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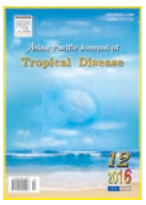

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Antibacterial activity of *Vitex parviflora* A.Juss. and *Cyanthillium cinereum* (L.) H.Rob. against human pathogens

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ABSTRACT

This study investigated the antibacterial activities of methanolic crude extracts of *Vitex parviflora* (*V. parviflora*) leaves and stem and *Cyanthillium cinereum* (*C. cinereum*) leaves and roots. Crude methanolic extracts of *V. parviflora* and *C. cinereum* were tested for their antibacterial activities against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The leaf and stem extracts of *V. parviflora* and root extracts of *C. cinereum* exhibited high antibacterial activity against *Staphylococcus aureus*. Increasing concentration of the *V. parviflora* leaves and roots crude extracts resulted in an increasing anti-staphylococcal activity as exhibited by increasing diameters of zone of inhibition. Root extracts of *C. cinereum* exhibited inverse dose-response relationship. The anti-staphylococcal activity was the highest at the lowest concentration (25 mg/mL) and it decreased as the concentration increased. This study established the anti-staphylococcal activity of methanolic crude extracts of *V. parviflora* leaves and stem and *C. cinereum* roots. This study also validated the use of these plants in folkloric medicines in the indigenous communities in the Philippines.

1. Introduction

Plants are widely used in healthcare all over the world. The World Health Organization estimated that around 80% of the total world population still rely on traditional medicines for their primary healthcare[1]. Eighty percent of the 122 plant-derived drugs were related to their original ethnopharmacological purpose. Plants that are used in traditional medicines have an advantage in drug discovery because of their long-term use by humans. Bioactive compounds from these plants have high tendency to have low toxicity to humans[2]. Natural products from medicinal plants have been discovered to possess antimicrobial properties against human pathogens[3]. In the Philippines, plants are used as alternative treatment for some common infectious bacterial diseases. Several studies have validated the antibacterial activities of the Philippine medicinal plants that are commonly used in traditional medicine[4-6].

Vitex parviflora (*V. parviflora*), locally known in the Philippines as "molave" or "mulawin", is a medium-sized to fairly large tree. It is most commonly found in regions with wet and dry seasons. It is used as medicinal herb in the Philippines. Ayta communities in Pampanga, Philippines utilize leaf and stem of *V. parviflora* as insect repellent[7]. In the province of Zamboanga, Philippines, its barks and roots are used to treat toothache, irregular menstruation, goiter, ulcer, anemia and acidity[8].

Cyanthillium cinereum (*C. cinereum*) is a weed commonly present in tropical and subtropical regions of the world. This weed is locally known as "kulantro"[9]. Previous study showed that *C. cinereum* has antioxidant protective effects against oxidative damage to biological molecules such as lipids and DNA. It also showed high efficiency in inhibiting hemolysis of erythrocytes and mild dose-dependent cytotoxic activity against human breast carcinoma[10]. The leaves of *C. cinereum* exhibited analgesic, antipyretic and anti-inflammatory properties[11].

The present study aimed to evaluate the antibacterial activities of methanolic crude extracts of *V. parviflora* leaves and stem and *C. cinereum* leaves and roots against several human bacterial pathogens. Results obtained in this study may contribute to the growing information about the indigenous materials that can be used for drug discovery.

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2. Materials and methods

2.1. Collection of plant specimens

V. parviflora and *C. cinereum* were collected from Tubo-tubo, Dinalupihan, Bataan in December 2015. Samples of the functional parts of these plants were collected for taxonomic identification. The habitat, morphological characteristics and reproductive parts of the plants were photographed and noted. Voucher specimens were preserved and submitted to the Mr. Danilo Tandang from the Botany Division of the National Museum of the Philippines for identification and authentication.

2.2. Extraction of methanol crude extracts

Air-dried plant samples were ground using a blender. Plants were soaked in 95% methanol for two days. Supernatant was obtained through filtration using Whatman filter paper. Extracts were pooled and concentrated under reduced pressure at 40 ℃ using a rotary evaporator yielding a certain amount of crude extract. Dried extracts were dissolved in 95% methanol prior to antimicrobial assay to obtain final concentrations of 100 mg/mL, 50 mg/mL and 25 mg/mL. Crude extracts were stored in 4 ℃ refrigerator until used.

2.3. Inoculum preparation

Two Gram-positive bacteria, *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*) and two Gram-negative bacteria *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) were used in this study. These bacterial strains were obtained from the Department of Medical Microbiology, College of Public Health at the University of the Philippines Manila. Prior to testing for antimicrobial activity, test organisms were reactivated by transferring them into a nutrient agar slant and incubating them at 37 ℃ for 24 h. Fresh cultures were then transferred to nutrient broth. The bacterial suspension was compared and adjusted to the turbidity of 0.5 McFarland standard giving a final inoculum concentration of 1.5×10^8 CFU/mL.

2.4. Antimicrobial assay

A sterile cotton swab was dipped into the bacterial culture and was spread evenly over the surface of Mueller-Hinton agar plate. Antibiotic discs that were known to be susceptible to each test microorganism were used as positive controls. Gentamycin for *S. aureus*, amikacin for *B. subtilis* and ciprofloxacin for *E. coli* and *P. aeruginosa* were the positive control used. The negative control used was 95% methanol. Different concentrations of the plant crude extracts were used in this assay specifically 100 mg/mL, 50 mg/mL and 25 mg/mL. The plant crude extracts were pipetted onto a sterile 6 mm paper disk. Mueller-Hinton agar plates preloaded with discs with respective extract and test organism were incubated at 37 ℃ for 24 h. Antimicrobial activities were evaluated by measuring the diameters of zones of inhibition in millimeters against the test organism using vernier caliper. All the tests were carried out in triplicate and their means were recorded.

2.5. Statistical analyses

The data were expressed as mean ± SD. One-way ANOVA and Tukey honest significant difference *post-hoc* analysis were carried out to test for any significant difference between the means using SPSS 24.0 (SPSS Inc., Chicago, USA). Differences between means at $P \leqslant 0.05$ level were considered significant.

3. Results and discussion

Results obtained from this study demonstrated anti-staphylococcal activity of *V. parviflora* and *C. cinereum* (Table 1). This study showed antibacterial activities of both leaves and stem of *V. parviflora* against *S. aureus*. Anti-staphylococcal activity of methanolic crude extracts of *V. parviflora* demonstrated a direct dose-response relationship. Increasing concentration of crude extracts of the *V. parviflora* leaves and stem (25 mg/mL, 50 mg/mL and 100 mg/mL) resulted in an increasing anti-staphylococcal activity as exhibited by increasing diameters of zone of inhibition. *V. parviflora* leaves exhibited low antibacterial activity against *E. coli*. It did not inhibit the growth of other bacteria (*B. subtilis* and *P. aeruginosa*) used in this study.

Table 1
Antibacterial activity of methanol extract of *V. parviflora* and *C. cinereum* using disc diffusion method.

Methanol extract	Concentration	Zone of inhibition (mm)			
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
V. parviflora leaves	100 mg/mL	15.70 ± 1.20	–	6.33 ± 0.09	–
	50 mg/mL	13.37 ± 1.68	–	6.47 ± 0.25	–
	25 mg/mL	10.97 ± 0.95	–	6.70 ± 0.22	–
	Positive control	27.07 ± 1.02	24.67 ± 2.88	48.97 ± 1.68	28.53 ± 4.32
	Negative control	–	–	–	–
V. parviflora stem	100 mg/mL	13.83 ± 2.58	–	–	–
	50 mg/mL	9.07 ± 0.66	–	–	–
	25 mg/mL	6.53 ± 0.12	–	–	–
	Positive control	27.70 ± 2.28	25.87 ± 2.16	47.00 ± 3.18	25.37 ± 1.96
	Negative control	–	–	–	–
C. cinereum leaves	100 mg/mL	–	–	6.80 ± 0.05	–
	50 mg/mL	6.47 ± 0.17	–	6.77 ± 0.09	–
	25 mg/mL	–	–	6.57 ± 0.24	–
	Positive ccontrol	29.20 ± 1.91	27.80 ± 1.91	47.70 ± 3.16	26.50 ± 1.87
	Negative control	–	–	–	–
C. cinereum roots	100 mg/mL	13.40 ± 0.60	–	–	–
	50 mg/mL	13.37 ± 0.81	–	–	–
	25 mg/mL	16.93 ± 1.80	–	–	–
	Positive control	27.03 ± 1.19	24.57 ± 1.84	44.70 ± 1.56	27.37 ± 0.97
	Negative control	–	–	–	–

A previous study conducted by Ragasa *et al.*[12] isolated retusin, a flavone, from chloroform extract of *V. parviflora* leaves. Retusin showed low activity against *P. aeruginosa* and no activity against *E. coli*, *S. aureus* and *B. subtilis*. Another study isolated terpenes and sterols from *V. parviflora* and these phytochemicals exhibited antimutagenic properties using micronucleus test[13]. Flavonoids, triterpenoids, lignans and iridoid glycosides have been isolated from *V. parviflora*[14]. These phytochemicals possibly contribute to the antibacterial activity of crude extracts from *V. parviflora*. Plant-derived flavonoids such as flavone, chromanones, chalcones and their derivatives are among the most common natural products with a broad spectrum of antibacterial activity[15]. Several isolated triterpenoids also exhibited significant antibacterial activity against Gram-positive bacteria[16].

There are previous studies that documented the therapeutic potential of *C. cinereum* against different diseases. One recent study showed antimicrobial activity of *C. cinereum* ethyl acetate fractions against human bacterial pathogens[10,17]. This study showed high antibacterial activity of methanolic crude extracts of *C. cinereum*

roots against *S. aureus* and mild antibacterial activity against *E. coli* (Table 1). There was an inverse dose-response relationship in the anti-staphylococcal activity of *C. cinereum* roots. The anti-staphylococcal activity of *C. cineremeum* roots was the highest at the lowest concentration (25 mg/mL) and it decreased as the concentration increased. It did not exhibit antibacterial activity against the other bacteria (*B. subtilis* and *P. aeruginosa*) used in this study.

Phytochemical analysis of petroleum ether, ethanol and aqueous extracts of *C. cinereum* revealed that it contains several phytochemicals such as alkaloids, phenols, tannins, saponins, steroids, glycosides, flavonoids, carbohydrates, phlorotannins and terpenoids[18]. These phytochemicals and secondary metabolites have been documented to exhibit antimicrobial activities[19,20].

However, statistical analysis revealed that the difference between the antibacterial activity of the crude extracts tested in this study and the antibiotics used as positive control was statistically significant. Although the methanolic crude extracts of *V. parviflora* and *C. cinereum* showed significantly lower antibacterial activity compared to commercial antibiotics, they might contain novel bioactive compounds present in low concentration. Further purification and isolation of compounds might increase the antibacterial activity of these plants. These same compounds on further purification might demonstrate increased antimicrobial activity.

4. Conclusion

This study established the anti-staphylococcal activity of methanolic crude extracts of *V. parviflora* leaves and stem and *C. cinereum* roots. This study also validated the use of these plants in folkloric medicines in the indigenous communities in the Philippines. Further investigations such as determination of microbial inhibitory concentration, bioassay-guided isolation and purification of compounds should be performed in *V. parviflora* and *C. cinereum* to support the use of these plants as novel and alternative sources of antibacterial agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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