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 strain 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 (Leconte) Airy Shaw (Laureaceae) exhibited the highest antibacterial activity (MIC = 24–128 µg/mL) followed by extracts of Betula alnoides Buch-Ham. e. × D. Don (Betulaceae), Arctocyclus pedunculata (L.) Miq. (Rutaceae), Croton alpinus A. Chev. ex Dagnep. (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.

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. 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
exhibited considerable antibacterial activity. Twenty-four plant extracts had the antibacterial activity. 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]. Flavonones 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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| No. | Plant species | Family | MIC (µg/mL)* |
|-----|---------------|--------|--------------|
|     |               |        | Bacillus cereus | Bacillus subtilis | Staphylococcus aureus |
| 1.  | Barleria priotitis L. | Acanthaceae | 75±26 | 128±0 | 512±0 |
| 2.  | Pygysstricta kunthiana (Wall. ex Nees) B. Hansen | Acanthaceae | 597±209 | – | – |
| 3.  | Alangium chinense (Lour.) Harms | Alangiaceae | 174±66 | – | – |
| 4.  | Rhiz chinensis Mill. | Anacardiaceae | 32±0 | 32±0 | 427±132 |
| 5.  | Toxicoderrn succedaneum (L.) Kuntze | Anacardiaceae | 102±0 | 768±200 | 102±0 |
| 6.  | Annona reticulata L. | Annonaceae | 64±0 | 64±0 | 384±140 |
| 7.  | Acorus gramineus Soland. ex Ait. | Araceae | 1195±18 | 1877±18 | 2048±0 |
| 8.  | Amorphophallus paeonifolius (Dennst.) Nicolson | Araceae | 128±0 | 256±0 | – |
| 9.  | Cryptolepis buchanani Roem. and Schult. | Asclepiadaceae | 341±132 | – | – |
| 10. | Chromolaena odorata (L.) King and Robinson | Asteraceae | – | – | 768±280 |
| 11. | Exapatorium fortunei Turcz. | Asteraceae | 394±140 | – | – |
| 12. | Tithonia diversifolia (Hems.) 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 (Will) Seem. ex K. Schum. | Bignoniaceae | 384±140 | – | – |
| 15. | Spathodea campanulata P. Beauv. | Bignoniaceae | 384±140 | – | – |
| 16. | Plumbago zeylanica L. | Plumbaginaceae | 192±70 | – | – |
| 17. | Canarium trandum 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 indica L. | Combretaceae | 102±4 | 512±0 | 102±40 |
| 21. | Rourea harmandiana Pierre | Conneraceae | 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. | Alibizia vialeana Pax | Fabaceae | 192±70 | – | – |
| 29. | Cassia siamea Lam. | Fabaceae | 768±280 | – | – |
| 30. | Castanopsis ceratocarpa Rehd. and Wils. | Fabaceae | 256±0 | 512±0 | 768±280 |
| 31. | Indigofera galgoides DC. | Fabaceae | 192±70 | – | – |
| 32. | Lithocarpus ducapaceae Hickel and A. Camus | Fabaceae | 384±140 | – | – |
| 33. | Milletia setigera Dunn | Fabaceae | 384±140 | – | – |
| 34. | Lysonotus chinii Chun ex W.T. Wang | Gesneriaceae | 149±52 | 512±0 | 683±264 |
| 35. | Caryodophyndon bavensis (Leomonte) Airy Shaw | Lauraceae | 64±0 | 128±0 | 64±0 |
| 36. | Litsea verticillata Hance | Lauraceae | 1195±18 | 1365±529 | – |
| 37. | Barringtonia macracanthya (Jack) Kurz | Lecythidaceae | 102±40 | 299±105 | 597±209 |
| 38. | Magnolia coco (Lour.) DC | Magnoliaceae | 384±140 | – | – |
| 39. | Mangiellia insignis (Wall.) Bl | Magnoliaceae | 768±280 | – | – |
| 40. | Stachyphrynium placentarium (Lour.) | Marcantaceae | 768±280 | – | – |
|     | Clausager and Borchs. | | | | |
| 41. | Chisocheton cunningianus subsp. balanae (C.D.C)Mabb. | Meliaceae | 171±66 | – | – |
| 42. | Melia azedarach L. | Meliaceae | 136±529 | 2048±0 | 1195±18 |
| 43. | Toona sureni (Blume) Merr. | Meliaceae | 341±132 | – | – |
| 44. | Kneema mixta W. J. de Wilde | Myristicaceae | 683±264 | – | – |
| 45. | Myrsine linearis (Lour.) Poir. | Myrsinaceae | 597±209 | 853±264 | 683±264 |
| 46. | Olax impricata Roxb. | Oleaceae | 1195±18 | 597±209 | 102±40 |
| 47. | Piper henryphyllum Miq. | Piperaceae | 136±529 | – | – |
| 48. | Rhamnus longipes Merr. and Chun | Rhamnaceae | 512±0 | 256±0 | 256±0 |
| 49. | Ziziphus atrospenis Pierre | Rhamnaceae | 192±70 | – | – |
| 50. | Carallia brachiata (Lour.) Merr. | Rhzophoraceae | 341±132 | 1195±18 | – |
| 51. | Coposspalatia flavescens Korth. | Rubiaceae | 128±0 | 256±0 | – |
| 52. | Acronychia pedunculata (L.) Miq. | Rutaceae | 75±26 | 256±0 | 171±66 |
| 53. | Clausena indica (Dalz) Oliv. | Rutaceae | 384±140 | – | – |
| 54. | Glycosmis petelogii Guill. | Rutaceae | 192±70 | – | – |
| 55. | Luvunga sarmentosa (Blume) Kurz | Rutaceae | 192±70 | – | – |
| 56. | Zanthoxyllum nitidum (Roxb.) DC. | Rutaceae | 341±132 | 853±264 | 512±397 |
| 57. | Schrophularia ningpoensis Hemsl. | Scrophulariaceae | 102±40 | 1707±529 | 136±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 hypstocida Miq. | Sterculiaceae | 768±280 | – | – |
| 62. | Schima surpepa Gardner and Champ. | Theaceae | – | 102±40 | 384±140 |
| 63. | Aphanaestheaspina (Thunb.) Planch. | Ulmaceae | 768±280 | – | – |
| 64. | Holoptelea integrifolia Rodb. Planch. | Ulmaceae | 384±140 | – | – |
| 65. | Callicarpa dichotoma (Lour.) Rauwch. | Verbenaceae | 256±0 | 256±0 | 683±264 |
| 66. | Tecctonia grandis L. | Verbenaceae | 256±0 | 384±140 | – |
|     | Streptomyceu sulfate (positive control) | – | 5±0 | 2.5±0 | 5±0 |

*All plant extracts were inactive against two Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) at maximum tested concentration (2048 µg/mL). *Values are mean±SD of two experiments with three replicates each, *Inactive at maximum tested concentration (2048 µg/mL).
CONFLICT OF INTERESTS
All authors declare that they have no competing interests.

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