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.
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 °C 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 °C 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 °C 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 °C 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 ≤ 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 methanic 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>
<thead>
<tr>
<th>Methanol extract</th>
<th>Concentration</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. parviflora leaves</td>
<td>100 mg/mL</td>
<td>15.70 ± 1.20</td>
</tr>
<tr>
<td></td>
<td>50 mg/mL</td>
<td>13.37 ± 1.68</td>
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<tr>
<td></td>
<td>25 mg/mL</td>
<td>10.97 ± 0.95</td>
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<tr>
<td>Positive control</td>
<td>27.07 ± 1.02</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>V. parviflora stem</td>
<td>100 mg/mL</td>
<td>13.83 ± 2.58</td>
</tr>
<tr>
<td></td>
<td>50 mg/mL</td>
<td>9.07 ± 0.66</td>
</tr>
<tr>
<td></td>
<td>25 mg/mL</td>
<td>6.53 ± 0.12</td>
</tr>
<tr>
<td>Positive control</td>
<td>27.37 ± 2.28</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>C. cinereum leaves</td>
<td>100 mg/mL</td>
<td>13.40 ± 0.60</td>
</tr>
<tr>
<td></td>
<td>50 mg/mL</td>
<td>13.37 ± 0.81</td>
</tr>
<tr>
<td></td>
<td>25 mg/mL</td>
<td>16.93 ± 1.80</td>
</tr>
<tr>
<td>Positive control</td>
<td>27.03 ± 1.19</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>–</td>
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</table>

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 bacterial[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 methanic 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. cinereum 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.

Acknowledgments

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References