



RESEARCH ARTICLE

Antibacterial activities of ethanolic extract of four species of Rutaceae family

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ABSTRACT

In this study, the antibacterial activity of ethanolic extract from the leaves of four Rutaceae species, including *Acronychia pedunculata*, *Clausena excavata*, *Glycosmis pentaphylla* and *Luvunga scandens*, were performed using the agar disk diffusion method for the first time. The ethanolic extracts from the leaves of *A. pedunculata* and *G. pentaphylla* were able to resist against all six bacterial strains with zones of inhibition for *Bacillus cereus* (17.3±2.1 mm, 20.8±1.0 mm) *Staphylococcus aureus* (8.5±0.5 mm, 17.6±0.3 mm) *Escherichia coli* (16.7±2.1 mm, 15.3±1.2 mm), *Pseudomonas aeruginosa* (11.7±0.6 mm, 14.0±1.7 mm), *Salmonella enteritidis* (22.3±0.6 mm, 24.6±0.5 mm) and *Salmonella typhimurium* (9.5±0.9 mm, 8.3±0.6 mm). On the other hand, the ethanolic extract of *C. excavata* leaf was resistant to *B. cereus* (12.3±0.6 mm), *S. aureus* (11.6±0.5 mm), *E. coli* (11.5±2.1 mm), *P. aeruginosa* (10.6±0.3 mm) while *B. cereus* (8.2±0.3 mm), *S. aureus* (9.3±0.6 mm), *E. coli* (8.5±0.5 mm) and *S. typhimurium* (8.3±0.6 mm) were inhibited by the ethanolic extract of *L. scandens* leaf. This study could provide necessary information for further application of these species in medicine.

Introduction

Medicinal plants are rich source of antimicrobial agents. However, the potential of vascular plants as a source for a new medicine is still largely unexplored. Only a small percentage have been recorded phytochemically among the estimated about 500000 species (1) in which the medicinal aspects and antimicrobial agents of Rutaceae plants were investigated by some previous studies (2, 3).

The Rutaceae consists of 156 genera and over 1800 species, which are widely distributed throughout the tropical and subtropical regions, particularly in Southeast Asia (4). Members of Rutaceae are important for their natural resources providing many useful products, such as food, medicines, spices and essential oil etc (5, 6). Many biologically-active compounds, including saponins, steroids, cardiac glycosides, alkaloids and flavonoids were isolated from Rutaceae species (7). Furthermore, the

antimicrobial activities of their extracts from different solvents have been reported in previous studies (8–10).

Vietnam has been home to a diversity of Rutaceae species with over 100 species recorded (11, 12). Furthermore, Binh Chau-Phuoc Buu Nature Reserve is the only remaining sandy forest along the coastline of Vietnam for species of the Dipterocarpaceae family, which is located in Xuyen Moc District, Ba Ria-Vung Tau Province, Vietnam. In the report of the Management Board of Binh Chau-Phuoc Buu Nature Reserve, this area has 796 plant species belonging to 142 genera. To date, there are many studies about this area which research and evaluate the diversity of plant species or record the new species in this area for the flora of Vietnam (11, 12). However, their bioactivity is still unknown. The present study envisages to study the antibacterial activities of ethanolic extracts from the leaves of four species, including *Acronychia pedunculata*, *Clausena excavata*,

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Glycosmis pentaphylla and *Luvunga scandens* collected from Binh Chau-Phuoc Buu Nature Reserve, Xuyen Moc District, Ba Ria-Vung Tau Province, Vietnam providing necessary information for further application of these species.

Materials and Methods

Plant materials

Specimens of following species viz., *Acronychia pedunculata*, *Clausena excavata*, *Glycosmis pentaphylla* and *Luvunga scandens* (Fig. 1) were collected from Binh Chau-Phuoc Buu Nature Reserve. Taxonomic identification was done using morphological vegetative and reproductive characters following the aforementioned literature (11-12). All vouchered specimens were deposited at the Herbarium of Binh Chau-Phuoc Buu Nature Reserve (Table 1). Furthermore, the ecological

attributes of four studied species were also presented in Table 1.

Bacterial strains

In this study, six bacterial strains were used to determine the antibacterial activity of ethanol extract from the leaves of four species, including two Gram-positive bacteria (*Bacillus cereus* (ATCC 11774) and *Staphylococcus aureus* (ATCC 25923) and four Gram-negative bacteria (*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enteritidis* (ATCC 13976), *Salmonella typhimurium* (ATCC 13311)). These bacterial strains were obtained from the microbiology collection of Department of Biotechnology, Institute of Food and Biotechnology, Industrial University of Ho Chi Minh City, Vietnam. All bacterial strains were preserved at -20°C in 20% glycerol solution. Before the analysis, the strains were cultured in Luria-Bertani broth at 37°C for 24 hrs (5, 9).



Fig. 1. Four studied species in habitat. A. *Acronychia pedunculata*, B. *Clausena excavata*, C. *Glycosmis pentaphylla*, D. *Luvunga scandens*.

Table 1. Detailed information of four specimens in Binh Chau-Phuoc Buu Nature Reserve

| Vouchered specimens | Scientific names | Collected sites | Ecological attributes |
|---------------------|-------------------------------|----------------------------|--|
| Le VS 351 | <i>Acronychia pedunculata</i> | 10°32'55"N; 107°29'12"E | Evergreen forests, growing with <i>Melaleuca</i> , <i>Hopea</i> , <i>Madhuca</i> , on sandy or sandy soil. |
| Le VS 352 | <i>Clausena excavata</i> | 10°32'47"N; 107°28'57"E | Semi-evergreen or evergreen forests |
| Le VS 353 | <i>Glycosmis pentaphylla</i> | 10°32'46"N; 107°30'27"E | Growing on Grassland at forest edge |
| Le VS 354 | <i>Luvunga scandens</i> | 10°32'46"N; 107°30'27"E | Semi-evergreen or evergreen forests |

Extraction procedure

Samples of fresh leaves were sliced and dried at 50°C until constant weight. The samples were then pulverized into a fine powder, 50 gm of which was immersed in 450 ml of ethanol 98% for five weeks at room temperature. The ethanolic extract was filtered through Whatman filter paper, and subsequently concentrated in reduced pressure at 60°C to obtain the brown medicinal paste (13). To ensure the absolute absence of ethanol in the paste, sublimation dryer was used.

Antibacterial activities

The antibacterial assay was conducted according to the standard method (14). The bacteria were cultured in Luria-Bertani (LB) Broth until a turbidity of 0.5 McFarland standards was reached. Subsequently, 100 µl of bacterial suspensions were spread on the surface of sterile Mueller Hinton plate, on which sterile 6 mm diameter discs were placed. 20 µl of the sample was pipetted into each disc. The plates were maintained at 4°C for 2 hrs to allow extract diffuse completely into the medium before being incubated at 37°C for 24 hrs. The antibacterial activity of the sample was then determined by measuring the diameter of the inhibition zone. Sterilized distilled water was used as negative control while Gentamycin antibiotic discs (supplied by Nam Khoa BioTek, Vietnam) was a positive control (14).

Data analysis

The experiments were performed in triplicates. The results were presented as mean ± standard deviation (SD) from triplicate analyses, and the differences among experimental groups were determined by Fisher's least significant difference (LSD) procedure using Statgraphics Centurion XV software (Statpoint Technologies Inc, Virginia, USA) with the criterion of statistical significance was set as $p < 0.05$.

Results

The antibacterial activity of ethanolic extracts from leaves of four studied species was evaluated by measuring the diameter of inhibition zone against tested bacteria (Table 2 and Fig. 2). The ethanolic extracts of two species, including *A. pedunculata* and *G. pentaphylla* resisted against six tested bacterial strains while others could inhibit the growth of four strains. Among the four species, the diameters of inhibition zones of ethanol extract of *A. pedunculata* leaves against *S. enteritidis*, *B. cereus*, *E. coli*, *P. aeruginosa*, *S. typhimurium* and *S. aureus* were

22.3±0.6 mm, 17.3±2.1 mm, 16.7±2.1 mm, 11.7±0.6 mm, 9.5±0.9 mm and 8.5±0.5 mm respectively. Furthermore, the ethanolic extracts of *G. pentaphylla* exhibited a strong antibacterial activity against *S. enteritidis* (24.6±0.5 mm), followed by *B. cereus*, (20.8±1.0 mm), *S. aureus* (17.6±0.3 mm), *E. coli* (15.3±1.2 mm), *S. typhimurium* (14.8±0.7 mm) and *P. aeruginosa* (14.0±1.7 mm). On the other hand, the best antibacterial activity of *C. excavata* was shown in undiluted ethanolic extract with zones of inhibition for *B. cereus* (12.3±0.6 mm), while the inhibition zones of *S. aureus*, *E. coli* and *P. aeruginosa* were 11.6±0.5 mm, 11.5±0.5 mm and 10.6±0.3 mm respectively. Finally, the *L. scandens* sample showed the antibacterial activity against *S. aureus*, *E. coli*, *S. typhimurium* and *B. cereus* in which their zones of inhibition were 9.3±0.6 mm, 8.5±0.5 mm, 8.3±0.6 mm and 8.2±0.3 mm respectively.

The antibacterial activities of ethanolic extract from leaves of four studied species were proved to be able to inhibit strong antibacterial activity against all tested bacteria in which many specimens had approximate inhibition zones as compared those of positive control (Table 3). Accordingly, the antibacterial activity of *A. pedunculata* leaves were shown in ethanolic extract with zones of inhibition for *B. cereus* (17.3±2.1 mm), *E. coli* (16.7±2.1 mm) whereas those of positive control were 18.3±0.6 mm, 17.8±1.0 mm respectively. Furthermore, the antibacterial activity of ethanolic extract of some specimens were higher than positive control with gentamycin discs. For instance, the diameters of inhibition zones of ethanolic extract from *G. pentaphylla* leaves against *B. cereus*, *S. enteritidis* and *S. aureus* were 20.8±1.0 mm, 24.6±0.5 mm, 17.6±0.3 mm respectively while those of positive control were 19.7 ±1.2 mm, 23.0±1.7 mm, 15.2±0.3 mm respectively. Similarly, the antibacterial effect of *A. pedunculata* leaves was stronger than in ethanolic extract with zones of inhibition for *S. enteritidis* (22.3±0.6 mm) positive control (21.3±0.6 mm).

Discussion

The antibacterial activities of the ethanolic extracts from the leaves of four species in present study, *A. pedunculata*, *C. excavata*, *G. pentaphylla* and *L. scandens* are limited. This paper is the first research showing the antibacterial activity of ethanolic extracts from the leaves of these species. However, certain previous studies showed the antimicrobial activities of the extracts from some different solvents from other species belonging to Rutaceae (8-9, 10, 15,

Table 2. The inhibition zone of ethanol extract from the leaves of four studied species against six tested bacteria.

| Tested bacteria | Growth inhibition zone (mm) | | | |
|-----------------------|-----------------------------|-----------------------|-----------------------|----------------------|
| | <i>A. pedunculata</i> | <i>C. excavata</i> | <i>G. pentaphylla</i> | <i>L. scandens</i> |
| <i>B. cereus</i> | 17.3±2.1 ^c | 12.3±0.6 ^b | 20.8±1.0 ^d | 8.2±0.3 ^a |
| <i>E. coli</i> | 16.7±2.1 ^c | 11.5±2.1 ^b | 15.3±1.2 ^c | 8.5±0.5 ^a |
| <i>P. aeruginosa</i> | 11.7±0.6 ^a | 10.6±0.3 ^a | 14.0±1.7 ^b | - |
| <i>S. enteritidis</i> | 22.3±0.6 ^a | - | 24.6±0.5 ^b | - |
| <i>S. typhimurium</i> | 9.5±0.9 ^a | - | 14.8±0.7 ^b | 8.3±0.6 ^a |
| <i>S. aureus</i> | 8.5±0.5 ^a | 11.6±0.5 ^b | 17.6±0.3 ^c | 9.3±0.6 ^a |

Different lowercase letters in the same row denote significant differences ($p < 0.05$) between various extracts. (-): No inhibition.

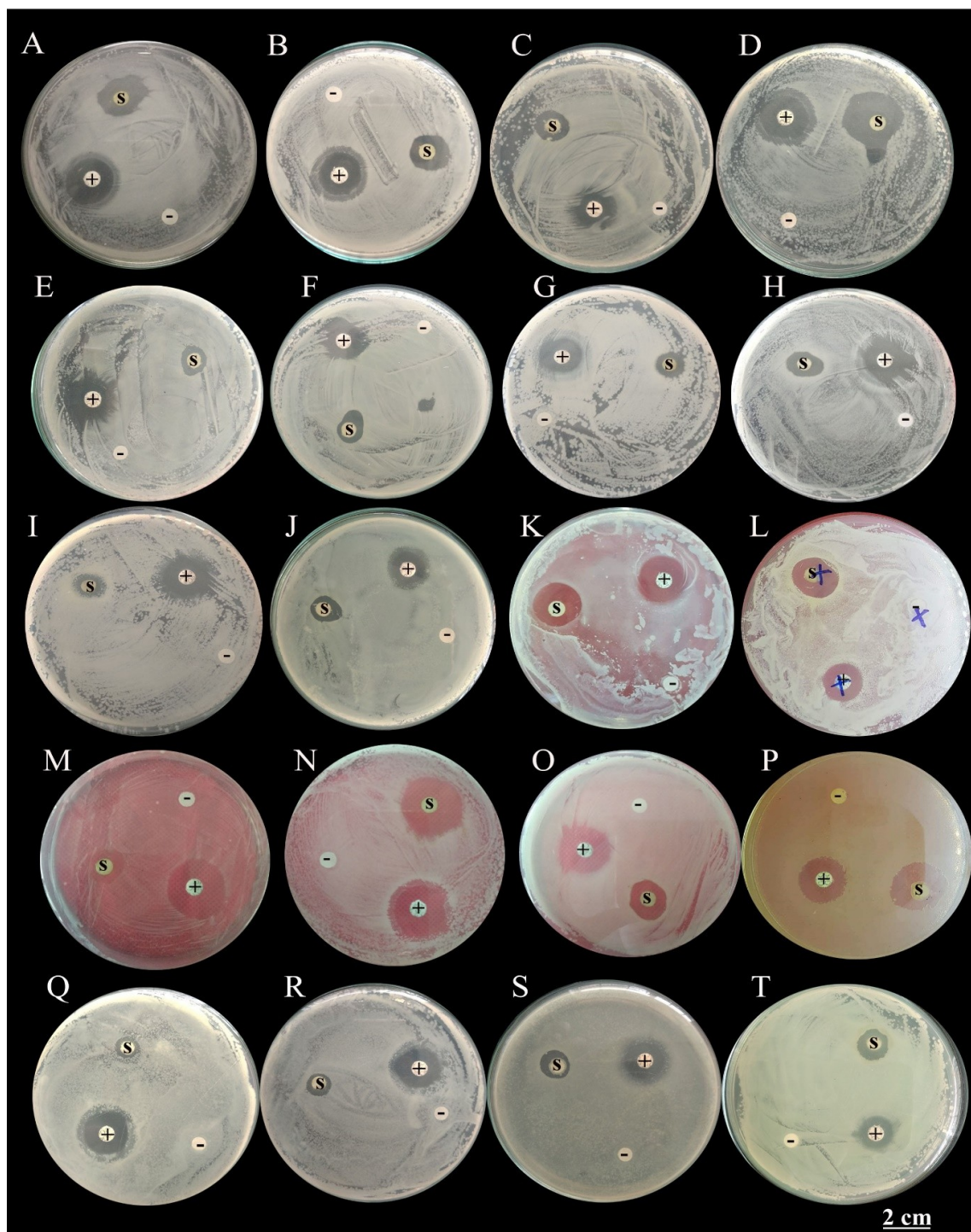


Fig. 2. Antibacterial activity of ethanol extract of four studied species against tested bacteria. *A. pedunculata* (A. *B. cereus*, B. *E. coli*, C. *P. aeruginosa*, D. *S. enteritidis*, E. *S. typhimurium*, F. *S. aureus*); *C. excavata* (G. *B. cereus*, H. *E. coli*, I. *P. aeruginosa*, J. *S. aureus*); *G. pentaphylla* (K. *B. cereus*, L. *E. coli*, M. *P. aeruginosa*, N. *S. enteritidis*, O. *S. typhimurium*, P. *S. aureus*); *L. scandens* (Q. *B. cereus*, R. *E. coli*, S. *S. typhimurium*, T. *S. aureus*). (-) Negative control with sterilized distilled water, (+) Positive control with discs containing gentamicin, (S) Samples.

Table 3. The inhibition zone of positive control with gentamycin antibiotic discs.

| Tested bacteria | Growth inhibition zone (mm) | | | |
|-----------------------|-----------------------------|------------------------|------------------------|------------------------|
| | <i>A. pedunculata</i> | <i>C. excavata</i> | <i>G. pentaphylla</i> | <i>L. scandens</i> |
| <i>B. cereus</i> | 18.3±0.6 ^{bc} | 17.8±0.3 ^{ab} | 19.7 ±1.2 ^c | 16.7±0.6 ^a |
| <i>E. coli</i> | 17.8±1.0 ^b | 16.8±0.8 ^{ab} | 15.5±0.5 ^a | 16.6±0.6 ^{ab} |
| <i>P. aeruginosa</i> | 14.0±0.0 ^a | 14.2±0.3 ^a | 17.7±0.6 ^c | 16.3±0.6 ^b |
| <i>S. enteritidis</i> | 21.3±0.6 ^{ab} | 19.6±1.2 ^a | 23.0±1.7 ^b | 21.5±0.5 ^{ab} |
| <i>S. typhimurium</i> | 16.1±0.8 ^a | 18.2±0.8 ^b | 17.8±0.3 ^b | 15.5±0.9 ^a |
| <i>S. aureus</i> | 13.6±0.6 ^a | 15.5±0.6 ^b | 15.2±0.3 ^b | 13.0±0.9 ^a |

Different lowercase letters in the same row denote significant differences ($p < 0.05$) between various extracts.

16). For instance, the hexane, chloroform, ethyl acetate, methanol and acetone extracts from *Glycosmis pentaphylla* leaf could be resistant to *S. aureus*, *E. coli* and *S. pneumoniae* (10). Similarly, the chloroform and ethyl acetate extracts from the leaves of three Rutaceae species (*Esenbeckia grandiflora*, *Pilocarpus spicatus* and *Galipea simplicifolia*) were able to resist against *Escherichia coli* and *Staphylococcus aureus* (9). Furthermore, the methanolic extract isolated from the stem bark of *Teclea afzelii* were resistant to 4 Gram-negative bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*), 2 Gram-negative bacteria strains (*Staphylococcus aureus* and *Bacillus subtilis*) (15). Moreover, the acetone and ethanol extracts of *Coleonema album* were resistant to some bacteria, including *E. coli*, *B. subtilis*, *E. faecalis*, *P. aeruginosa*, *S. aureus*, *M. smegmatis*, *M. tuberculosis* (8). Studies revealed that the dichloromethane, ethyl acetate and methanol extract of *L. scandens* were resistant to *S. aureus*, *B. cereus*, *E. faecalis* and *E. coli* (16).

Many previous studies have demonstrated that chemical compounds which isolated from studied specimens in this study have been proved to be resistant to many bacterial strains (1, 17-19). Accordingly, the extract of *A. pedunculata* contained polyphenol, triterpene alcohols and acetophenones. These components had the ability to inhibit the activity of *Salmonella enterica* and *Staphylococcus epidermidis* (17-18). Similarly, another study has shown that 10 chemical compounds, including coumarins, dentatin, nordentatin, clausenidin, xanthoxyletin carbazole derivatives, 3-formylcarbazole, mukonal, 3-methoxy-carbonylcarbazole, murrayanine, 2-hydroxy-3-formyl-7-methoxycarbazole and clauszoline J isolated from *C. excavata* were resistant to *Mycobacterium tuberculosis* (19). Furthermore, *G. pentaphylla* contain important bioactive chemical constituents, including alkaloids, flavonoids, saponins, steroids, terpenoides, polysaccharides and tannins which could inhibit the growth of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pneumonia* (1).

Conclusion

In this study, the antibacterial activity of ethanolic extract from the leaves of four Rutaceae species were firstly investigated. The ethanolic extracts from the leaves of *A. pedunculata* and *G. pentaphylla* were able to resist against all six bacterial strains, such as *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia*

coli, *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *S. typhimurium*. The ethanolic extract of *C. excavata* leaf was resistant to *B. cereus*, *S. aureus*, *E. coli*, *P. aeruginosa* while *B. cereus*, *S. aureus*, *E. coli* and *S. typhimurium* were inhibited by the ethanolic extract of leaves of *Luvunga scandens*. The antibacterial activity of leaves of *A. pedunculata* had approximate inhibition zones as compared those of positive control whereas diameters of inhibition zones of ethanolic extract from *G. pentaphylla* and *A. pedunculata* were higher than positive control. This study could provide necessary information for further application of these species.

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Authors' contributions

The present study was designed by Hong Thien Van. The samples were collected by Van Son Le, a staff of Binh Chau-Phuoc Buu Nature Reserve. All authors performed experiments and handled the research data. Data analysis was conducted by Hong Thien Van. Hong Thien Van drafted the manuscript and resolved all the queries of reviewers.

Conflict of interests

No conflict of interest was declared by the authors.

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