bacteria was evaluated by agar disc diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. Data (mean $\pm$ SD, n = 3) were analyzed by one-way ANOVA with Duncan's test using SPSS v20 (p<0.05). **Results:** Phytochemical analysis revealed the presence of phenolics, flavonoids, alkaloids and terpenoids in the ethanol extract. The extract demonstrated considerable antioxidant activity with strong DPPH radical scavenging capacity. Antibacterial assays showed inhibitory activity against *Acinetobacter baumannii*, *Burkholderia pseudomallei*, carbapenem-resistant Enterobacteriaceae (CRE), multidrug-resistant *Klebsiella pneumoniae* (MDR-K) and *Proteus mirabilis*, with inhibition zone diameters ranging from 7 to 11 mm. The lowest MIC value was observed against *A. baumannii* (1.95 mg/mL), while the lowest MBC value was detected against *B. pseudomallei* (31.25 mg/mL). No detectable antibacterial activity was observed against *Enterococcus faecalis* and colistin-resistant *Pseudomonas aeruginosa*. **Conclusion:** The new findings demonstrated that *F. fragrans* Roxb. flower extract possesses promising phytochemical constituents with antioxidant and antibacterial properties. The extract may serve as a potential natural source of bioactive compounds for herbal medicine and future antimicrobial applications against antibiotic-resistant bacteria.

Key words: *Fagraea fragrans* Roxb., antioxidant activity, antibacterial activity, antibiotic-resistant bacteria, flower extract

Citation: Charoensopharat, K., S. Rattanasuk and K. Wechgama, 2026. Phytochemical profiling, antioxidant potential and antibacterial activity of *Fagraea fragrans* Roxb. flower extract against antibiotic-resistant bacteria. Pak. J. Biol. Sci., 29: 401-410.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotic-resistant bacteria have become a major global public health threat, reducing treatment efficacy and increasing morbidity, mortality and healthcare costs. This crisis has been further exacerbated by the overuse, misuse and improper disposal of antibiotics and it has been projected that antimicrobial resistance could cause up to 10 mL deaths annually by 2050 if effective intervention strategies are not developed¹. Consequently, the search for novel antibacterial agents from natural sources, particularly medicinal plants, has gained increasing urgency². Plant-derived bioactive compounds are especially attractive because they offer chemical diversity, multiple mechanisms of action and the potential for reduced adverse effects compared with many synthetic drugs³. The rapid spread of multidrug-resistant pathogens has intensified interest in phytochemicals as promising alternatives or adjuncts to conventional antibiotics. Many plant-derived compounds have demonstrated substantial activity against drug-resistant bacterial strains and may delay the development of resistance because of their structural complexity and multifaceted modes of action⁴. In addition to their antimicrobial potential, numerous phytochemicals exhibit antioxidant activity, thereby offering a dual therapeutic advantage in conditions where oxidative stress and microbial infection coexist⁵. In particular, polyphenolic compounds, which are widely distributed in the plant kingdom, are recognized for their potent antioxidant and antimicrobial properties and may therefore provide an integrated strategy for addressing both oxidative damage and bacterial resistance⁶.

Medicinal plants are rich sources of biologically active secondary metabolites, such as phenolics, flavonoids, alkaloids and terpenoids, many of which exhibit antioxidant and antimicrobial properties. These compounds may inhibit bacterial growth, reduce survival and interfere with virulence mechanisms, highlighting their potential as alternative therapeutic agents against antibiotic-resistant bacteria⁷. The growing prevalence of multidrug-resistant pathogens, commonly defined as organisms non-susceptible to at least one agent in three or more antimicrobial categories, further underscores the need for alternative treatment strategies⁸. This sustained global interest in natural products highlights their value as a rich reservoir of novel therapeutic agents in the era of escalating antimicrobial resistance⁹. Natural products derived from plants have attracted considerable attention because they may provide safer, more accessible and structurally diverse compounds for pharmaceutical development. Secondary metabolites such as terpenoids,

alkaloids and polyphenols exhibit a broad spectrum of biological activities and are therefore regarded as important templates for the discovery of new antimicrobial agents⁵. Importantly, many of these compounds act through multiple mechanisms, including direct antibacterial effects, disruption of virulence pathways, membrane damage and synergistic interactions with existing antibiotics, thereby helping to circumvent conventional resistance mechanisms¹⁰. Their chemical complexity may also reduce the likelihood of rapid resistance development, further supporting their pharmaceutical potential¹¹.

Fagraea fragrans Roxb., also known as Tembusu, is a tropical plant of medicinal and ecological importance and has long been recognized in Southeast Asian Ethnobotany for its traditional medicinal uses¹². It has reportedly been used in traditional preparations, including herbal teas, for the management of various ailments. Preliminary phytochemical screening has suggested the presence of secondary metabolites such as flavonoids and phenolic compounds, which are commonly associated with antioxidant and antimicrobial activities. Despite this potential, the flower of *F. fragrans* Roxb. has received limited scientific attention and its phytochemical composition and biological activities remain insufficiently documented, particularly in relation to antibiotic-resistant bacteria. This knowledge gap highlights the need for further investigation into the antioxidant and antipathogenic properties of *F. fragrans* Roxb. flower extract. Therefore, the present study aimed to evaluate the total phenolic content, total flavonoid content, antioxidant activity and antibacterial potential of *F. fragrans* Roxb. flower extracts against selected antibiotic-resistant bacterial strains. This work seeks to contribute to the identification of novel plant-derived antimicrobial agents and to support broader efforts to address the growing challenge of antibiotic resistance.

MATERIALS AND METHODS

Study area: The research was conducted from April 2025 to Sep 2025 at the Microbiology Laboratory, Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et, Thailand.

Plant sample collection: Flowers of *Fagraea fragrans* Roxb. were collected from Roi Et Rajabhat University, Roi Et Province, Thailand, in April 2025 (Fig. 1). The plant specimens were deposited in the Vascular Plant Herbarium, Mahasarakham University (VMSU), Kantharawichai District, Maha Sarakham Province, Thailand, under voucher specimen number

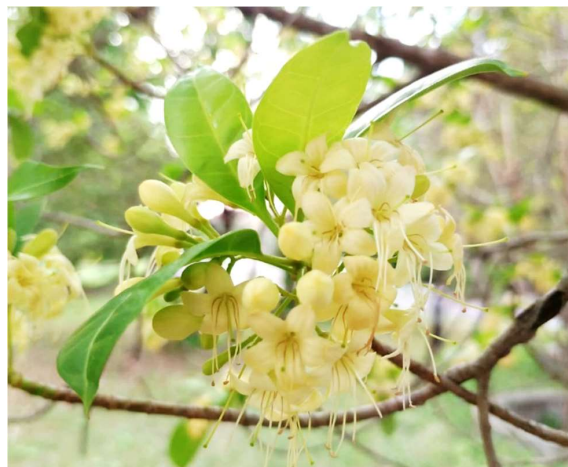


Fig. 1: *Fagraea fragrans* Roxb. flower

Kanlayani02. The flower sample was washed thoroughly to remove dust and impurities, then dried in a hot-air oven at 50°C for 72 hrs or until completely dry. The dried samples were subsequently ground into a fine powder using an herbal grinder. Fifty grams of powdered *F. fragrans* Roxb. flower sample were transferred into Erlenmeyer flasks and extracted with 500 mL of ethanol. Extraction was performed by shaking at room temperature at 150 rpm for 3 hrs. The mixtures were filtered through Whatman No. 1 filter paper and the filtrates were concentrated to dryness using a hot-air oven at 50°C. The percentage yield of each extract was calculated and the crude extracts were dissolved in Dimethyl Sulfoxide (DMSO) to obtain a final concentration of 500 mg/mL. All extracts were stored at -20°C until analysis. The extraction yield was calculated using the following equation¹³⁻¹⁵:

$$\text{Yield (\%)} = \frac{\text{Weight of crude extract obtained}}{\text{Weight of powdered plant sample used for extraction}} \times 100$$

Determination of total phenolic content: Total phenolic content (TPC) was determined using the Folin-Ciocalteu method with gallic acid used as the standard¹⁶. Briefly, 5 g of finely ground sample was extracted with 80% methanol at a ratio of 1:4 and shaken at 150 rpm at 35°C for 24 hrs. The extract was then filtered and 300 µL of the filtrate was mixed with 2.25 mL of Folin-Ciocalteu reagent. After standing at room temperature for 5 min, 2.25 mL of 6% Na₂CO₃ was added. The mixture was shaken thoroughly and kept in the dark at room temperature for 90 min. Absorbance was measured at 725 nm and TPC was calculated from a gallic acid standard curve.

Determination of total flavonoid content: Total flavonoid content (TFC) was analyzed using a modified method with quercetin as the standard¹⁶. Briefly, 0.5 mL of the extract were mixed with 0.15 mL distilled water and 0.15 mL of 5% NaNO₂. After incubation at room temperature for 6 min, 0.3 mL of 10% AlCl₃ were added and the mixture was left for another 5 min. Then, 1 mL of 1 M NaOH was added. Absorbance was measured at 725 nm and TFC was calculated using a quercetin standard curve.

In vitro antioxidant activity assay: Antioxidant activity was evaluated using the DPPH radical scavenging assay, modified from Butsat *et al.*¹⁶. Briefly, 0.10 mL of the extract was mixed with 1.90 mL of DPPH solution. The mixture was shaken thoroughly and incubated in the dark at room temperature for 30 min. Absorbance was then measured at 517 nm and antioxidant activity was calculated based on a trolox standard curve. The percentage of inhibition was calculated using the following equation:

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

Where A_{DPPH} is the absorbance of the DPPH control and A_{sample} is the absorbance of the sample after reaction with DPPH

Bacterial strain and culture conditions: The antibiotic-resistant bacterial strains used in this study, *Acinetobacter baumannii*, *Burkholderia pseudomallei*, carbapenem-resistant Enterobacteriaceae (CRE), *Enterococcus faecalis*, multidrug-

esistant *Klebsiella pneumoniae* (MDR-K) and *Proteus mirabilis*, colistin-resistant *Pseudomonas aeruginosa* (CRP) were obtained from the Department of Microbiology, Roi Et Hospital, Roi Et, Thailand. Pathogenic bacteria were cultured by selecting a single colony and inoculating it into nutrient broth (NB), followed by incubation at 37°C with shaking at 150 rpm for 24 hrs. The bacterial suspension was then adjusted to OD600 = 0.1 before the experiments¹⁷.

Antibacterial activity by agar disc diffusion assay:

Antibacterial activity was determined using the agar disc diffusion method. Briefly, 100 µL of each bacterial suspension adjusted to OD600 = 0.1 was spread onto nutrient agar (NA) plates. Sterile paper discs (5 mm in diameter) were placed on the inoculated agar surface and 10 µL of ethanolic *F. fragrans* Roxb. flower extract was applied onto the discs. DMSO was used as the negative control. The plates were left undisturbed for 10 min to allow complete diffusion of the extracts and then incubated at 37°C for 24 hrs. The diameter of the inhibition zone was measured in millimeters. All tests were performed in triplicate.

Determination of minimum inhibitory concentration and minimum bactericidal concentration:

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *F. fragrans* Roxb. flower extracts were determined using a 96-well microplate assay. Briefly, 100 µL of NB was added to each well and 100 µL of the extract was added to the first well. Two-fold serial dilutions were then prepared from wells 1 to 10. Subsequently, 100 µL of each bacterial suspension was added to the respective wells. The plates were incubated at 37°C for 24 hrs. To determine MIC and MBC values, 50 µL of INT solution (4 mg/mL) was added to each well and the plates were further incubated at 37°C for 30 min. The MIC was defined as the lowest concentration at which a color change to pink was observed after the addition of INT, compared with the dark purple control. The MBC was defined as the lowest concentration at which no color change of the INT solution occurred¹⁸.

Statistical analysis: All experiments were carried out in triplicate and the results were expressed as Mean ± Standard Deviation. Data were analyzed using One-Way Analysis of Variance (ANOVA) and differences among means were determined by Duncan's multiple range test at a significance level of $p < 0.05$. Statistical analysis was performed using SPSS version 20.

RESULTS AND DISCUSSION

Total phenolic content, total flavonoid content and antioxidant activity of *Fagraea fragrans* Roxb. flower extract:

The flower extract of *F. fragrans* Roxb. contained appreciable levels of phenolic and flavonoid compounds and exhibited strong antioxidant activity. The total phenolic content was 105.53 ± 2.34 mg GAE/100 g dry weight, while the total flavonoid content was substantially higher at 360.06 ± 7.89 mg QE/100 g dry weight (Table 1). The higher flavonoid level suggests that flavonoids may represent one of the major antioxidant-associated phytochemical groups in the flower extract. In addition, the extract showed a high DPPH radical scavenging activity of $81.06 \pm 0.84\%$, indicating a strong *in vitro* free radical scavenging capacity. These findings suggest that the antioxidant potential of *F. fragrans* Roxb. flower extract may be closely associated with its phytochemical composition, particularly its flavonoid-rich fraction. The present result is consistent with previous studies reporting that elevated flavonoid content is strongly correlated with enhanced antioxidant activity in plant extracts¹⁹. Similarly, *F. fragrans* Roxb. fruit extracts have previously shown notable antioxidant activity, with one isolated compound demonstrating particularly strong radical scavenging potential²⁰.

The antioxidant effect observed in the present study may also be attributed to the combined action of multiple phytochemical constituents, including flavonoids, terpenoids and saponins, which are widely recognized for their antioxidant properties²¹. The strong antioxidant profile of *F. fragrans* flower also supports its possible value as a natural source of bioactive compounds for nutraceutical and pharmaceutical applications. This interpretation is supported by previous evidence showing that antioxidant-rich plant extracts may also possess relevant antibacterial potential against pathogenic microorganisms²². For example, extracts of Spanish jasmine have been reported to exhibit potent antioxidant activity, with an EC50 value of 13.0 ± 0.06 µg/mL, together with significant antibacterial activity against Gram-negative bacteria such as *Vibrio parahaemolyticus* and several *Staphylococcus* strains, including drug-resistant isolates²³. Phenolic compounds have long been recognized for their diverse biological properties, including antioxidant, antiviral, antibacterial, anticancer, anti-inflammatory and anti-allergic activities²⁴. Their antioxidant effects are primarily associated with their ability to donate hydrogen atoms or electrons to reactive oxygen species, thereby neutralizing free radicals and reducing oxidative stress²⁵. Flavonoids, as an important subgroup of phenolic compounds, contribute substantially to this activity through the action of hydroxyl

Table 1: Total phenolic content, total flavonoid content and DPPH radical scavenging activity of *Fagraea fragrans* Roxb. flower extract

Extract	Total phenolic content (mg GAE/100 g dry wt)	Total flavonoid content (mg QE/100 g dry wt)	DPPH radical scavenging activity (% inhibition)
<i>Fagraea fragrans</i> flower	105.53 ± 2.34	360.06 ± 7.89	81.06 ± 0.84

Table 2: Antibacterial activity of *Fagraea fragrans* Roxb. flower extracts against antibiotic-resistant bacteria determined by agar disc diffusion assay

Bacterial strain	Inhibition zone (mm)
<i>Acinetobacter baumannii</i>	11 ^a
<i>Burkholderia pseudomallei</i>	11 ^a
Carbapenem-resistant Enterobacteriaceae (CRE)	7 ^b
<i>Enterococcus faecalis</i>	-
Multidrug-resistant <i>Klebsiella pneumoniae</i> (MDR-K)	10 ^a
Colistin-resistant <i>Pseudomonas aeruginosa</i> (CRP)	-
<i>Proteus mirabilis</i>	8 ^b

Values followed by different superscript letters within the same column are significantly different at $p < 0.05$ and - indicates no detectable activity

groups and conjugated double bonds, which enable them to scavenge reactive species and inhibit radical formation²⁶. In addition, the reducing power of phenolic compounds, particularly flavonoids, may help terminate free radical chain reactions by converting unstable radicals into more stable products²⁷.

Antibacterial activity of *Fagraea fragrans* Roxb. flower extracts by agar disc diffusion assay:

The antibacterial activity of the ethanol extract of *F. fragrans* Roxb. flowers against selected antibiotic-resistant bacterial strains was evaluated using the agar disc diffusion assay and the results are presented in Table 2. The extract exhibited varying degrees of antibacterial activity depending on the tested bacterial strain, indicating selective inhibitory effects against both Gram-negative and Gram-positive antibiotic-resistant pathogens. The inhibition zone diameters ranged from 7 to 11 mm, demonstrating moderate antibacterial potential of the extract. Among the tested bacteria, the strongest antibacterial activity was observed against *A. baumannii* and *B. pseudomallei*, both of which exhibited inhibition zone diameters of 11 mm (Fig. 1). These results suggest that the ethanol extract contained bioactive compounds capable of suppressing the growth of highly problematic opportunistic pathogens associated with severe nosocomial infections and antimicrobial resistance. The extract also showed considerable inhibitory activity against multidrug-resistant MDR-K, with an inhibition zone diameter of 10 mm. This finding is particularly noteworthy because MDR-K is recognized as an important pathogen associated with resistance to multiple classes of antibiotics, posing major therapeutic challenges in clinical settings.

Moderate antibacterial activity was detected against *P. mirabilis* and CRE, with inhibition zone diameters of 8 and 7 mm, respectively. Although the inhibitory effects against these strains were comparatively lower than those observed

for *A. baumannii* and *B. pseudomallei*, the results still indicate that the extract possesses broad-spectrum antibacterial properties against clinically important resistant bacteria. The relatively smaller inhibition zones observed against CRE may be associated with the complex resistance mechanisms of this bacterial group, including carbapenemase production, reduced membrane permeability and active efflux systems, which can limit the susceptibility of bacterial cells to plant-derived antimicrobial compounds. In contrast, no detectable antibacterial activity was observed against *E. faecalis* and CRP under the experimental conditions used in this study. The absence of inhibition against these strains may reflect intrinsic resistance mechanisms, including reduced cell membrane permeability, biofilm formation and multidrug efflux pumps, which are commonly associated with resistance to both conventional antibiotics and natural antimicrobial agents. Particularly, CRP is known for its highly impermeable outer membrane and adaptive resistance mechanisms, which may reduce the effectiveness of phytochemical compounds present in the extract.

Statistical analysis demonstrated significant differences in inhibition zone diameters among susceptible bacterial strains ($p < 0.05$), as indicated by different superscript letters within the same column. These findings suggest that the antibacterial activity of the ethanol extract was strain-dependent and may be influenced by differences in bacterial cell wall structure, resistance mechanisms and susceptibility to phytochemical constituents.

Minimum inhibitory concentration of *Fagraea fragrans* Roxb. flower extracts:

The minimum inhibitory concentration (MIC) values of *F. fragrans* Roxb. flower ethanol extract against antibiotic-resistant bacteria are presented in Table 3. The extract inhibited five of the seven tested bacterial strains, with MIC values ranging from 1.95 to 7.8 mg/mL. The lowest MIC value was observed against *A. baumannii* at 1.95 mg/mL,

Table 3: Minimum inhibitory concentration (MIC) of *Fagraea fragrans* Roxb. flower extracts against antibiotic-resistant bacteria

Bacterial strain	MIC value (mg/mL)
<i>Acinetobacter baumannii</i>	1.95 ^a
<i>Burkholderia pseudomallei</i>	3.9 ^b
Carbapenem-resistant Enterobacteriaceae (CRE)	3.9 ^b
<i>Enterococcus faecalis</i>	-
Multidrug-resistant <i>Klebsiella pneumoniae</i> (MDR-K)	3.9 ^b
Colistin-resistant <i>Pseudomonas aeruginosa</i> (CRP)	-
<i>Proteus mirabilis</i>	7.8 ^c

Values followed by different superscript letters within the same column are significantly different at $p < 0.05$ and -: Indicates no detectable activity

Table 4: Minimum bactericidal concentration (MBC) of *Fagraea fragrans* flower extracts against antibiotic-resistant bacteria

Bacterial strain	MBC value (mg/mL)
<i>Acinetobacter baumannii</i>	62.5 ^b
<i>Burkholderia pseudomallei</i>	31.25 ^a
Carbapenem-resistant Enterobacteriaceae (CRE)	125 ^c
<i>Enterococcus faecalis</i>	-
Multidrug-resistant <i>Klebsiella pneumoniae</i> (MDR-K)	62.5 ^b
Colistin-resistant <i>Pseudomonas aeruginosa</i> (CRP)	-
<i>Proteus mirabilis</i>	250

Values followed by different superscript letters within the same column are significantly different at $p < 0.05$ and -: Indicates no detectable activity

indicating that this strain was the most susceptible to the ethanol extract. This result is consistent with the agar disc diffusion assay, in which *A. baumannii* also showed one of the largest inhibition zones. The extract showed similar inhibitory effects against *B. pseudomallei*, CRE and MDR-K, with MIC values of 3.9 mg/mL. These results suggest that the ethanol extract possessed comparable antibacterial potency against these clinically important resistant bacteria. Although the inhibition zones differed slightly among these strains, the MIC results indicate that the same extract concentration was required to inhibit visible bacterial growth.

The highest MIC value was recorded against *P. mirabilis* at 7.8 mg/mL, suggesting that this strain was less susceptible to the extract than the other inhibited bacteria. This finding agrees with the relatively smaller inhibition zone observed in the disc diffusion assay. In contrast, no detectable inhibitory activity was observed against *E. faecalis* and CRP under the tested conditions, indicating that these strains may possess stronger intrinsic or acquired resistance mechanisms against the bioactive constituents present in the extract. The MIC results confirmed that *F. fragrans* Roxb. flower ethanol extract exhibited strain-dependent antibacterial activity, with the strongest inhibitory effect against *A. baumannii*, followed by *B. pseudomallei*, CRE and MDR-K.

Minimum bactericidal concentration of *Fagraea fragrans* Roxb. flower extracts:

The minimum bactericidal concentration (MBC) values of *F. fragrans* Roxb. flower ethanol extract against antibiotic-resistant bacteria are presented in Table 4. The extract showed bactericidal activity against five of the seven tested bacterial strains, with MBC values ranging

from 31.25 to 250 mg/mL. The lowest MBC value was observed against *B. pseudomallei* at 31.25 mg/mL, indicating that this strain was the most susceptible to the bactericidal effect of the extract. The extract showed bactericidal activity against *A. baumannii* and MDR-K at 62.5 mg/mL, suggesting a comparable killing effect against these two resistant pathogens. A higher MBC value was recorded against CRE at 125 mg/mL, indicating lower bactericidal sensitivity. The highest MBC value was observed against *P. mirabilis* at 250 mg/mL, suggesting that this strain required the greatest extract concentration for bacterial killing and was the least susceptible among the inhibited strains.

No detectable bactericidal activity was observed against *E. faecalis* and CRP under the tested conditions, which is consistent with the absence of inhibition in the disc diffusion and MIC assays. Statistical analysis indicated significant differences in MBC values among the susceptible strains ($p < 0.05$), confirming that the bactericidal effect of the ethanol extract was strain-dependent. The MBC results demonstrated that *F. fragrans* flower ethanol extract possessed measurable bactericidal activity against several antibiotic-resistant bacteria, particularly *B. pseudomallei*, *A. baumannii* and MDR-K.

The present study demonstrated that the ethanol extract of *F. fragrans* Roxb. flowers possesses promising antioxidant and antibacterial activities against several clinically important antibiotic-resistant bacteria. Although *F. fragrans* has traditionally been used in herbal medicine and previous studies have reported the phytochemical constituents of different plant parts, scientific information regarding the antibacterial activity of its flower extract against

multidrug-resistant pathogens remains limited²⁸. Therefore, this study helps address an important research gap by providing evidence of the antibacterial potential of *F. fragrans* Roxb. flowers against resistant bacterial strains, including *A. baumannii*, MDR-K, CRP and *B. pseudomallei*. The observed antibacterial activity suggests that the flower extract may contain bioactive phytochemicals capable of inhibiting the growth of resistant pathogens that are increasingly associated with therapeutic failure and hospital-acquired infections.

The antibacterial assays revealed that the ethanol extract exhibited varying inhibitory effects depending on the bacterial species tested. Among the susceptible strains, *A. baumannii* showed the lowest MIC value, whereas *B. pseudomallei* exhibited the lowest MBC value, indicating high sensitivity to the extract. These findings are particularly important because both pathogens are recognized as serious opportunistic bacteria associated with multidrug resistance and high mortality rates²⁹. The antibacterial activity observed in the present study is comparable to previous reports describing the efficacy of plant-derived extracts against resistant bacteria. For example, ethanolic plant extracts and natural products have demonstrated inhibitory effects against MDR-K, *A. baumannii* and other pathogenic bacteria, although their antibacterial potency varies considerably depending on plant species, extraction methods and phytochemical composition¹⁵. Similar antibacterial activities have also been reported for extracts of *Garcinia mangostana* and *Melaleuca cajuputi*, which showed inhibitory effects against resistant bacterial strains³⁰. The present findings therefore support the growing body of evidence indicating that medicinal plants remain valuable sources of antibacterial compounds for combating antimicrobial resistance.

The ability of the *F. fragrans* Roxb. extract to inhibit Gram-negative bacteria is particularly noteworthy because Gram-negative pathogens generally exhibit greater resistance to antimicrobial agents due to the presence of an outer lipopolysaccharide membrane, reduced membrane permeability, efflux pumps and enzymatic degradation systems³¹. Despite these protective mechanisms, the extract demonstrated measurable inhibitory and bactericidal activities against several Gram-negative resistant pathogens, suggesting that the phytochemicals present in the extract may interfere with bacterial membrane integrity or other essential cellular processes. Previous studies have reported that plant-derived compounds, particularly flavonoids and phenolic compounds, can exert antibacterial effects through several mechanisms, including disruption of bacterial cell membranes, inhibition of nucleic acid synthesis, interference

with enzyme activity and suppression of protein synthesis. Therefore, the phenolics, flavonoids, alkaloids and terpenoids identified in the present study may act synergistically and contribute to the observed antibacterial activity of the extract³².

In addition to antibacterial activity, the ethanol extract exhibited considerable antioxidant capacity, which may further enhance its therapeutic potential. Antioxidant compounds are important because oxidative stress is closely associated with inflammation, cellular damage and disease progression. The antioxidant activity observed in this study is likely associated with the phenolic and flavonoid constituents identified in the extract, as these compounds are widely recognized for their free radical scavenging properties. Several medicinal plants rich in phenolics and flavonoids have similarly demonstrated both antioxidant and antibacterial activities, suggesting that these bioactive compounds contribute to multiple pharmacological effects simultaneously³³. The coexistence of antioxidant and antibacterial properties in *F. fragrans* Roxb. flowers therefore increases their potential value for herbal medicine and pharmaceutical applications. Although the extract demonstrated promising antibacterial activity, no detectable inhibitory effect was observed against *E. faecalis* and CRP. This lack of susceptibility may be associated with intrinsic and acquired resistance mechanisms, including biofilm formation, reduced permeability and highly efficient efflux systems, which commonly limit the effectiveness of both conventional antibiotics and plant-derived compounds. Similar observations have been reported in other studies involving resistant *P. aeruginosa*, which is recognized as one of the most difficult pathogens to control due to its complex resistance mechanisms³⁴. Nevertheless, the activity observed against other resistant strains highlights the selective antibacterial potential of *F. fragrans* Roxb. extract.

The present findings suggest that *F. fragrans* Roxb. flowers represent a promising candidate for further pharmacological investigation. Future studies should focus on bioactivity-guided fractionation, compound isolation, spectroscopic characterization and mechanistic evaluation to identify the specific constituents responsible for the observed biological activities. In addition, *in vivo* efficacy, toxicity and safety assessments are necessary to validate the therapeutic potential of the extract and support its development as a natural antimicrobial agent. Such investigations are essential for expanding the limited scientific knowledge regarding *F. fragrans* Roxb. and for advancing the development of plant-based therapeutic strategies against emerging antibiotic-resistant pathogens.

CONCLUSION

Fagraea fragrans Roxb. flower demonstrated promising phytochemical, antioxidant and antibacterial properties against selected antibiotic-resistant bacteria. The extract contained appreciable amounts of total phenolics and, notably, a relatively high level of total flavonoids, which may contribute to its strong DPPH radical scavenging activity. Among the tested solvent extracts, the ethanol extract showed the broadest and most potent antibacterial activity, with the lowest MIC and MBC values against several resistant bacterial strains. These findings indicate that *F. fragrans* Roxb. flower is a promising natural source of bioactive compounds with potential application as an antioxidant and antibacterial agent. Further studies should focus on the isolation and characterization of the active constituents, elucidation of their mechanisms of action and evaluation of their safety and efficacy in *in vivo* systems to support future pharmaceutical or nutraceutical development.

SIGNIFICANCE STATEMENT

This study provides new scientific evidence demonstrating the antioxidant and antibacterial activities of *F. fragrans* Roxb. flower extract against clinically important antibiotic-resistant bacteria. The findings address the limited information available regarding the medicinal potential of *F. fragrans* Roxb. flowers and highlight their potential as a natural source of bioactive phytochemicals, including phenolics and flavonoids. The extract exhibited inhibitory and bactericidal activities against multidrug-resistant pathogens such as *A. baumannii*, MDR-K and CRE. These results support the potential application of *F. fragrans* Roxb. in herbal medicine and provide a foundation for future studies involving bioactive compound isolation, mechanism-of-action analysis and development of plant-based antimicrobial agents to combat antibiotic-resistant infections.

ACKNOWLEDGMENT

This research received funding support from the Fundamental Fund of Rajabhat Maha Sarakham University through the National Science, Research and Innovation Fund (NSRF), Thailand. Grant No. FRB690009/0208.

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