Advances in Bioresearch
Adv. Biores., Vol 9 (5) September 2018: 01-06

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.9.5.16

Advances in Bioresearch

ORIGINAL ARTICLE

Philippine Ethnobotanicals Inhibit Formation of Coagulase in *Staphylococcus aureus*

James Ryan G. Vias[1], Wilson R. Jacinto[2] And Khristina G. Judan Cruz[1*]
[1]Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija Philippines
[2] Biological Sciences Department, De La Salle University-Dasmarinas, City of Dasmarinas, Cavite, Philippines

ABSTRACT
This study screened the quorum sensing inhibition (QSI) activities of ethnobotanicals Cestrum nocturnum, Sarcandra glabra, Derris elliptica, Oreocnide trinervis, Ayapana triplinervis, Alstonia scholaris, Ageratina adenophora collected from the ancestral domain of the Igorot community of Imugan, Nueva Vizcaya, Philippines against Staphylococcus aureus PNCM 1582. Ethanol extracts of Sarcandra glabra, Derris elliptica, Ayapana triplinervis, Ageratina adenophora and Oreocnide trinervis exhibited QSI against Staphylococcus aureus PNCM 1582 through the tube coagulase assay. The results show a considerable potential of the ethnobotanicals as sources of compounds that can inhibit quorum sensing.
Keywords: *Quorum sensing inhibition,* Coagulase in *Staphylococcus aureus*

Received 04.04.2018 Revised 18.06.2018 Accepted 16.08.2018

How to cite this article:
Vias, J.R.G., Jacinto, W.R. and Judan Cruz, K. G. Philippine Ethnobotanicals Inhibit Formation of Coagulase in *Staphylococcus aureus*. Adv. Biores. Vol 9 [5] September 2018: 01-06.

INTRODUCTION

Bacterial cells communicate, regulate and perform cellular activities through quorum sensing (QS) [1]. Through the production and use of signaling molecules called autoinducers (AI), QS controls genes that allow bacterial cells to regulate various processes of bacterial cells such as bioluminescence, sporulation, competence, biofilm formation, antibiotic production, and virulence factor secretion [2] in a synchronized, uniform matter which makes colonization and disease progression much more effective [3, 4].

The continuing increase of highly resistant bacteria has undermined the ability of newer generations of antibiotics [5]. One of these bacteria is *Staphylococcus aureus*. This bacterium causes superficial skin lesions, osteomyelitis, endocartis and furunculosis. *S. aureus* is one of the leading causes of nocosomial infections of surgical wounds in hospitals [6]. Quorum sensing regulates various virulence factors in *S. aureus* [7] which makes the bacterium exist in two phenotypes, an adhesive colonizer phenotype that is tolerated by the host, and an invasive infective phenotype which damages the tissues of the host and is responsible for the manifestation of disease [8, 9].

A way to stop, control and interfere with bacteria is done through quorum sensing inhibition (QSI) or anti-quorum sensing (AQS). QSI aims to interfere the communication system that bacteria use, which may have been a reason bacteria are developing resistance against antibiotics [10]. Quorum sensing inhibition can be done in a number of ways [11] such as application of compounds that can inhibit quorum sensing. These compounds may come from either synthetic forms or natural extracts from organisms [12]. Plants and phytochemicals are now being studied and screened for their potential to combat quorum sensing in bacteria, especially by the antimicrobial-resistant strains. The search for quorum sensing inhibitors provides new information on the usage of natural compounds for remedy of health conditions, as well as the potential to control and handle diseases caused by pathogens through inhibition or interference of the communication between bacterial cells [13].

The production of secondary metabolites in plants has played a key-role in their survival in environments loaded with bacteria [14]. These phytochemicals synthesized by plants limit the microbes' abilities to

colonize and spread virulence [15] and has been reported to have disease-controlling potential by altering the genes responsible for the pathogenesis and virulence through interference with QS [16]. Since then, many plants have been tested for their potential as quorum sensing inhibitors.

Ethnobotanicals are plants that are part of a particular culture and region used by indigenous people. These plants play roles in local healing processes and the knowledge of these plants are handed from generation to generation [17,18]. Ethnobotanicals have shown therapeutic properties and are being studied for the discovery and development of new drugs [19]. While some ethnobotanicals have shown antibacterial properties [20, 21], the properties of these plants to interfere with QS are yet to be explored because only few studies regarding ethnobotanicals as quorum sensing inhibitors have been published [22]. Ethnobotanicals in the Philippines are not well known because these plants can only be found and are only utilized in the areas of ethnic communities as therapeutic agents for various health conditions. These plants may show potential for the development of new drugs since they are locally used for their medicinal properties, and may also be sources of quorum sensing inhibitors.

The ethnobotanicals of the Igorot community of Imugan, Sta. Fe, Nueva Vizcaya have been studied, and has exhibited different medicinal properties such as anti-inflammatory [21], analgesic [21, 23], antibacterial [21], anti-diabetic [24], anti-oxidant [25], anti-gout [26] and antipyretic [23]. Recently, these ethonobotanicals were found to inhibit quorum sensing in two bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus* through various virulence [27,28,29]. This may contribute in the search of combating diseases against antibacterial-resistant bacteria through quorum sensing inhibition. This study may provide additional information and ways in utilizing plant extracts in the continuous search for the controlling of microbial infections by reducing the risk of developing resistance.

MATERIALS AND METHODS

Collection of Plant Samples

Ethnobotanical plant samples *Cestrum nocturnum*((Dama de Noche), *Sarcandra glabra* (Hag-ob), *Oreocnide trinervis* (La-latan), *Derris elliptica* (Opay), *Alstonia scholaris* (Palay), *Ageratina adenophora* (Panawel) and*Ayapana triplinervis* (Pantaleon) were collected along a trail of Mt. Imanduyan, Brgy. Imugan, Sta. Fe, Nueva Vizcaya, elevated 1092 meters above sea level. Brgy. Imugan is geographically located between the Cordillera Central and Sierra Madre mountain ranges.

Leaf samples used in this study were collected at Mount Imanduyan, Brgy. Imugan, Sta. Fe, Nueva Vizcaya. Mature leaf samples were collected by handpicking during the day, placed in clean, sealed plastic bags and transported to the laboratory for processing. The place, time, season and the name of the collector were recorded.Confirmation of the pre-identified collected plants was done by an expert botanist at the National Museum of the Philippines in Manila. Vegetative parts of the specimens were collected and pressed for authentication of the correct species.

Ethanol Extraction Procedure

The collected leaves were washed and rinsed in running tap water for complete elimination of foreign matters on the surface. Second rinsing using distilled water followed next, and then final rinsing with 70% ethanol (v/v) was done afterwards. Dried plant materials were pulverized to fine, sand-like particles using a blender [30].

Fifty (50) grams of finely ground leaves of each plant was soaked in 500 ml of 95% ethanol in a stoppered flask for 72 hours. The resulting mixture was then filtered using Whatman no.1 filter paper and the solvent completely removed using a rotary evaporator [30]. The extracts were then weighed and stored in tightly stoppered sterile amber bottles [31] at temperatures between 0-5 ˚C.

Sterilization of the extracts was done, first by centrifugation of the mixture at 10, 000 x g for 30 minutes, and filtration with a membrane filter with a pore diameter of 0.45 µm The sterility of the extracts was monitored by inoculating 100 µl in brain heart infusion agar (BHIA) from time to time. The sterile extracts was stored at 2-8 °C prior to use [31].

Confirmation of Test Bacterial Characteristics

Staphylococcus aureus stock cultures were revived in brain-heart infusion broth (BHIB), and inoculation in mannitol salt agar (MSA) was performed to check identity. Working stock cultures were grown and maintained in BHIB test tubes at 5°C.

Phenotypic Detection of Quorum Sensing Inhibition in *S. aureus* PNCM 1582 through Tube Coagulase Assay

The rabbit plasma was rehydrated to reconstitute according to the instructions provided in the kit (Scharlau Lyophilized Rabbit Plasma Art No. 064-PLA-CO). About 260 µl of rabbit plasma was placed in an Eppendorf tube followed by inoculation of 100µl of *S. aureus* culture. Under aseptic conditions, 40µl of each plant extract was added to the mixture of *S. aureus* culture and plasma, and was then incubated at

35-37°C during a period between 4 to 24 hours. The formation of a coagulum indicates production of coagulase, and, hence, negative for QSI. QSI is present in the extracts if coagulation did not form in the plasma after 4 to 24 hours.

RESULTS AND DISCUSSION

Phenotypic Detection of Quorum Sensing Inhibition in *S. aureus* PNCM 1582 through Tube Coagulase Assay

QSI was exhibited by inhibiting the coagulation of the rabbit plasma. Ethanol extracts of *D. elliptica, A. triplinervis, O. trinervis, A. adenophora,* and *S. glabra* showed inhibition of the phenotypic expression of coagulase by *S. aureus*, indicating presence of quorum sensing inhibition activity.

Staphylococcus aureus is a deadly pathogen to humans. It affects nearly 30% of the adult population [9], in which resistant strains causes outbreaks in hospitals and can be epidemic [32].*S. aureus* expresses many cell-surface associated and extracellular proteins that have potential as virulence factors [6]. Coagulase is an extracellular protein produced by *S. aureus*, which promotes fibrin formation in human plasma by non-enzymatic activation of prothrombin [33].

Quorum sensing in gram-positive bacteria is encoded by a peptide-based quorum sensing system, specifically by the accessory gene regulator (*agr*) locus [34]. Pathogenesis of *S. aureus* has virulence progressing in two discrete stages. Initially, the production of adhesins and surface proteins during exponential growth occurs followed by increased toxin production, which leads to damage of tissues and bacterial spread [35]. One of the virulence factors produced by *S. aureus* is coagulase.

Coagulase is synthesized during exponential growth [36, 37] wherein the gene *coa* is responsible for coagulase expression in *S. aureus* [38] wherein the protein's action is to create a barrier of plasma as a means of protection against phagocytes [6]. After exponential growth, extracellular proteins such as serine protease, nuclease, lipase, fibrinolysin, α-, β-, and δ-hemolysin, are produced, as well as toxins, toxic shock syndrome toxin 1 and enterotoxin [39]. In other words, coagulase creates a defensive mechanism against phagocytes while allowing the bacterial cells to secrete the virulence factors that causes infectious diseases in its host.As coagulase, along with other surface proteins, are secreted during the exponential growth, it could be suggested that the extracts of the ethnobotanicals *D. elliptica, A. triplinervis, O. trinervis, A. adenophora,* and *S. glabra* may have affected the pathogenicity of *S. aureus* by suppressing the phenotypic expression of the gene *coa.*

Previous studies initially screened the extracts of these ethnobotanicals against *Pseudomonas aeruginosa* and *Staphylococcus aureus* virulence factors*.* Extracts of *Oreocnide trinervis, Cestrum nocturnum* and *Ayapana triplinervis* exhibited QSI activity through inhibition of pyocyanin production in *P. aeruginosa*. Additionally, extracts of *Bidens pilosa, C. nocturnum, Sarcandra glabra, O. trinervis*, *Derris elliptica, Alstonia scholaris, A. triplinervis* and *Ageratina adenophora* interfered with the swarming motility of *P. aeruginosa,* an activity regulated by quorum sensing. The production of α-hemolysin in *S. aureus* was also inhibited by the extracts of *C. nocturnum, S. glabra, O. trinervis, D. elliptica, A. scholaris, A. adenophora* and *A. triplinervis*. Morever, DNase assay employed in *S. aureus* and extracts of *C. nocturnum, O. trinervis,* and *A. triplinervis* showed inhibition of QS in the said bacterium [27]. All methanolic extracts of the ethnobotanicals also inhibited swarming motility of *P. aeruginosa* while extracts of *D. elliptica* and *O. trinervis* manifested effect on the production of DNase[28]. N-hexane extracts of the same ethnobotanicals against *P. aeruginosa* and *S. aureus*were also subjected to the same assays.In *P. aeruginosa*, swarming motility was inhibited by *P. pentandrum, O. trinervis, D. elliptica, A. scholaris, A. adenophor*a and *A. triplinervis* while *B. pilosa, C. nocturnum*and *S. glabra*showed higher decrease in swarming motility. In the α-hemolysin assay, all extracts showed presence of QSI against *S. aureus* [29].

Some ethnobotanicals used in this study have been reported to contain certain phytochemicals which may have acted on the quorum sensing activity in *S. aureus*. Most of the compounds found in the ethnobotanicals are among those found to have quorum sensing inhibition in bacteria. *S. glabra, D. elliptica, A. adenophora,* and *A. triplinervis* showed anti-QS activities in both assays. *S. glabra* was found to contain coumarins, flavonoids, rosmarinic acid, and sesquiterpenoids [40,41]. *D. elliptica,* on the other hand, was found to have tannins, saponins, terpenoids, and alkaloids [42], while *A. adenophora* contains sesquiterpenes, chlorogenic acid [43] alkaloids, glycosides, saponins and coumarins [44, 45]. Furthermore, *A. triplinervis* have been found to have coumarin, tannins, flavonoids, phenols, terpenes and alkaloids [46, 47].

The extracted compounds of plant materials are dependent on the nature of the extracting solvent [48] wherein, ethanol has been proven of extracting tannins, polyphenols, flavonol, terpenoids, and alkaloids [49, 50]. However, the identification of the specific compounds extracted using ethanol in this study is yet to be verified. It is difficult to indicate the nature and identity of the active compound/s present in the

extracts due to the novelty of some species. The mechanisms of the phytochemicals effective for QSI are yet to be considered [51].

Quorum sensing inhibition activity does not kill or inhibit bacterial growth, rather, it can be able to control and eradicate infections caused by pathogens [52] by targeting quorum sensing in various methods. Most of the ethanolic extracts of the ethnobotanicals have exhibited quorum sensing inhibition activity in both bacteria, thus, these plants can be potential sources of new drugs in this perspective of combating bacterial infections.

The ethnobotanicals utilized in this study are currently in use of the Igorot community of Imugan for its medicinal and toxic properties, and have not yet been widely cultivated and domesticated. Several researches on these ethnobotanicals point out to its remarkable pharmacological potential as quorum sensing inhibitors against *P. aeruginosa* and *S. aureus* virulence factors using different extraction solvents. [27, 28, 29]. The findings of this study can contribute to the development of ways to fight antibiotic-resistant bacteria, hence, decreased use of antibiotics to prevent the growth of resistant strains.

CONCLUSION

Ethanol extracts of *D. elliptica, A. triplinervis, O. trinervis, A. adenophora,* and *S. glabra* showinhibition of the phenotypic expression of coagulase by *S. aureus*, indicating presence of quorum sensing inhibition activity.

REFERENCES

1. Rasmussen, T. B. and Givskov, M. (2006). Quorum-sensing inhibitors as anti-pathogenic drugs. Int J Med Microbiol. 296(2-3):149-61.
2. Williams, P. and Camara M. (2009). Current Opinion in Microbiology. 12(2):182–191. doi:10.1016 / j.mib.2009.01.005.
3. Tay, S. B. and Yew W.S. (2013). Development of Quorum-Based Anti-Virulence Therapeutics Targeting Gram-Negative Bacterial Pathogens Int. J. Mol. Sci. 14(8): 16570-16599; doi:10.3390/ijms140816570.
4. Ng, W. and BASSLER, B.L.. (2009). Bacterial Quorum-Sensing Network Architectures. Annu Rev Genet.; 43: 197–222.doi: 10.1146/annurev-genet-102108-134304.
5. Levy, S. B. (2002). The Antibiotic Paradox: How the Misuse of Antibiotics Destroys their Curative Powers. Perseus Publishing; Cambridge, MA.
6. Foster, T. J., (1996). *Staphylococcus*.Medical Microbiology, 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston.
7. Dunny, G. M. and Leonard B.A.B. (1997). Cell-cell communication in gram-positive bacteria. Annual Review of Microbiology, 51: 527-564.
8. Njoroge, J. and Sperandio, V. (2009). Jamming bacterial communication: New approaches for the treatment of infectious diseases. EMBO Mol Med. 1:201-10.
9. George, E.A. and Muir, T.W. (2007). Molecular mechanisms of agr: *Quorum sensing* in virulent staphylococci. Chembiochem. 2007;8:847-55.
10. Bolgelmez-Tinaz, G. (2013). Disruption of bacterial cell-to-cell communication (Quorum Sensing): a promising novel way to combat bacteria-mediated diseases. MÜSBED. ; 3(3): 159-163doi: 10.5455 /musbed. 20130910085643.
11. Smith, R. S. and IGLEWSKI, B.H. (2003). *Pseudomonas aeruginosa* quorum-sensing systems and virulence. Curr Opin Microbiol. 6(1):56-60.
12. Clatworthy, A. E., Pierson, E. and Hung, D.T. (2007).Targeting virulence: a new paradigm for antimicrobial therapy. Nature Chemical Biology 3, 541–548 doi:10.1038/nchembio.2007.24.
13. Domingo, D. and López, M. (2003). Plantas Con Acción Antimicrobiana (Plants with Antimicrobial Action). Revista Espaňola de Quimioterapia. 16 (4): 385-393.
14. Vattem, D. A., Mihalik, K., Crixell, S. H. and McLean R.J.C. (2007). Dietary phytochemicals as quorum sensing inhibitors. Fitoterapia. 78(4): 302–310 doi:10.1016/j.fitote.2007.03.009.
15. Quave, C. L., Pieroni, A. and Giusti, M.E. (2011). Cross-Cultural Ethnobiology in the Western Balkans: Medical Ethnobotany and Ethnozoology Among Albanians and Serbs in the Pešter Plateau, Sandžak, South-Western Serbia. Human Ecology. 39: 333-349.
16. Packiavathy, I. A. S. V., Ravi, A.V., Agilandeswari, P., Musthafa, K. S. and Pandian, S.K. (2012). Antibiofilm and quorum sensing inhibitory potential of *Cuminum cyminum* and its secondary metabolite methyl eugenol against Gram negative bacterial pathogens. Food Res. Int. 45: 85–92.
17. Abe, R. and Ohtani, K. (2012). An ethnobotanical study of medicinal plants and traditional therapies on Batan Island, the Philippines. Journal of Ethnopharmacology. 145(2):554–565. doi:10.1016/j.jep.2012.11.029.
18. Gbadamosi, I.T. (2012). Evaluation of Antibacterial Activity of Six Ethnobotanicals Used in the Treatment of Infectious Diseases in Nigeria. Botany Research International 5 (4): 83-89, 2012 ISSN 2221-3635 © IDOSI Publications, 2012; doi: 10.5829/idosi.bri.2012.5.4.22.
19. Sheng-JI, P. (2001).Ethnobotanical Approaches of Traditional Medicine Studies: Some Experiences From Asia. Pharmaceutical Biology. 39(1):74-79.

20. Hernández, T., Canales, M., Avila, J. G., Duran, A., Caballero, J., Romo De Vivar, A. and Lira, R. (2003). Ethnobotany and Antibacterial Activity of Some Plants Used in Traditional Medicine of Zapotitlán de las Salinas, Puebla (México). Journal of Ethnopharmacology. 88: 181–188.
21. Judan Cruz, J.K. Gabriel, C. M. and Abella, E.A. (2018).Biological Activities Of Philippine Ethnotoxic Plants. Int J Bio Pharm Allied Sc. May, 2018, 7(5): 709-718.
22. Adonizio, A. L., Downum, K., Bennett, B. C. and Mathee K. (2006). Anti-Quorum Sensing Activity of Medicinal Plants in Southern Florida. Journal of Ethnopharmacology. 105(3):427-435.
23. Ruiz, J. C. V., Gajeton, M. B., Gabriel, R. J. C., Lictawa, M. J. C., Lucas, E. R. and Cruz, K.J. (2015). Analgesic and antipyretic potential of an ethnobotanical plant, *Alstonia scholaris* L. in mice. Journal of Agricultural Technology. 11(2): 563-565.
24. Alfonso, F. R., David, E. S. and Cruz, K.J. (2015). α-Glucosidase Inhibitory Activity of Selected Ethnobotanicals of Imugan, Nueva Vizcaya. An Undergraduate Thesis, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija.
25. Divina, R. B. K. M., David, E. S. and Cruz, K.J. (2015). Antioxidant Screening of Ethnobotanicals of Imugan, Nueva Vizcaya, Philippines. An Undergraduate Thesis, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija.
26. Jose, K. J. C., David, E. S. and Cruz, K.J. (2015). Xanthine Oxidase Inhibition of the Selected Ethnobotanicals of Imugan, Nueva Vizcaya. An Undergraduate Thesis, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija.
27. Benito, G. B. A., CRUZ, K. J. and Jacinto, W.R. (2015). Determination of Quorum Sensing Inhibition in the Ethanolic Ethnobotanical Extracts of the Igorot Community of Imugan Ancestral Domain. A Masteral Thesis. Department of Biological Sciences, College Of Arts And Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija.
28. Barrogo, K., Cruz, K.J. and Jacinto, W.R.. (2015). Detection of Quorum Sensing Inhibition in *Pseudomonas aeruginosa* and *S. aureus* Using Methanolic Extracts of the Imugan Ancestral Domain. A Masteral Thesis. Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija.
29. Padilla, K. A. V., Cruz, K.J. and Jacinto, W.R.. (2015). Quorum Sensing Inhibition In N-Hexane Extracts Of Ethnobotanicals Of Imugan Ancestral Domain. A Masteral Thesis. Department Of Biological Sciences, College Of Arts And Sciences, Central Luzon State University, Science City Of Munoz, Nueva Ecija.
30. Tan, L. Y., Yin, W. F. and Chan, K.G. (2013). *Piper nigrum*, *Piper betle* and *Gnetumgnemon* – Natural Food Sources with Anti-Quorum Sensing Properties. Sensors. 13: 3975-3985.doi:10.3390/s130303975.
31. Srisawat, S. (2007). Effect of Some Thai Medicinal Plant Extracts on Antibacterial Activity of Periodontopathic Bacteria and Their Anti-Inflammatory Activity and Toxicity to Gingival Connective Tissue Fibroblast. Prince of Songkla University. Retrieved from http://kb.psu.ac.th /psukb/handle/2553/1492.
32. Lyon, B. R. AND Skurray, R. (1987). Antimicrobial resistance in *Staphylococcus aureus*: genetic basis. Microbiol Reviews. 51:88.
33. Zajdel, M., Wegrzynowicz, Z., Sawecka, J., Jeljaszewicz, J. And Pulverer, G. (1976). Mechanism of action of staphylocoagulase. J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections, 549-575.
34. Novick, R. P. and Geisinger, E. (2008). Quorum Sensing in Staphylococci. Annual Review in Genetics. 42:541–564. doi: 10.1146/annurev.genet.42.110807.091640.
35. Tamber S., Reyes, D., Donegan, N. P., SCHWARTZMAN, J. D., CHEUNG, A. L. and MEMMI, G. (2010). The Staphylococcus-Specific Gene *rsr* Represses *agr* and Virulence in *Staphylococcus aureus.* Infection and Immunity. 4384-4391.
36. Engels, W., Kamps, M. And Van Boven, C. P. A.. (1978). Influence ofvcultivation conditions on the production of staphylocoagulase by *Staphylococcus aureus* 104. J. Gen. Microbiol. 109:237-243.
37. Movitz, J. (1976). Formation of extracellular protein A by *Staphylococcus aureus*. Eur. J. Biochem. 68:291-299.
38. Lebeau C., Vandenesch, F., Greenland, T., Novick, R. P. And Etienne, J. (1994). Coagulase expression in *Staphylococcus aureus* is positively and negatively modulated by an agr-dependent mechanism. J. Bacteriol. 176(17):5534-5536.
39. ABBAS-ALI, B. and COLEMAN, G. (1977). The characteristics of extracellular protein secretion by *Staphylococcusaureus* (Wood 46) and their relationship to the regulation of a-toxin formation. J. Gen. Microbiol. 99:277-282.
40. Hu, X. R. (2010). Study on the Chemical Constituents of *Sarcandra glabra*. Retrieved on June 12, 2015 from http://www.globethesis.com/?t=1114360275975402.
41. Feng, S., Xu, L., Wu, M., Hao, J., Qiu, S. X. and Wei, X. (2010). A new coumarin from *Sarcandra glabra.* Fitoterapia. 81(6):472-474. doi:10.1016/j.fitote.2009.12.009.
42. Espiritu, H. M., Abella, E. A. and Parayao, A.M. (2014). Antibacterial Assay, Cytotoxicity Testing, and Phytochemical Screening of Selected Ethnotoxic Plants Collected at Imugan, Santa Fe, Nueva Vizcaya. An Undergraduate Thesis, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija.
43. Proksch, P., Wray, V., Isman, M. B. And Rahaus, I.(1990). Ontogenetic Variation of Biologically Active Natural Products in *Ageratina adenophora*. Phytochemistry. 29(2): 453-457 doi:10.1016/0031-9422(90)5096-X

44. Baral, B. and Maharjan, B.L. (2011). Antagonistic Characteristics and Phytochemical Screening of Invasive Alien Species of Nepal Himalaya. International Journal of Pharmaceutical and Biological Archives. 2(5):1444-1450 ISSN 0976-3333.
45. Rajeswary M. and Govindarajan, M. (2013). Mosquito larvicidal and Phytochemical Properties of *Ageratina adenophora* (Asteraceae) Against Three Important Mosquitoes. J Vector Borne Dis, 50: 141-143
46. Stuart, G. (2014). Philippine Medicinal Plants. Ayapana, *Ayapana triplinervis* Vahl. Philippine Alternative Medicine. Retrieved from StuartXchange http://www.stuartxchange.org/Ayapana.html
47. Facknath, S. and Lalljee, B. (2008). Study of Various Extracts of A*yapana triplinervis* for Their Potential in Controlling Three Insect Pests of Horticultural Crops. Tropicultura. 26(2):119-124.
48. Sultana, B., Anwar, F. and M. Ashraf. (2009) Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. Molecules. 14: 2167-2180 doi:10.3390/molecules14062167.
49. Tiwari, P., Kumar, B., Kaur, M., Kaur G. and Kaur, H. (2011). Phytochemical Screening and Extraction: A Review. Internationale Pharmaceutica Sciencia. 1(1): 98-106
50. Cowan, M. M. (1999). Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews 12(4): 564-582.
51. Zahin, M., Hasan, S., Aqil, F., Ahmad Khan, M. S., Husain, F. M. and Ahmad, I. (2010). Screening of Certain Medicinal Plants From India for Their Anti-Quorum Sensing Activity. Indian Journal of Experimental Biology. 48: 1219-1224.
52. Song, C., Ma, H., Zhao, Q., Song, S. and Jia, Z. (2012). Inhibition of Quorum Sensing Activity by Ethanol Extract of *Scutellaria baicalensis.* Georgi. J Plant PatholMicrobiol S7:001. doi:10.4172/2157-7471.S7-001.