INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 15, No. 2, p. 33-41, 2019

RESEARCH PAPER OPEN ACCESS

The *agrA* expression linked to quorum sensing-mediated coagulase formation using Philippine Ilongot ethnobotanicals against *Staphylococcus aureus*

Gabrielle T. Salamanca, Somar Israel D. Fernando, Khristina G. Judan Cruz*

Department of Biological Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines

Key words: *Staphylococcus aureus*, Quorum sensing, Coagulase, Ilongot, Ethnobotanicals, *agrA*.

http://dx.doi.org/10.12692/ijb/15.2.33-41 Article published on August 09, 2019

Abstract

Staphylococcal infections are among the most common hospital-acquired infections worldwide. Quorum sensing in bacterium is vital to their pathogenicity and the use of quorum sensing inhibition (QSI) compounds reveals opportunities to target virulence without developing resistance and suggests a strategic approach to develop drugs against pathogenic bacteria. Ethnobotanical crude extracts (ECEs) of the Ilongot community of Aurora province, Philippines were tested for the QSI activity against coagulase formation in the reference strain *Staphylococcus aureus*. Antibacterial testing of extracts was done as pre-screening for coagulase formation. Extracts were evaluated through tube coagulase assay for coagulase formation. Molecular confirmation of quorum sensing inhibition as affected by the ECEs was done through gene expression analysis of *agrA*. Antibacterial activity was observed in *Urena lobata* leaves, *Dillenia philippinensis* leaves and *Diplazium esculentum*. Coagulation was inhibited in tubes treated with crude extracts of *Dillenia philippinensis* bark, *Stachytarpeta jamaicensis* leaves, and *Eleusine indica* roots therefore, exhibiting QSI activities against *S. aureus*. *S. jamaicensis* leaves. *E. indica* roots and *D. philippinensis* bark significantly downregulated *agrA* therefore, confirming the QSI activity. The results indicate the potential of these ethnobotanicals for therapeutic approach in inhibiting bacterial virulence without developing resistance.

* **Corresponding Author:** Khristina G. Judan Cruz ✉ kjcruz@clsu.edu.ph

Introduction

Staphylococcus aureus is known as a lethal pathogenic bacteria that possesses virulence factors such as toxins like Panton–Valentine leukocidin (PVL); adhesins, immunomodulators and enzymes like coagulase (Watkins *et al.*, 2012). This pathogen is also the leading cause of nosocomial infections, endocarditis and bacteremia (Boucher *et al.*, 2010) while severe manifestations could cause pyomyositis, osteomyelitis, necrotizing pneumonia, sepsis and necrotizing fasciitis (Bassetti *et al.*, 2011; Changchien *et al.*, 2011; Kechrid *et al.*, 2011; Kreienbuehl *et al.*, 2011; Burdette *et al.*, 2012).

One of the factors that contribute to *S. aureus* virulence is the quorum-sensing controlled secretion of coagulase (Rasmussen and Givskov, 2006). Coagulase is a polypeptide that binds to and activates prothrombin that converts fibrinogen to fibrin of muscular tissue and induce clotting of plasma or blood (Cheng *et al.*, 2010). This enzyme binds tightly to the surface of the pathogen and coats its surface with fibrin upon contact with blood, this fibrin clot protects the bacterium from phagocytosis and enables the it to isolate itself from other defenses of the host, therefore helping the said pathogen to become resistant to human immune response (DeLeo *et al.*, 2009; Tortora *et al.*, 2013).

The discovery of quorum-sensing (QS) and quorum sensing inhibition (QSI) provides a promising potential to control pathogenicity in *S. aureus*. The use of QSI agents is a viable approach to disrupt the cellular communication and therefore preventing the pathogen to become virulent (Smith and Iglewski, 2003).

One group of plants, particularly the ethnobotanicals, can be potentially tapped to develop of QSI agents due to their numerous bioactive compounds with medicinal properties (Damle and Sharon, 2017). A few ethnobotanicals in the Philippines have shown to control virulence factors in pathogenic bacteria (Barrogo *et al.*, 2015; Limos *et al.*, 2015; Cruz *et al.*, 2018) and thus, the search of new ethnobotanicals from ethnic communities in the Philippines as potential QSI agents has been gaining interest. This study screened the ethnobotanicals from the Philippine Ilongot community for their potential in QSI against coagulase secretion, with molecular confirmation through agrA expression in *S. aureus*.

Materials and methods

Collection of plant samples

Plants included in this study were based on the survey done by Balberona *et al.*(2018). The plant samples collected from the Ilongot-Egongot community in Sierra Madre Mountains of Bayanihan, Maria Aurora, Aurora, Philippines include *Hydrocotyle vulgaris* leaves, *Mikania micrantha* leaves, *Dillenia philippinensis* bark and leaves, *Ceiba pentandra* leaves, *Cymbopogon winterianus* leaves, *Cassia alata* leaves, *Urena lobata* leaves, *Premna odorata* bark and leaves, *Stachytarpeta jamaicensis* leaves, *Elusine indica* roots and leaves, *Diplazium esculentum* whole plant and *Phyllanthus urinaria* whole plant.

Preparation of ethanol extracts

Samples were cleaned by rinsing in running tap water, disinfected with 70% (v/v) and rinsed in distilled water three times. Washed plant materials were cut into small pieces (2-3 cm) and air dried. Dried plant materials were ground to fine particles using sterile electric grinder. Fifty (50) grams of each ground dried plant material were soaked in 500 ml of 80% ethanol in a stoppered flask for 72 hours.

The mixture was then filtered using Whatman no.1 filter paper and the solvent was completely removed using a rotary evaporator. Sterilization of the extracts were accomplished through centrifugation of the mixture at 10,000 x g for 30 minutes, and then filtration using a membrane filter with a pore diameter of 0.45 µm(Srisawat, 2007).

Preparation of bacterial culture

The stock cultures were revived using Mueller-Hinton Broth (MHB). Working stock cultures were grown and maintained on Mueller-Hinton Agar (MHA) at 5 ˚C.

Antibacterial assay

Disc diffusion method for antimicrobial susceptibility testing was carried out to assess the presence of antibacterial activities of the ethnobotanical extracts (Rezaei *et al.*, 2011). The 24-hour culture of *S. aureus* in MHB were adjusted to 0.5 McFarland standard. Different sterile swabs were dipped into the bacterial culture and was aseptically swabbed onto the surface of Muller Hinton agar plates evenly. Twenty µl of each treatment namely: the ethnobotanical extracts, Vancomycin (positive control), and distilled water (negative control) were pipetted on different sterile discs placed on empty sterile petri dishes and were allowed to stand for 30 minutes. The discs were placed equidistantly on the Mueller-Hinton agar surface using different sterile forceps. Every petri dish contained three extracts, one positive and one negative control. The setup was done in triplicates. The plates were incubated at 37°C for 24 hours. The zone of inhibition was measured after 12 and 24 hours prior to incubation. Extracts with antibacterial properties were eliminated for the succeeding tube coagulase assay.

Tube coagulase assay

The rabbit plasma was rehydrated to reconstitute according to the instructions provided in the kit (Scharlau Lyophilized Rabbit Plasma Ast No. 064-PLA-CO). The rehydrated plasma (195 µl) was placed in an Eppendorf tube and inoculated with 75 µl of *S. aureus* culture. Thirty (30) µl of each extract were aseptically added to the mixture and then incubated at room temperature for 24 hours. The formation of coagulum would indicate the production of coagulase, and hence, negative for QSI.

RNA extraction

The tubes with positive results for QSI from the coagulase assay were extracted for RNA. Samples were lysed and homogenized in TRIzol™ Reagent by adding 1 ml of TRIzol™ Reagent and incubated for 10 minutes to permit complete dissociation of the nucleoprotein complex. Chloroform (0.2 ml) was added per 1 ml of TRIzol™ Reagent used for lysis, and then the tubes were securely capped and were incubated for 5 minutes. The samples were then centrifuged for 15 minutes at 14,000 rpm at 4 ˚C.

The aqueous phase containing the RNA was transferred to a new tube by angling the tube at 45˚ and pipetting the solution out. For the precipitation of the RNA, 500 uL of isopropanol was added to the aqueous phase and incubated for 10 minutes and then centrifuged for 10 minutes at 12,000 × g at 4°C.

The supernatant was discarded with a micropipette. For the washing of the RNA, the pellet was re-suspended in 1 mL of 75% ethanol and vortexed then centrifuged for 5 minutes at 7500 × g at 4°C.

The supernatant was discarded with a micropipette. The RNA pellets were air dried for 5–10 minutes. For the solubilizing the RNA, the pellet was re-suspended in 50 µL of RNase-free water and incubated in a heat block set at 60°C for 10–15 minutes. The RNA was stored at –80°C freezer until use.

qRT-PCR for agrA expression

The expression of *agrA* in *S. aureus* treated with extracts positive for QSI was determined to evaluate QSI activity through qRT-PCR analysis.

The qRT-PCR program were as follows: incubation at 42°C for 5 min for reverse transcription; 1 cycle at 95°C for 2 min; then 45 cycles at 95°C for 20 s, 55°C for 20 s and 72°C for 20 s. KAPA One Step RT-PCR kit (KAPA Biosystems) was used for amplifications consisting of a mixture of 2.6 HPLC water, 5.0 µl KAPA Universal Mix, 0.2 µl dUTP, 0.5 µl of reconstituted forward and reverse primers (16S rRNA Staph 756F 5'-AACTCTGTTATTAGGGAAGAACA-3', Staph 750R 5'-CCACCTTCCTCCGGTTTGTCACC-3' (McClure *et al.*, 2006); *agrA*F 5'-CTACAAAGTTGCAGCGATGGA-3', *agrA*R 3'-TGGGCAATGAGTCTGTGAGA-5' (Sully *et al.*, 2014)), 0.2 µl RT Mix, and 1.0 µl RNA template. 16S rRNA was used as the internal control. Relative quantification of the amplified transcripts was done through Bio-Rad CFX96TM Real-Time System Thermal Cycler.

Statistical analysis

The Ct values of *agrA* expression analysis was done using LIVAK method of 2(-ΔΔCC(t)) then statistical analysis was done through dependent T-test at 5% level of significance.

Results and discussion

Antibacterial Activity of ECEs against S. aureus

A total of 15 ethnobotanical crude extracts (ECEs) were tested for their antibacterial activity against *S. aureus* (Table 1). The antibacterial assay served as screening of plant extracts for the succeeding virulence assay to rule out antibacterial-mediated decrease in virulence factor production. Three crude extracts, namely *U. lobata* leaves, *D. philippinensis* leaves and *D. esculentum* showed inhibition of *S. aureus* with mean values of 7.98 mm, 8.91 mm and 15.22 mm inhibition respectively.

Other studies on these plants also reported positive antibacterial activity against a range of pathogenic bacteria.

Table 1. Quorum sensing inhibition properties of ECEs against *S. aureus*.

Ethnobotanical crude extract	Coagulase formation	QSI
Hydrocotyle vulgaris	+	-
Mikania micrantha leaves	+	-
Dillenia philippinensis bark	-	+
Ceiba pentandra leaves	+	-
Cymbopogan winterianus leaves	+	-
Cassia alata	+	-
Urena lobata L. leaves	with antibacterial activity	with antibacterial activity
Dillenia philippinensis leaves	with antibacterial activity	with antibacterial activity
Premna odorata bark	+	-
Stachytarpeta jamaicensis leaves	-	+
Eleusine indica roots	-	+
Premna odorata leaves	+	-
Diplazium esculentum	with antibacterial activity	with antibacterial activity
Eleusine indica leaves	+	-
Phyllanthus urinaria	+	-

Legend (+) = Positive/Present; (-) = Negative/Absent.

Three flavonoid compounds isolated from *U. lobata* leaves, such as kaempferol 1, quercetin 2, and 3-O-β-D-(6″-O-trans-p coumaroyl)-α-L-glucopyranosyl-kaempferol 3 (tiliroside), showed strong antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Bacillus polyxyma* and *Candida albicans* (Adeloye *et al.*, 2007). Triterpenes present in *D.philippinensis* leaves were reported to possess significant antimicrobial property (Ragasa *et al.*, 2009). *D. esculentum* displayed strong antimicrobial activity against *S. aureus* with 11.33 mm zone of inhibition along with *Salmonella typhi*, and it was found that the extracts, when mixed in equal proportion with the antibiotic Tetracycline, were proven to be more effective against bacteria than the antibiotic alone (Amit *et al.*, 2011; Akter *et al.*, 2014). Proven to have antibacterial properties, these ethnobotanical extracts did not continue with the anti-quorum sensing assay.

Phenotypic detection of Quorum Sensing Inhibition activity of ECEs Against S. aureus

One way of testing the QSI property of the crude extracts is with tube coagulase assay since *S. aureus*, under active quorum-sensing condition, secretes coagulase. Coagulase is known to be an extracellular polypeptide that binds to and activates prothrombin that converts fibrinogen to fibrin of muscular tissue and induce clotting of plasma or blood (Cheng *et al.*, 2010).

Table 2. Phytochemicals present in the ethnobotanicals with QSI activities.

Ethnobotanical crude extract	Phytochemicals/Bioactive compounds	Reference
Dillenia philippinensis	Alkaloids, flavonoids, tannins and triterpenes	(Ragasa *et al.*, 2009; Barcelo, 2015)
Stachytarpeta jamaicensis	Alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, steroids and glycoside	(Idu *et al.*, 2007; Sasidharan *et al.*, 2007; Thangiah, 2019)
Eleusine indica	Flavonoids, cyanogenetic glucoside, albuminoids, and phenolic compounds	(Alaekwe *et al.*, 2015; Desai *et al.*, 2017)

In this assay, the plasma must not clot nor coagulate in order to be positive for QSI activity. Results of the assay exhibited that the following tubes with the crude extracts of *Dillenia philippinensis* bark, *Stachytarpeta jamaicensis* leaves and *Eleusine indica* roots showed no clot, thus, were phenotypically positive for QSI activity against *S. aureus*(Table 1).

Molecular Detection of Quorum Sensing Inhibition of ECEs against S. aureus

The mean Ct values shown in Figure 1 revealed that the expression of *agrA* treated with the crude extracts of *S. jamaicensis* leaves (4.439), *E. indica roots* (2.785), and *D. philippinensis* bark (1.022) were significantly lower than the control (10.065), therefore confirming the QSI activity.

Bacteria, such as *S. aureus*, undergo significant physiological rearrangements as their population density increases (Camilli and Bassler, 2006). The corresponding gene regulatory changes are controlled by quorum-sensing systems whereas in *S. aureus*, expression of several virulence factors is regulated by the *agr* system, which is composed of two divergent operons P3, and P2 where the target gene, *agrA* is found (Queck *et al.*, 2008). This Gram-positive bacterial pathogen causes a wide variety of lethal and hostile infections in humans. The pathogenic success of *S. aureus* can be attributed to the various array of virulence factors, including a large number of cell-surface bound proteins such as adhesins, fibrinogen/fibronectin binding proteins that are expressed during colonization of the host, and secreted proteins such as haemolysins, proteases, lipases that are required for acute infections (Dunman *et al.*, 2001; Cheung *et al.*, 2011). The *agr* system manages the phenotypic change in *S. aureus* during infection, which turns the bacterial pathogen from adhesive and colonising to tissue damaging and invasive (Novick and Geisinger, 2008).*AgrA* protein is the response regulator of the *S. aureus* quorum sensing system and is required for the activation of these phenotypic changes (Koenig *et al.*, 2004). Therefore, the downregulation of *agr* operon or specifically, the *agrA* expression which are important components the pathogenicity of the bacterium could hinder the pathogenic success of *S. aureus*, at least during the acute stage of its infection (Cheung *et al.*, 2011).

Natural products are highly valued due to their therapeutic effects on numerous diseases caused by pathogens and are being studied for their bioactivities both for therapeutic and ecological roles. Humans and animals possess immune systems which naturally responds as protection against invaders, while plants, on the other hand, which lack these immune systems may have evolved to produce their own QSI agents that can be utilized for defense against QS pathogens (Koh *et al.*, 2013). The downregulation of *agrA* may be due to the numerous secondary metabolites present in the plant extracts tested. Alkaloids, flavonoids and tannins present in the alcoholic extracts of *D. philippinensis* fruit and *E. indica* (Barcelo, 2015; Alaekwe *et al.*, 2015) possess QSI activities against multiple virulence factors of clinically isolated *P. aeruginosa* (Mutungwa *et al.*, 2015). Rajkumari *et al.* (2018) reported that triterpenes exhibit significant attenuation in the production of quorum sensing regulated virulence factors which are found in *D. philippinensis*. Moreover, secondary metabolites such as flavonoids

and tannins are present in *S. jamaicensis* leaves (Idu *et al.*, 2007; Sasidharan *et al.*, 2007). Listed in Table 2 are the secondary metabolites found in the plants reported to have shown QSI activity against *S. aureus*.

Pharmacological Potential of the Ilongot Ethnobotanical Extracts Against QS in S. aureus

The emergence of diseases caused by bacterial pathogens coupled with multi-drug resistance are one of most pressing concerns of the public health sector. Thus, increasing demand for novel means of controlling infectious diseases caused by these pathogens addresses continual development of drug resistance (Kalia *et al.*, 2007). The growing popularity of quorum-sensing systems in pathogenic bacteria give rise to a number of possibilities in manipulating these regulatory mechanisms because many bacterial pathogens use quorum sensing to regulate virulence while studies and strategies are now being designed to interfere or disrupt with these signaling systems through the use of secondary metabolites (Adonizio *et al.*, 2008; Song *et al.*, 2012; Tan *et al.*, 2013).

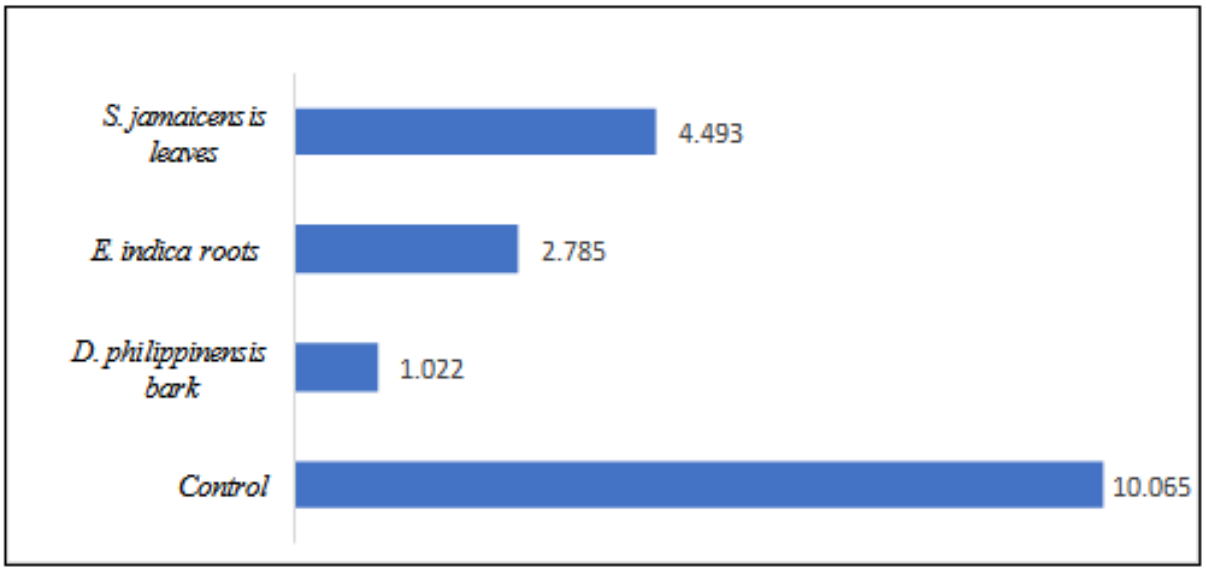

Fig. 1. Mean Ct values of *agrA* in *S. aureus* with ECEs. All extracts showed significantly lowered expression of *agrA*.

The ethnobotanicals of the Ilongot community of Aurora, Philippines, specifically, the *D. philippinensis* bark, *S. jamaicensis* leaves and *E. indica* roots may be used in development of therapeutic strategies such as QSI through battling invasive bacterial pathogens such as *S. aureus* by enabling the inhibition of pathogenesis of bacteria such as the coagulase formation through disrupting their communication by downregulating *agrA*. These findings provide evidences in controlling pathogenic bacteria such as *S. aureus* and can therefore potentially be considered and developed as natural anti-pathogenic drug.

References

Adeloye A, Akinpelu D, Ogundaini OA, Obafemi C. 2007. Studies on Antimicrobial, Antioxidant and Phytochemical Analysis of *Urena lobata* Leave Extract. Journal of Physical and Natural Sciences **1,** 1-9.

Adonizio AL, Downum K, Bennett BC, Mathee K. 2006. Anti-Quorum Sensing Activity of Medicinal Plants in Southern Florida. Journal of Ethnopharmacology **105(3),** 427-435.

Akter S, Hossain MM, Ara I, Akhtar P. 2014. Investigation of *In vitro* Antioxidant, Antimicrobial and Cytotoxic Activity of *Diplazium esculentum* (Retz.) Sw. International Journal of advances in Pharmacy, Biology and Chemistry **3(3),** 723-733.

Alaekwe IO, Ajiwe VI, Ajiwe A, Aningo G. 2015.

Phytochemical and Anti–microbial screening of the Aerial Parts of *Eleusine indica*. International Journal of Pure & Applied Bioscience **3**(1), 257-264.

Amit S, Sunil K, Sp B, Arvind N. 2011. Antibacterial activity of Diplazium esculentum (Retz.) Sw. Pharmacognosy Journal **3(21),** 77-79.

Balberona AN, Noveno JJ, Angeles MGB, Santos RI, Cachin E. 2018. Ethnomedicinal Plants Utilized by the Ilongot-Eǵongot Community of Bayanihan, Maria Aurora, Aurora, Philippines. International Journal of Agricultural Technology **14(2),** 145-159.

Barcelo RC. 2015. Phytochemical Screening and Antioxidant Activity of Edible Wild Fruits in Benguet, Cordillera Administrative Region, Philippines. Electronic Journal of Biology **11(3),** 80-89.

Barrogo K, Cruz KJ, Jacinto WR. 2015. Quorum Sensing-Inhibition Activities of Philippine Ethnobotanicals against Virulence Factors in *Pseudomonas aeruginosa***.** International Journal of Biosciences **13,** 173-182.

Bassetti M, Trecarichi EM, Mesini A, Spanu T, Giacobbe DR, Rossi M, Shenone E, Pascale GD, Molinari MP, Cauda R, Viscoli C, Tumbarello M. 2011. Risk Factors and Mortality of Healthcare-Associated and Community-Acquired *Staphylococcus aureus* bacteraemia. Clininical Microbiology and Infection **18(9),** 862-869.

Boucher H, Miller LG, Razonable RR. 2010. Serious Infections Caused by Methicillin-Resistant *Staphylococcus aureus*. Clinical Infectious Diseases **51,** S183–S197.

Burdette SD, Watkins RR, Wong KK, Mathew SD, Martin DJ, Markert RJ. 2012. *Staphylococcus aureus* Pyomyositis Compared with Non-*Staphylococcus aureus* Pyomyositis. Journal of Infection **64(5),** 507-512.

Camilli A, Bassler BL. 2006. Bacterial Small-Molecule Signaling Pathways. Science **311,** 1113–1116.

Changchien CH, Chen YY, Chen SW, Chen WL, Tsay JG, Chu C. 2011. Retrospective Study of Necrotizing Fasciitis and Characterization of its Associated Methicillin-Resistant *Staphylococcus aureus* in Taiwan. BMC Infectious Diseases **11(297),** 1-11.

Cheng AG, McAdow M, Kim HK, Bae T, Missiakas DM, Schneewind O. 2010. Contribution of Coagulases towards *Staphylococcus aureus* Disease and Protective Immunity. PLoS Pathogens **6(8),** 19-20.

Cheung GY, Wang R, Khan BA, Sturdevant DE, Otto M. 2011. Role of the Accessory Gene Regulator agr in Community-Associated Methicillin-Resistant *Staphylococcus aureus* Pathogenesis. Infection and Immunity **79,** 1927–1935.

Cruz KG, Gatchalan JJC, Jacinto WR. 2018. Philippine Ethnobotanicals Inhibit Quorum Sensing-Controlled Biofilm Formation in *Pseudomonas aureginosa*. International Journals of Biology, Pharmacy and Allied Sciences **7(4),** 527-537.

Damle S, Sharon K. 2017. Phytochemical Studies of *Ixora coccinea* Linn – An Ethnobotanical Plant from Karwar district. International Journals of Biology, Pharmacy and Allied Sciences **6(7),** 1403-1415.

DeLeo FR, Diep BA, Otto M. 2009. Host Defense and Pathogenesis in *Staphylococcus aureus* Infections. Infectious Disease Clinics of North America **23(1),** 17–34.

Desai AV, Patil VM, Patil SS, Yeligar VC, Patil SV. 2017. Phytochemical Investigation of *Eleusine indica* for In-vivo Diuretic and In-vitro Anti-urolithiatic Activity. World Journal of Pharmaceutical Research **6(8),** 1292-1304.

Dunman PM, Murphy E, Haney S, Palacios D, Tucker-Kellogg G, Wu S, Brown EL, Zagursky RJ, Shales D, Projan SJ. 2001. Transcription Profiling-Based Identification of *Staphylococcus aureus* Genes Regulated by the agr and/or sarA Loci. Journal of Bacteriology **183(24),** 7341-7353.

Idu M, Omogbai EKI, Aghimien GE, Amaechina F, Timothy O, Omonigho SE. 2007. Preliminary Phytochemistry, Antimicrobial Properties and Acute Toxicity of *Stachytarpheta jamaicensis* (L.) Vahl. Leaves. Trends in Medical Research **2(4),** 193-198.

Kalia VC, Rani A, Lal S, Cheema S, Raut CP. 2007. Combing Databases Reveals Potential Antibiotic Producers. Expert Opinion on Drug Discovery **2(2),** 211-224.

Kechrid A, Pérez-Vázquez M, Smaoui H, Hariga D, Rodríguez-Baños M, Vindel A, Baquero F, Cantón R, Del Campo R. 2011. Molecular Analysis of Community-Acquired Methicillin-Susceptible and Resistant *Staphylococcus aureus* Isolates Recovered from Bacteraemic and Osteomyelitis Infections in Children from Tunisia. Clinical Microbiology and Infection **17(7),** 1020-1026.

Koenig RL, Ray JL, Maleki SJ, Smeltzer MS, Hurlburt BK. 2004. *Staphylococcus aureus* AgrA Binding to the RNAIII-agr Regulatory Region. Journal of Bacteriology **186(22),** 7549-7555.

Koh CL, Sam CK, Yin WF, Tan L, Krishnan T, Chong Y, Chan KG. 2013. Plant-Derived Natural Products as Sources of Anti-Quorum Sensing Compounds. Sensors **13(5),** 6217–6228.

Kreienbuehl L, Charbonney E, Eggimann P. 2011. Community-Acquired Necrotizing Pneumonia Due to Methicillin-Sensitive *Staphylococcus aureus* Secreting Panton-Valentine Leukocidin: A Review of Case Reports. Annuals of Intensive Care **1,** 1-7.

Limos GBB, Jacinto WR, Cruz KJ. 2015. Philippine Ethnobotanicals Inhibit Virulence Factors in *Staphylococcus aureus*. International Journal of Biosciences **13,** 178-187.

McClure J, Conly JM, Lau G. 2006. Novel Multiplex PCR Assay for Detection of the Staphylococcal Virulence Marker Panton-Valentine Leukocidin Genes and Simultaneous Discrimination of Methicillin-Susceptible From Resistant Staphylococci. Journal of Clinical Microbiology **44,** 1141-1144.

Mutungwa W, Alluri N, Majumdar M. (2015). Anti-Quorum Sensing Activity of Some Commonly Used Traditional Indian Spices. International Journal of Pharmacy and Pharmaceutical Sciences **7,** 80-83.

Novick RP, Geisinger E. 2008. Quorum Sensing in Staphylococci. Annual Review of Genetics **42,** 541-564.

Queck SY, Jameson-Lee M, Villaruz AE, Bach TL, Khan BA, Sturdevant DE, Ricklefs SM, Li M, Otto M. 2008. RNAIII-Independent Target Gene Control by the agr Quorum-Sensing System: Insight Into the Evolution of Virulence Regulation in *Staphyloccocus aureus*. Molecular cell **32(1),** 150-158.

Ragasa C, Alimboyoguen AB, Shen C. 2009. Antimicrobial Triterpenes from *Dillenia philippinensis*. The Philippine Scientist **46(1),** 78-87.

Rajkumari J, Borkotoky S, Murali A, Suchiang K, Mohanty SK, Busi S. 2018. Attenuation of Quorum Sensing Controlled Virulence Factors and Biofilm Formation in *Pseudomonas aeruginosa* by Pentacyclic Triterpenes, Betulin and Betulinic Acid. Microbial Pathogenesis **118,** 48–60.

Rasmussen TB. Givskov M. 2006. Quorum-Sensing Inhibitors as Anti-Pathogenic Drugs. International Journal of Medicinal Microbiology **296(2-3),** 149-61.

Rezai A, Oyong GG, Borja VB, Inoue M, Abe T, Tamamura R, Nagatsuka H, Setsu K, Buery RR. 2011. Molecular Screening of Anti-Quorum Sensing Capability of *Salvadora persica* on *Enterococcus faecalis*. Journal of Hard Tissue Biology **20(2),** 115-124.

Sasidharan S, Latha L, Y, Zuraini Z, Suryani S, Sangetha S, Shirley L. 2007. Antidiarrheal and Antimicrobial Activities of *Stachytarpheta jamaicensis* Leaves. Indian Journal of Pharmacology **39(5),** 245.

Smith RS, Iglewski BH. 2003. *Pseudomonas aeruginosa* Quorum-Sensing Systems and Virulence. Current Opinion in Microbiology **6(1),** 56-60.

Song C, Ma H, Zhao Q, Song S, Jia Z. 2012. Inhibition of Quorum Sensing Activity by Ethanol Extract of *Scutellaria baicalensis* Georgi. Journal of Plant Pathology and Microbiology **72,** 443.

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.

Sully EK, Malachowa N, Elmore BO, Alexander SM, Femling JK, Gray BM, DeLeo FR, Otto M, Cheung AL, Edwards BS, Sklar LA, Horswill AR, Hall PR, Gresham HD. 2014. Selective Chemical Inhibition of agr Quorum Sensing in *Staphylococcus aureus* Promotes Host Defense With Minimal Impact on Resistance. PLoS Pathogens **10(6),** 1-14.

Tan LY, Yin WF, Chan KG. 2013. *Piper nigrum*, *Piper betle* and *Gnetum gnemon*-Natural Food Sources with Anti-Quorum Sensing Properties. Sensors **13(3),** 3975-3985.

Thangiah A. 2019. Phytochemical Screening and Antimicrobial Evaluation of Ethanolic-Aqua Extract of *Stachytarpheta jamaicensis* (L.) vahl Leaves Against Some Selected Human Pathogenic Bacteria. Rasyan Journal of Chemistry **12(1),** 300-307.

Tortora GJ, Funke BR, Case CL. 2013. Microbiology: An Introduction (11 ed.). Glenview, IL Pearson Education Inc.

Wielders CLC, Fluit AC, Brisse S, Verhoef J, Schmitz FJ. 2002. mecA Gene is Widely Disseminated in *Staphylococcus aureus* Population. Journal of Clinical Microbiology **40(11),** 3970-3975.