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Research Article

SCREENING ANTIBACTERIAL ACTIVITY OF VIETNAMESE PLANT EXTRACTS AGAINST HUMAN PATHOGENIC BACTERIA

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ABSTRACT

Objectives: Infectious diseases caused by bacteria are a leading cause of death worldwide. Hence, the objectives of the study are aimed to evaluate the antibacterial activity against five human pathogenic bacteria of methanolic extracts from 66 plants collected from Vietnam.

Methods: The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of methanol extracts of 66 plant species against five bacterial strains.

Results: In this study, all the plant extracts were active against at least one train with MIC values ranging from 24 to 2048 µg/mL. Twenty-five plant extracts were active against all three Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis,* and *Staphylococcus aureus*). Of these, the extracts of *Macaranga trichocarpa* (Rchb. f. and Zoll.) Mull. Arg. (*Euphorbiaceae*), *Calophyllum inophyllum* L. (*Clusiaceae*) and *Caryodaphnopsis baviensis* (*Lecomte*) Airy Shaw (*Lauraceae*) exhibited the highest antibacterial activity (MIC =24–128 µg/mL), followed by extracts of *Betula alnoides* Buch.-Ham. e × . D. Don (*Betulaceae*), *Acronychia pedunculata* (L.) Miq. (*Rutaceae*), *Croton alpinus* A. Chev. ex Gagnep. (*Euphorbiaceae*) (MIC =64–256 µg/mL). Furthermore, the extract of *Rhus chinensis* Mill. (*Anacardiaceae*) and *Annona reticulata* L. (*Annonaceae*) exhibited potent antibacterial activity against the two *Bacillus* species (MIC =32–64 µg/mL).

Conclusion: Results of this study reveal that plant extracts from Vietnam have highly antibacterial activity against Gram-positive bacteria. These results suggest that Vietnamese plant extracts may be a rich source of antibacterial drugs.

Keywords: Screening, Antibacterial, Human pathogenic bacteria, Vietnamese plant extracts.

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INTRODUCTION

Infectious diseases remain a major health concern, being the second leading cause of death worldwide, and remain a dominant feature of domestic and international public health considerations in the 21st century [1,2]. Bacterial infections are prevalent in developing countries due to factors such as inadequate sanitation, poor hygiene, and overcrowded living conditions [3]. Antibiotics have proven to be powerful drugs for control of infectious diseases and remain one of the most important discoveries in modern medicine [4]. At present, the world is facing the widespread emergence of bacterial resistance to antibiotics [2]. Antibiotic resistance has been recognized by the World Health Organization as the greatest threat in the treatment of infectious diseases [4].

To combat antibiotic resistance, the development of new antibacterial agents that suppress bacterial resistance mechanisms is necessary. Plants have traditionally provided a source of new chemicals, and numerous clinical studies have demonstrated the therapeutic value of molecules of plant origin [4]. Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants as traditional antibiotics [5]. Indeed, higher plant-derived products represent ~25% of drugs in current clinical use [4].

Considering the therapeutic potential of plants, the aim of the present study was to evaluate the *in vitro* antibacterial activity of Vietnamese plant extracts against five human pathogenic bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis,* and *Staphylococcus aureus,* which cause food poisoning and various infections in the community and in hospitals [6,7].

METHODS

Plant materials and extraction

A total of 68 plant leaves and branches were collected from different locations in Vietnam in 2012. Plant species were identified by Associate Prof. Xuan Phuong Vu, Dr. The Bach Tran and Dr. The Cuong Nguyen from the Institute of Ecology and Biological Resources, Vietnam. Voucher specimens have been preserved in the Herbarium of the Department of Phytochemistry and Research and Development Center of Bioactive Compounds, Vietnam Institute of Industrial Chemistry. Plant extracts were prepared as described in our previous study [8]. Briefly, air-dried and powdered aerial parts of the plant species (10 g) were extracted twice with 100 mL of methanol for 48 h at room temperature. Extracts were filtered and the filtrates were evaporated to dryness using a rotary evaporator, and then stored at −20°C until further use. Then, for the antibacterial activity assays, the extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL and stored at 4°C as stock solutions.

Bacterial culture

Five bacterial species, comprising three Gram-positive (*B. cereus* American Type Culture Collection [ATCC] 21768, *B. subtilis* ATCC 6633, and *S. aureus* ATCC 6538) and two Gram-negative (*E. coli* ATCC

25922 and *P. aeruginosa* ATCC 9027) strains, were purchased from the (ATCC; Manassas, VA, USA). Bacterial strains were cultured on nutrient agar plates and incubated aerobically at 37°C for 24 h. For *in vitro* antibacterial assays, the bacteria were cultured in nutrient broth (NB) and incubated aerobically at 37°C for 24 h, and the bacterial suspension was adjusted to 10^5 cfu/ml.

Antibacterial assay

The antibacterial activities of plant extracts were evaluated by broth microdilution assays and minimum inhibitory concentration (MIC) values were determined. Twofold serial dilutions of each plant extract were added to the wells of sterile 96-well plates containing 100 µl of inoculated NB medium. The final concentrations of the plant extracts were 15.6–2000 µg/ml and the final bacterial density was 10^5 cfu/ml. DMSO (2%) was used as a negative control and did not affect bacterial growth; streptomycin sulfate was used as a positive control. The plates were incubated at 37°C for 24 h. The MIC values were determined as the lowest concentration that completely inhibited the growth of bacteria.

For weakly soluble plant extracts, the MIC was determined following addition of 20 µl of 0.2 mg/ml iodonitrotetrazolium chloride (Sigma-Aldrich, Korea) to the test wells and incubation at 37°C for 3 h. Viable bacteria reduce this yellow dye to pink [8]. Experiments were conducted twice in triplicate for each extract against individual bacterial species at all test concentrations.

RESULTS AND DISCUSSION

A total of 68 plant extracts were evaluated for antibacterial activity against five human pathogenic bacteria using the broth microdilution method. The plant extracts exhibited considerable antibacterial activity against the Gram-positive bacteria. *B. cereus* exhibited the greatest susceptibility to all of the plant extracts, followed by *B. subtilis* and *S. aureus*. Twenty-four plant extracts had the antibacterial activity against all three Gram-positive bacteria. Of these, the extracts of *Macaranga trichocarpa*, *Calophyllum inophyllum*, and *Caryodaphnopsis baviensis* had the highest antibacterial activity (MICs 31.3–125 µg/ml). Extracts of *Acronychia pedunculata, Betula alnoides, Croton alpinus, Garcinia cowa,* and *Annona reticulata* showed slightly weaker antibacterial activity (MICs 62.5–250 µg/ml). To the best of our knowledge, this is the first report of antibacterial activity of *C. alpinus*. In contrast, antibacterial activities and phytochemistry have been described for *A. pedunculata* [9], *B. alnoides* [10], *G. cowa* [11-13], and *A. reticulata* [14,15]. Furthermore, the extract of *Rhus chinensis* exhibited potent antibacterial activity against *Bacillus* species with MIC values of 31.3 µg/ml (Table 1). Conversely, most of the test plant extracts were inactive against the two Gram-negative bacteria. Only the extract of *C. inophyllum* at a concentration of 2000 µg/ml completely inhibited the growth of *E. coli* (Table 1).

In the following sections, the plants showing the greatest activities against Gram-positive bacteria are discussed in terms of their known phytochemical components, together with biological and pharmacological activities.

C. inophyllum

All parts of *C. inophyllum* have considerable therapeutic properties, such as antiseptic, expectorant, diuretic, astringent, and purgative effects [16]. The fruit oil is beneficial in treatment of rheumatism, gonorrhea, and itching, the gum extracted from the stems is used to treat ulcers, and a decoction from the bark is used for hemorrhage and ulcers [17]. Oil of seeds and roots is used to treat wounds and scabies [16]. Phytochemical components of this plant have been reported previously [17]. Extracts from leaves and other parts exhibited antibacterial activities against various bacteria as described in the previous reports [16,18]. In our study, the methanol extract of leaves and branches of *C. inophyllum* had marked antibacterial activity against Gram-positive bacteria (MICs 31.3–62.5 µg/ml) and at 2000 µg/ml completely inhibited the growth of *E. coli* (Table 1).

C. baviensis

In this study, the methanol extract of *C. baviensis* exhibited significant antibacterial activity against Gram-positive bacteria (MICs 62.5–125 µg/ml) (Table 1). At present, there is lack of scientific documentation on the pharmacological and biological activities, as well as the phytochemical components, of *C. baviensis*. To the best of our knowledge, isolation of compounds from this plant has been reported only by Anh *et al*. [19], and this is the first report of antibacterial activity of an extract of *C. baviensis*.

M. trichocarpa

The methanol extract of *M. trichocarpa* showed the strongest antibacterial activity against three Gram-positive bacteria among the 68 plant extracts tested in this study (MICs 31.3–62.5 µg/ml) (Table 1). Leaves of some *Macaranga* species are used in folk medicine to treat swellings, cuts, sores, boils, and bruises. This genus is reported to be a rich source of isoprenylated, geranylated, and farnesylated flavonoids. Flavonoids and stilbenes are major constituents and most likely responsible for the activities of plants of this genus [20]. Flavanones and dihydrochalcones have been reported to show antibacterial activities against various bacterial species, including *B. subtilis*, *S. aureus*, *E. coli,* and *P. aeruginosa* [21]. However, the methanol extract of *M. trichocarpa* did not inhibit the growth of *E. coli* and *P. aeruginosa* at the maximum concentration tested (2000 µg/ml) (Table 1).

R. chinensis

This plant has long been used by practitioners of folk medicine in Asia. *R. chinensis* plant parts, particularly the galls on its leaves, *Galla chinensis*, have preventative and therapeutic effects on diverse ailments, including diarrhea, dysentery, rectal and intestinal cancer, diabetes mellitus, sepsis, oral diseases, and inflammation. Phytochemical studies on *R. chinensis* have demonstrated that it contains high levels of two phenolic compounds, gallic acid and methyl gallate. Recent studies revealed that *R. chinensis* compounds possess strong antiviral, anticancer, hepatoprotective, and antioxidant activities. Extracts from *G. chinensis* inhibited several bacteria, including *B. cereus*, *B. subtilis*, *S. aureus, E. coli,* and others (MICs 0.5–8 mg/ml) [22]. In our study, the methanol extract of *R. chinensis* exhibited considerable antibacterial activity against the Gram-positive bacteria, especially against the two *Bacillus* species (MIC 31.3 µg/ml) (Table 1).

CONCLUSION

The Vietnamese plant extracts investigated in this study significantly suppressed the growth of Gram-positive bacteria. Discovery of biological activities in Vietnamese plants is a new venture. Although the antibacterial activities of some highly antibacterial plant extracts have been reported, *C. baviensis* and *C. alpinus* extracts have not previously been reported to show potent antibacterial activities.

Our results provide important information on the antibacterial activities of Vietnamese plant extracts to medical plant consumers, pharmacologists and researchers. Some Vietnamese plant extracts have potential for application as natural antibacterial agents and can be used for the development of new antibacterial drugs.

To develop new plant-derived antibacterial agents, further studies are necessary to isolate and characterize the active components from the antibacterial plants. Furthermore, additional research on combinations of the antibacterial components or plants with other antimicrobial agents would be useful to enhance their antibacterial potency.

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Table 1: Antibacterial activities of plant extracts against human pathogenic bacteria[a]

No.	Plant species	Family	MIC (μg/mL)[b]		
			Bacillus cereus	*Bacillus subtilis*	*Staphylococcus aureus*
1.	*Barleria prionotis* L.	*Acanthaceae*	75±26	128±0	512±0
2.	*Ptyssiglottis kunthiana* (Wall. ex Nees) B. Hansen	*Acanthaceae*	597±209	–[c]	–
3.	*Alangium chinense* (Lour.) Harms	*Alangiaceae*	171±66	–	–
4.	*Rhus chinensis* Mill.	*Anacardiaceae*	32±0	32±0	427±132
5.	*Toxicodendron succedaneum* (L.) Kuntze	*Anacardiaceae*	1024±0	768±280	1024±0
6.	*Annona reticulata* L.	*Annonaceae*	64±0	64±0	384±140
7.	*Acorus gramineus* Soland. Ex Ait.	*Araceae*	1195±418	1877±418	2048±0
8.	*Amorphophallus paeoniifolius* (Dennst.) Nicolson	*Araceae*	128±0	256±0	–
9.	*Cryptolepis buchananii* Roem. and Schult.	*Asclepiadaceae*	341±132	–	–
10.	*Chromolaena odorata* (L.).King and Robinson	*Asteraceae*	–	–	768±280
11.	*Eupatorium fortunei* Turcz.	*Asteraceae*	384±140	–	–
12.	*Tithonia diversifolia* (Hemsl.) A. Gray	*Asteraceae*	–	512±0	768±280
13.	*Betula alnoides* Buch.-Ham. ex D. Don	*Betulaceae*	64±0	64±0	256±0
14.	*Markhamia stipulata* (Wall.) Seem. Ex K. Schum.	*Bignoniaceae*	384±140	–	–
15.	*Spathodea campanulata* P. Beauv.	*Bignoniaceae*	384±140	–	–
16.	*Plumbago zeylanica* L.	*Plumbaginaceae*	192±70	–	–
17.	*Canarium tramdenum* Dai and Yakovl.	*Burseraceae*	384±140	–	–
18.	*Calophyllum inophyllum* L.	*Clusiaceae*	32±0	53±17	64±0
19.	*Garcinia cowa* Roxb.	*Clusiaceae*	256±0	256±0	384±140
20.	*Quisqualis indicca* L.	*Combretaceae*	1024±0	512±0	1024±0
21.	*Rourea harmandiana* Pierre	*Connaraceae*	768±280	–	–
22.	*Hopea chinensis* (Merr.) Hand. Mazz	*Dipterocarpaceae*	48±18	512±0	128±0
23.	*Croton alpinus* A. Chev. ex Gagnep.	*Euphorbiaceae*	128±0	128±0	192±70
24.	*Endospermum chinense* Benth.	*Euphorbiaceae*	384±140	–	–
25.	*Euphorbia atoto* Forst. f.	*Euphorbiaceae*	384±140	–	–
26.	*Macaranga trichocarpa* (Rchb. f. and Zoll.) Mull. Arg.	*Euphorbiaceae*	64±0	24±9	24±9
27.	*Mallotus yunnanensis* Pax and K. Hoffm.	*Euphorbiaceae*	768±280	–	–
28.	*Albizia vialeana* Pierre	*Fabaceae*	192±70	–	–
29.	*Cassia siamea* Lam.	*Fabaceae*	768±280	–	–
30.	*Castanopsis ceratacantha* Rehd. and Wils.	*Fabaceae*	256±0	512±0	768±280
31.	*Indigofera galegoides* DC.	*Fabaceae*	192±70	–	–
32.	*Lithocarpus ducampii* (Hickel and A. Camus) A. Camus	*Fagaceae*	384±140	–	–
33.	*Millettia setigera* Dunn	*Fabaceae*	384±140	–	–
34.	*Lysionotus chingii* Chun ex W.T. Wang	*Gesneriaceae*	149±52	512±0	683±264
35.	*Caryodaphnopsis baviensis* (Lecomte) Airy Shaw	*Lauraceae*	64±0	128±0	64±0
36.	*Litsea verticillata* Hance	*Lauraceae*	1195±418	1365±529	–
37.	*Barringtonia macrostachya* (Jack) Kurz	*Lecythidaceae*	1024±0	299±105	597±209
38.	*Magnolia coco* (Lour.) DC	*Magnoliaceae*	384±140	–	–
39.	*Manglietia insignis* (Wall.) Bl.	*Magnoliaceae*	768±280	–	–
40.	*Stachyphrynium placentarium* (Lour.) Clausager and Borchs.	*Maranthaceae*	768±280	–	–
41.	*Chisocheton cumingianus* subsp. *balansae* (C. DC.)Mabb.	*Meliaceae*	171±66	–	–
42.	*Melia azedarach* L.	*Meliaceae*	1365±529	2048±0	1195±418
43.	*Toona sureni* (Blume) Merr.	*Meliaceae*	341±132	–	–
44.	*Knema mixta* W. J. de Wilde	*Myristicaceae*	683±264	–	–
45.	*Myrsine linearis* (Lour.) Poir.	*Myrsinaceae*	597±209	853±264	683±264
46.	*Olax imbricata* Roxb.	*Olacaceae*	1195±418	597±209	1024±0
47.	*Piper hymenophyllum* Miq.	*Piperaceae*	1365±529	–	–
48.	*Rhamnus longipes* Merr. and Chun	*Rhamnaceae*	512±0	256±0	256±0
49.	*Ziziphus attopensis* Pierre	*Rhamnaceae*	192±70	–	–
50.	*Carallia brachiata* (Lour.) Merr.	*Rhizophoraceae*	341±132	1195±418	–
51.	*Coptosapelta flavescens* Korth.	*Rubiaceae*	128±0	256±0	–
52.	*Acronychia pedunculata* (L.) Miq.	*Rutaceae*	75±26	256±0	171±66
53.	*Clausena indica* (Dalzell) Oliv.	*Rutaceae*	384±140	–	–
54.	*Glycosmis petelotii* Guill.	*Rutaceae*	192±70	–	–
55.	*Luvunga sarmentosa* (Blume) Kurz	*Rutaceae*	192±70	–	–
56.	*Zanthoxylum nitidum* (Roxb.) DC.	*Rutaceae*	341±132	853±264	512±397
57.	*Scrophularia ningpoensis* Hemsl.	*Scrophulariaceae*	1024±0	1707±529	1365±529
58.	*Brucea javanica* (L.) Merr.	*Simaroubaceae*	683±264	2048±0	384±140
59.	*Brucea mollis* Wall. ex Kurz	*Simaroubaceae*	768±280	–	–
60.	*Sonneratia caseolaris* (L.) Engl.	*Sonneratiaceae*	683±264	683±264	–
61.	*Sterculia hyposticta* Miq.	*Sterculiaceae*	768±280	–	–
62.	*Schima surpeba* Gardner and Champ.	*Theaceae*	–	1024±0	384±140
63.	*Aphananthe aspera* (Thunb.) Planch.	*Ulmaceae*	768±280	–	–
64.	*Holoptelea integrifolia* Roxb. Planch.	*Ulmaceae*	384±140	–	–
65.	*Callicarpa dichotoma* (Lour.) Raeusch.	*Verbenaceae*	256±0	256±0	683±264
66.	*Tectona grandis* L.	*Verbenaceae*	128±0	384±140	–
	Streptomycin sulfate (positive control)		5±0	2.5±0	5±0

[a]All plant extracts were inactive against two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) at maximum tested concentration (2048 μg/mL), [b]Values are mean±SD of two experiments with three replicates each, [c]Inactive at maximum tested concentration (2048 μg/mL).

CONFLICT OF INTERESTS

All authors declare that they have no competing interests.

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