PJHRD Philippine Journal of Health Research and Development

RESEARCH COMMUNICATION

Antibacterial property of leaf ethanol extracts of *Persea americana* Mill. variants against *Staphylococcus aureus*

Christian Paolo S. Vidal*, Mary Ann C. Sison, Evalyn A. Roxas

*Corresponding author's email address: csvidal@up.edu.ph

Department of Medical Microbiology, College of Public Health, University of the Philippines Manila, Manila, Philippines

ABSTRACT

Background: Antibacterial resistance poses a significant health and economic burden worldwide. Relatedly, plant-based drug discovery remains an important adjunct to modern pharmaceutical research and development. Plants have an extensive record of being used as a form of alternative healthcare. For instance, the leaves of *Persea americana* Mill. (avocado) were used by traditional medicinal healers in Africa and the Philippines to alleviate common ailments such as skin ulcers and diarrhea.

Objectives: To determine their antibacterial activity, leaves of different *P. americana* variants maintained at the National Plant Genetic Resources Laboratory, University of the Philippines Los Baños were subjected to disk diffusion assay.

Methodology: Four *P. americana* leaf ethanol extracts (Cardinal, Morado, Semil 1, and Semil 2 variants) were tested in a range of concentrations (5 mg to 5 µg) against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) using standard disc diffusion.

Results: At 5 mg, all four *P. americana* leaf variants exhibited an inhibitory activity against *S. aureus*. Furthermore, Semil 2 variant showed the strongest relative antibacterial potential with activity at lower concentrations of 1.25 mg and 0.31 mg. On the other hand, all four variants did not suppress *E. coli* growth at the concentrations used.

Conclusion: The results indicate the potential antibacterial activity of *P. americana* leaf variants against *S. aureus* which is consistent with their ethnomedicinal use as a skin ulcer treatment since *S. aureus* is a common etiologic agent of skin ulcers. Furthermore, the findings suggest the four *P. americana* leaf variants, particularly Semil 2, as possible sources of novel antibacterial compounds against *S. aureus*.

Keywords: *anti-bacterial agents, diarrhea, phytochemicals, plant extracts, skin diseases*

Introduction

Antibacterial resistance threatens to render existing antibiotics ineffective [1]. Failure of several classes of antibiotics to inhibit previously susceptible bacteria was already reported in tertiary hospitals in Asia, America, and India [2-4]. Accordingly, common pathogens like *Staphylococcus aureus* and *Escherichia coli* have been associated with greater hospital costs and worse clinical outcomes in patients [5-8]. Antibacterial-resistant infections cause at least 50,000 deaths annually in Europe and the United States and are estimated to result in a global economic loss of US $100 trillion by 2050 [9]. Hence, there is an urgency to discover novel antibacterial leads from different ecological fields such as plants [10].

Plants are recognized as an important source of medicines [11]. Extracts of various plant species were reported to possess several pharmaceutical properties which include bacterial growth inhibition [12-14]. The bioactivities of plant extracts are derived from their secondary metabolites such as phenols, alkaloids, and lectins, which are affected by environmental factors such as light, temperature, humidity, and soil quality, as well as by predation and pathogenesis [15-17]. Around 80% of the population of developing countries have relied on plants as a form of primary health care which further demonstrates their medicinal value [18].

An example of a plant used in ethnomedicine is *Persea americana*, also known as avocado. Its leaves were used by

traditional healers in Africa, America, and India to treat various diseases including skin ulcers, tuberculosis, and bronchitis [19-22]. These practices may be supported by the in vitro antibacterial activity of *P. americana* leaf alcohol extracts against *E. coli*, *S. aureus*, and *Mycobacterium tuberculosis*, among others [23-26]. In the Philippines, the metabolites of *P. americana* leaves are concentrated through boiling in hot water and consumed as a decoction to treat diarrhea [27]. Even though secondary metabolite production is affected by geographical differences and growth conditions, only one other study has been done to substantiate the ethnomedicinal applications of *P. americana* leaves in the Philippines [28]. In this study, the authors tested the leaf ethanol extracts of four Philippine *P. americana* variants using standard disc diffusion against *S. aureus* and *E. coli*—which are significant etiologic agents of skin infections and diarrhea, respectively [29,30]. The study recorded the antibacterial effects of four *P. americana* leaf variants (Cardinal, Morado, Semil 1, and Semil 2) in a range of concentrations (5 mg to 5 µg) against *S. aureus* and *E. coli*.

Methodology

Preparation of P. americana Leaf Ethanol Extracts

The preparation of leaf ethanol extracts was adapted from the methods of Das *et al.* (2010) [31]. *Persea americana* leaves in an amount of 2 kg were collected for Cardinal, Morado, Semil 1, and Semil 2 variants from the National Plant Genetic Resources Laboratory (NPGRL), University of the Philippines Los Baños (UPLB). The leaves were washed with distilled water and air-dried in a well-ventilated and shaded area for 3 days. Afterwards, the leaves were oven-dried at 40°C for 7 days to remove residual moisture. The dried leaves were milled to a fine powder and sent to the Institute of Pharmaceutical Sciences, National Institutes of Health University of the Philippines Manila for the preparation of leaf ethanol extracts in the following manner. For each *P. americana* variant, milled leaves were placed in a sterile glass container containing adequate 95% ethanol to submerge the sample (250 g leaf powder/2 L ethanol). The solutions were stirred every 12 hours for 3 days at room temperature. Each suspension was filtered using Whatman no. 1 filter paper, and the filtrates were dried in vacuo at 40°C. The leaf ethanol extracts were stored in dark amber-colored bottles at ~4°C prior to the antibacterial assay.

Growth and Standardization of Test Bacteria

The growth and standardization of bacteria were adapted from the methodology of the Clinical and Laboratory Standards Institute (CLSI) (2018a) [32]. The *Staphylococcus aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were obtained from the Department of Medical Microbiology, College of Public Health, University of the Philippines Manila. The stock cultures of the test bacteria were initially grown in Mueller Hinton broth (MHB) incubated at 36±1 °C for 24 hours and refrigerated at ~4 °C afterwards. Prior to the assay, overnight cultures of the test bacteria were prepared by streaking the stock cultures on fresh Mueller Hinton agar (MHA) plates and incubating them for 24 hours at 36±1 °C. The isolated colonies from the MHA plates were suspended in sterile 0.85% saline solution and visually adjusted to match the turbidity of a 0.5 McFarland standard, which is roughly equivalent to 10^8 colony-forming units per milliliter (CFU/ml).

Preparation of Discs

The preparation of discs was adapted from the methods of Das *et al.* (2010) [31]. For each *P. americana* variant, 100 mg of leaf ethanol extract was dissolved in 1 ml of 95% ethanol solution to make a stock solution. The stock solution was serially diluted four-fold with 95% ethanol to yield six test solutions with concentrations of 100 mg/ml, 25 mg/ml, 6.25 mg/ml, 1.5 mg/ml, 0.39 mg/ml, and 0.098 mg/ml. From the test solutions, 50 µl aliquots were impregnated into 6 mm blank discs (Bioanalyse) to yield a final weight of 5 mg, 1.25 mg, 0.31 mg, 78 µg, 20 µg, and 5 µg leaf extract per disc. Fifty microliters of 95% ethanol absorbed in a 6 mm blank disc was used as negative control. Discs were air-dried overnight. Commercial discs of 30 µg cefoxitin (Bioanalyse) and 10 µg ampicillin (Bioanalyse) were used as positive control for *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922), respectively. Positive controls were used due to the following reasons: (1) *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) are inherently sensitive to cefoxitin and ampicillin, respectively; (2) cefoxitin and ampicillin are primarily recommended in many clinical laboratories for routine susceptibility testing of *S. aureus* and *E. coli*, respectively [32].

Antibacterial Susceptibility Test

Standard disc diffusion was carried out according to the methodology set by CLSI (2018a) [32]. On separate MHA plates, standardized bacterial suspensions of *S. aureus* and *E. coli* were uniformly spread on the surface of MHA plates using a sterile cotton swab. The lids were left ajar for ~5 minutes to allow excess surface moisture to evaporate. Each bacterium was tested against twenty-four discs containing the leaf ethanol extracts (4 leaf variants x 6 concentrations), one positive control disc, and one negative control disc,

placed on the surface of MHA plates. The inoculated MHA with the discs were incubated for ~18 hours at 36±1 °C. Afterwards, the diameter of the zone around each disc showing no bacterial growth was measured to the nearest whole millimeter. Three replicates of disc diffusion were performed, and the mean and standard deviation of the zones of inhibition were reported.

Results

To determine the antibacterial activity of leaf ethanol extracts of four Philippine *P. americana* variants (Cardinal, Morado, Semil 1, and Semil 2) against *S. aureus* and *E. coli*, the authors conducted standard disc diffusion. At 5 mg, all four *P. americana* variants inhibited the growth of *S. aureus*. The inhibition zones ranged from 8 to 12 mm, with Semil 2 displaying the highest activity. Additionally, Semil 2 continued to show activity down to concentrations of 1.25 mg (12±0.58 mm zone of inhibition [ZOI]) and 0.31 mg (9±1 mm ZOI) against *S. aureus*, whereas the other variants did not. On the other hand, all four *P. americana* leaf variants exerted no antibacterial effect on *E. coli* at the concentrations tested. The positive control cefoxitin induced 30±1.15 mm ZOI against *S. aureus*, while ampicillin produced a 23±0.58 mm ZOI against *E. coli*. In contrast, the negative control of 95% ethanol produced no inhibition zone against both *S. aureus* and *E. coli* (Table 1). Both positive controls were effective at inhibiting their respective test bacterium, according to their susceptibility breakpoint criteria [33].

Discussion

The findings of this study indicate that Philippine *P. americana* leaf extracts inhibit the growth of *S. aureus* but not *E. coli* at the studied concentrations, producing zones of inhibition ranging from 8 to 12 mm at 5 mg concentration. Similarly, Ogundare and Oladejo (2014) reported that the leaf methanol extract of *P. americana* at 4 mg concentration inhibited the growth of *S. aureus* (6 mm ZOI) but failed to inhibit *E. coli* [24]. In contrast, Boadi *et al.* (2015) reported that the leaf methanol extract of *P. americana* inhibited the growth of both *S. aureus* (> 1 mm ZOI) and *E. coli* (> 0.8 mm

Table 1. *Zones of Inhibition of Leaf Ethanol Extracts of P. americana Variants at Different Concentrations against S. aureus and E. coli in Disc Diffusion.*

Antibacterial Agent	Zone of Inhibition (mm)	
	S. aureus	*E. coli*
P. americana Leaf Ethanol Extract (5 mg)		
Semil 2	12±1.73	0
Semil 1	8±1	0
Cardinal	8±1.15	0
Morado	9±0.58	0
P. americana Leaf Ethanol Extract (1.25 mg)		
Semil 2	12±0.58	0
Semil 1	0	0
Cardinal	0	0
Morado	0	0
P. americana Leaf Ethanol Extract (0.31 mg)		
Semil 2	9±1	0
Semil 1	0	0
Cardinal	0	0
Morado	0	0
P. americana Leaf Ethanol Extract (78 µg – 5 µg)		
Semil 2	0	0
Semil 1	0	0
Cardinal	0	0
Morado	0	0
Controls		
Cefoxitin (30 µg)	30±1.15	-
Ampicillin (10 µg)	-	23±0.58
95% Ethanol	0	0

Data are expressed as mean and standard deviation of three replicates (n = 3). Values are inclusive of the 6 mm disc used.

ZOI). Additionally, they observed that the antibacterial effect was greater for *S. aureus* compared to *E. coli* in terms of their inhibition zone diameters [25]. Another local study by Manuel *et al.* (2011) on Philippine *P. americana* corroborated this finding, with the leaf methanol extracts at 10 µg concentration showing greater inhibition zones for *S. aureus* (16±2.65 mm ZOI) compared to *E. coli* (8.33±0.58 mm ZOI) [28]. In comparison with this study, however, the previous study by Manuel *et al.* (2011) obtained *P. americana* samples from the mountain areas of the Cordillera Administrative Region, where the source of *P. americana* leaves can be subjected to uncontrolled environmental and/or anthropogenic factors. This may lead to some degree of variation in the secondary metabolite profile of the original plant source [34-36]. In this regard, this study likely yielded more reliable results by obtaining the *P. americana* specimens from a controlled environment at the NPGRL, UPLB; thus, ensuring the integrity of the plant source and reproducibility of study results. In sum, this study provides evidence that contributes to the effectiveness of Philippine *P. americana* leaf ethanol extracts in inhibiting the growth of *S. aureus*, but not *E. coli*. Possibly, the lack of an outer lipopolysaccharide layer predisposes Gram-positive bacteria more to the effects of the *P. americana* leaf extracts [25]. The presence of efflux pump systems in Gram-negative bacteria may also play a role in conferring antibacterial resistance to *P. americana* leaf extracts [37].

Various phytochemicals have been identified in *P. americana* leaves, which have been implicated as the source of their bioactivity [38]. For instance, the presence of anthraquinones, tannins, saponins, terpenoids, steroids, alkaloids, flavonoids, and glycosides was discovered in African *P. americana* leaf alcohol extracts [24,25]. The *Persea americana* leaves collected from the Cordillera Administrative Region of the Philippines possessed a similar phytochemical profile, albeit with the exception of alkaloids and cyanogenic glycosides [33]. The significance of secondary metabolite variation within the same plant species had been demonstrated in prior studies. For instance, the active anti-hyperglycemic agent in *Momordica charantia* leaves responsible for lowering blood sugar was only isolated from 1 out of 15 varieties that were screened in the Philippines [39]. Thus, the secondary metabolite profile of the same plant—and in extension its bioactivity—may differ across geographical areas. This variability in intra-species secondary metabolite profile may in part explain the conflicting reports from previous studies on the antibacterial activity of *P. americana* leaf extracts against *S. aureus* and *E. coli*.

The concentrations used in the study could also have affected the antibacterial activity of the *P. americana* leaf ethanol extracts against *E. coli*. Other studies have reported that *P. americana* leaves suppressed Gram-positive more than Gram-negative bacteria at similar concentrations [25,33]. In the study, the activity of all four variants against *S. aureus* was only observed at the highest concentration (5 mg). Hence, the low potency of the leaf ethanol extracts may explain their inactivity against *E. coli* at 5 mg and lower concentrations. Accordingly, a natural product could potentially contain the pure active component in an amount as little as 0.0003% of the dry cell weight used [40]. Hence, repeating antibacterial susceptibility testing using either higher concentrations of *P. americana* leaf ethanol extract or a purified bioactive component may present a more accurate assessment of its antibacterial effects. The crudeness of the leaf ethanol extract would also explain the disparity in activity between the *P. americana* leaf extracts and the positive controls (cefoxitin and ampicillin) since the controls used were pure substances. Nonetheless, the results of the study show that all four *P. americana* variants exhibit antibacterial activity against *S. aureus*. Thus, all four *P. americana* variants, particularly Semil 2, may represent possible sources of novel antibacterial compounds against *S. aureus*.

Conclusion

In summary, all four *P. americana* variants—Cardinal, Morado, Semil 1, and Semil 2—inhibited the growth of *S. aureus*, but not *E. coli*, at 5 mg concentration. *Staphylococcus aureus* is considered one of the major pathogens of humans and a significant cause of skin and soft tissue infections. Thus, the present findings contribute to the scientific basis of the ethnomedicinal practice of using *P. americana* leaves to treat skin ulcers. Furthermore, the study results suggest the significance of Philippine *P. americana* leaves as a possible source of a novel antibacterial compound against this important human pathogen.

Acknowledgements

The completion of this study would not have been possible without the help of various people and institutions. With their guidance and support, the authors were able to achieve the objectives of this study. The researchers would like to express their sincere gratitude to the following: the University of the Philippines Manila–Research Ethics Board for their evaluation and approval of the study protocol; the National Plant Genetic Resources Laboratory, Institute of

Plant Breeding, University of the Philippines Los Baños for their generous assistance and provision of the *P. americana* leaves for this study; and the University of the Philippines Manila College of Public Health (UPM CPH), Department of Medical Microbiology Faculty for their insights and comments that led to the improvement of the study. The authors are also thankful to Alice Alma C. Bungay, DVM, MVS, FVPH, DPCLAM; Azita Racquel G. Lacuna, RMT, DMM, MPH; Micaella C. Dato, DMM; and to the whole Department of Medical Microbiology, UPM CPH personnel, for their support and encouragement.

References

1. Frieri M, Kumar K, Boutin A. (2017) Antibiotic resistance. Journal of Infection and Public Health 10(4):369-378.
2. Latha T, Anil B, Manjunatha H, *et al.* (2019) MRSA: the leading pathogen of orthopedic infection in a tertiary care hospital, South India. African Health Sciences 19(1):1393-1401.
3. Lim WW, Wu P, Bond HS, *et al*. (2019) Determinants of methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence in the Asia-Pacific region: A systematic review and meta-analysis. Journal of Global Antimicrobial Resistance 16:17-27.
4. Fernandes MR, Moura Q, Sartori L, *et al.* (2016) Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the mcr-1 gene. Eurosurveillance 21(17).
5. Davis WT, Gilbert SR. (2018) Comparison of methicillin-resistant versus susceptible *Staphylococcus aureus* pediatric osteomyelitis. Journal of Pediatric Orthopaedics 38(5):e285-e291.
6. Jokinen E, Laine J, Huttunen R, *et al.* (2017) Comparison of outcome and clinical characteristics of bacteremia caused by methicillin-resistant, penicillin-resistant and penicillin-susceptible *Staphylococcus aureus* strains. Infectious Diseases 49(7):493-500.
7. Antonanzas F, Lozano C, Torres C. (2015) Economic features of antibiotic resistance: the case of methicillin-resistant *Staphylococcus aureus*. Pharmacoeconomics 33(4): 285-325.
8. Stewardson AJ, Allignol A, Beyersmann J, *et al*. (2016) The health and economic burden of bloodstream infections caused by antimicrobial-susceptible and non-susceptible *Enterobacteriaceae* and *Staphylococcus aureus* in European hospitals, 2010 and 2011: a multicentre retrospective cohort study. Eurosurveillance 21(33).
9. O'Neill JIM. (2014) Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Review on Antimicrobial Resistance 20:1–16.
10. Aminov R. (2017) History of antimicrobial drug discovery: Major classes and health impact. Biochemical Pharmacology 133:4–19.
11. Tamalene, MN, Uday UK, Bhakat RK, Vianti EVA, Bahtiar B, Suparman S. (2021) Utilization of mangrove plants as a source of Malaria medicine in North Maluku Province, Indonesia. Asian Journal of Ethnobiology 4(2).
12. Roozegar MA, Jalilian FA, Havasian MR, Panahi J, Pakzad I. (2016) Antimicrobial effect of Pistacia atlantica leaf extract. Bioinformation 12(1): 19.
13. Umar M, Mohammed IB, Oko JO, Tafinta IY, Aliko AA, Jobbi DY. (2016) Phytochemical analysis and antimicrobial effect of lemon grass (Cymbopogon citratus) obtained from Zaria, Kaduna State, Nigeria. Journal of Complementary and Alternative Medical Research 1(2):1-8.
14. Danish P, Ali Q, Hafeez MM, Malik A. (2020) Antifungal and antibacterial activity of aloe vera plant extract. Biological and Clinical Sciences Research Journal 2020(1).
15. Reymond P. (2004) A conserved transcript pattern in response to a specialist and a generalist herbivore. The Plant Cell 16(11):3132–3147.
16. Iven T, König S, Singh S, *et al*. (2012) Transcriptional activation and production of tryptophan-derived secondary metabolites in Arabidopsis roots contributes to the defense against the fungal vascular pathogen Verticillium longisporum. Molecular Plant 5(6):1389–1402.
17. Sampaio BL, Edrada-Ebel R, Da Costa FB. (2016) Effect of the environment on the secondary metabolic profile of Tithonia diversifolia: a model for environmental metabolomics of plants. Scientific Reports 6(1).
18. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. (1985) Medicinal plants in therapy. Bulletin of the World Health Organization 63(6):965–981.
19. Adeyemi O, Okpo S, Ogunti O. (2002) Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). Fitoterapia 73(5):375–380.
20. Pesewu GA, Cutler RR, Humber DP. (2008) Antibacterial activity of plants used in traditional medicines of Ghana with particular reference to MRSA. Journal of Ethnopharmacology 116(1):102–111.
21. Tabuti JRS, Kukunda CB, Waako PJ. (2010) Medicinal plants used by traditional medicine practitioners in

the treatment of tuberculosis and related ailments in Uganda. Journal of Ethnopharmacology 127(1):130–136.
22. Yasir M, Das S, Kharya MD. (2010) The phytochemical and pharmacological profile of *Persea americana* Mill. Pharmacognosy Reviews 4(7):77–84.
23. Tomani JC, Nkurunziza JP, Mukazayire MJ, Kanezeza R, Baziruwitonda E, Duez P. (2008) Antidiarrhea activity and preliminary phytochemical screening of Indigofera arrecta, Cyathula uncinulata, *Persea americana* and *Cupressus lusitanica*. Planta Medica 74(9).
24. Ogundare AO, Oladejo BO. (2014) Antibacterial activities of the leaf and bark extract of *Persea americana*. American Journal of Ethnomedicine 1(1):64–71.
25. Boadi NO, Saah SA, Mensah JK, Badu M, Addai-Arhin S, Mensah, MB. (2015) Phytoconstituents, antimicrobial and antioxidant properties of the leaves of *Persea americana* Mill cultivated in Ghana. Journal of Medicinal Plants Research 9(36):933–939.
26. Gómez-Cansino R, Guzmán-Gutiérrez SL, Campos-Lara MG, Espitia-Pinzón CI, Reyes-Chilpa R. (2017) Natural compounds from Mexican medicinal plants as potential drug leads for anti-tuberculosis drugs. Anais da Academia Brasileira de Ciencias 89(1):31–43.
27. Cortes-Maramba NP. (1998) Medicinal plants in the Philippines, In: Tomlinson TR, Akerele O (eds.). Medicinal Plants: Their Role in Health and Biodiversity, Pennsylvania: University of Pennsylvania Press, pp. 169–176.
28. Parlet CP, Brown MM, Horswill AR. (2019) Commensal staphylococci influence *Staphylococcus aureus* skin colonization and disease. Trends in Microbiology 27(6): 497-507.
29. Yang SC, Lin CH, Aljuffali IA, Fang JY. (2017) Current pathogenic *Escherichia coli* foodborne outbreak cases and therapy development. Archives of Microbiology 199(6): 811-825.
30. Das K, Tiwari RKS, Shrivastava DK. (2010) Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research 4(2):104–111.
31. Clinical and Laboratory Standards Institute. (2018a) M02-A13: Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard (13th Edition). Wayne, PA: Clinical and Laboratory Standards Institute.
32. Clinical and Laboratory Standards Institute. (2018b) M100-S28: Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Eighth Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute.
33. Manuel JF, Balangcod TD, Laruan LA, Patacsil MC, Martin SP. (2011) Suppression of growth of some medically important bacterial pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*) with plant extracts of selected indigenous semi-temperate medicinal plants in the Philippines. University of the Cordilleras Tangkoyob Research Journal 6(1):2-12.
34. Murch SJ, Haq K, Rupasinghe HPV, Saxena PK. (2003) Nickel contamination affects growth and secondary metabolite composition of St. John's wort (Hypericum perforatum L.). Environmental and Experimental Botany 49(3):251–257.
35. Ode PJ, Johnson SN, Moore BD. (2014) Atmospheric change and induced plant secondary metabolites—are we reshaping the building blocks of multi-trophic interactions? Current Opinion in Insect Science 5:57–65.
36. Ibrahim M, Chee Kong Y, Mohd Zain N. (2017) Effect of cadmium and copper exposure on growth, secondary metabolites and antioxidant activity in the medicinal plant sambung nyawa (Gynura procumbens (Lour.) Merr). Molecules 22(10):1623.
37. Marasini BP, Baral P, Aryal P, *et al.* (2015) Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. BioMed Research International 1–6.
38. Evbuomwan L, Inetianbor JE. (2017) Antibacterial activity of Persia americana leaf extracts against multidrug resistant bacterial isolates. AASCIT Journal of Bioscience 3(4):29–34.
39. The Manila Times. (2009) The plant whisperer.
40. Sakato K, Tanaka H, Mukai N, Misawa M. (1974) Isolation and identification of camptothecin from cells of Camptotheca acuminata suspension cultures. Agricultural and Biological Chemistry 38(1):217–218.