Journal of Applied Biology & Biotechnology Vol. 12(4), pp. 1-13, Jul-Aug, 2024
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2024.164825

Quorum sensing inhibition activities of Philippine ethnobotanicals against multidrug-resistant pathogens

Khristina G. Judan Cruz*

Department of Biological Sciences, Central Luzon State University, Philippines.

ARTICLE INFO

Article history:
Received on: December 10, 2023
Accepted on: March 20, 2024
Available online: May 20, 2024

Key words:
Ethnobotanicals,
Quorum sensing,
Virulence factors.

ABSTRACT

Alternative therapeutic approaches to target microbial multidrug resistance (MDR) have become an important thrust in research. One potential approach to manage antibiotic resistance and pathogenicity in MDR pathogens is through targeting quorum sensing (QS). Blocking the QS system does not affect bacterial growth while preventing bacteria from triggering virulence, reducing pathogenicity and selective pressure to survive, hence, reducing the resistance evolution. Recent ethnopharmacological research highlights the prospects of Philippine ethnobotanicals to discover novel molecules and approaches for targeting the QS system to control diseases and pathogens. In this review, two sets of Philippine ethnobotanicals utilized by ethnic communities - *Ikalahan* and *Ilongot-Eǵongot* – are highlighted as QS inhibitors. Inhibition of QS-linked virulence factor production such as violacein, biofilm, DNase, α-Hemolysin, swarming motility and coagulase in MDR pathogens *Pseudomonas aeruginosa, Staphylococcus aureus*, *Aeromonas hydrophila, Streptococcus agalactiae, Candida albicans* and one QS reporter bacteria, *Chromobacterium violaceum* by the ethnobotanical extracts are presented. All Philippine ethnobotanicals from the two ethnic communities included in this review showed inhibition on one or more virulence factors highlighting their activities against QS and emphasizes their potential for in-depth research on QS-based drug development for antipathogenesis.

1. INTRODUCTION

The global emergence of antimicrobial resistance (AMR) has become a serious health concern. Recent statistics reported AMR as the direct cause of 1.27 million deaths in 2019 with almost 5 million deaths due to complications of AMR [1], and these numbers are likely to intensify in the coming years. Aside from presenting urgent health concerns and high mortality rates, antimicrobial resistance has imposed major economic burdens on the world healthcare system with global economic impacts due to increasing treatment and diagnostic costs. It is foreseen that the global gross domestic product will decline by 3.8% and will lose $100 trillion by 2050 if the AMR crisis is not managed [2,3]. Multi-drug resistance (MDR) microbes have emerged as a result of persistent and prolonged antibiotic treatment and have become less responsive to commercial antibiotics. As antibiotics become less effective while more MDR pathogens emerge and spread globally, research efforts are urgently needed to produce novel, effective strategies to control antimicrobial resistance.

A potential alternative to manage antibiotic resistance and pathogenicity in MDR pathogens is through targeting quorum sensing (QS). QS is a communication system in microbes to coordinate metabolic activities for adaptation to environmental conditions through the regulation of specific genes [4,5]. This is made possible by the production and release of signal molecules called autoinducers [6,7] followed by a cascade of transcription regulation and gene expression through intricate signaling pathways [8]. This system is density-dependent, that is, the bacterial population must reach a threshold density to collectively alter gene expression patterns [9]. QS controls several physiological processes and virulence factors that enhance disease progression more effectively, and these include bioluminescence, biofilm formation, swarming motility, coagulase, and competence [10-12]. QS has attracted considerable interest as a mechanism in controlling pathogenesis in microbes as QS can be selectively blocked to control virulence. Blocking the QS system, called QS inhibition (QSI), does not affect bacterial growth while preventing bacteria from triggering virulence, thereby reducing pathogenicity. In this approach, bacteria are not exposed to selective pressure to survive, hence reducing the resistance evolution [13,14]. QSI represents a novel strategy to control pathogenic infections [15] and several studies have succeeded in demonstrating significant outcomes in terms of practical applicability. This suggests that QSI is a practical alternative to antibiotics [16].

Advancing trends in the control of antimicrobial resistance call for innovative and practical alternatives based on biological control approaches. Recent ethnopharmacological research highlights the potential of ethnobotanicals to discover novel molecules and

*Corresponding Author:
Khristina G. Judan Cruz,
Department of Biological Sciences,
Central Luzon State University, Philippines.
E-mail: kjcruz@clsu.edu.ph

approaches to control diseases and pathogens. Ethnobotanicals are plants of cultural and traditional significance, particularly in relation to their use by the ethnic community for a wide range of purposes [17,18]. Ethnobotany explores how ethnic cultures interact with and utilize these plant resources [19]. Ethnological practices are conventionally handed down through generations as traditions, and this includes medicinal practices using plants to manage diseases and ailments. Most ethnobotanicals are collected from the wild, mostly from geographically isolated areas, and as such, many remain untapped for their pharmacological prospects. This presents valuable resources for discovering and developing pharmacological strategies against microbial resistance and pathogenesis.

Plants and plant metabolites have shown actions on animal and microbial cells and displayed remarkable efficiency against a wide range of medical conditions. Through co-evolution with bacteria, plants developed an array of defenses through metabolites to protect themselves against infections. These antimicrobial metabolites have been shown to inhibit several pathogenic mechanisms in microbes. Phytochemicals have been proven to block QS systems by interfering with QS signals reducing their capacity to coordinate activities resulting in decreased virulence and pathogenicity [20]. Plant metabolites either resemble or mimic QS signals in bacteria that affect QS-related activities making this one of the most effective natural suppressors of QS communication systems [21].

The Philippines is a mega-biodiverse country that hosts two-thirds of the global biodiversity with around 80% of the world's plant and animal species. Despite its small size, the Philippines harbors higher biodiversity than any other country in the world. In terms of plant species, it ranks 5th in the world and hosts 5% of the world's flora [22]. The Philippines comprises an archipelago of more than 7000 islands. Its geography contributed to the country's exceptional level of endemism including at least 25 genera of plants and 50% terrestrial wildlife species. Half of the 52,177 described species in the Philippines are endemic making it one of the top ten most endemic countries in the world [23]. Despite being a top biodiversity hotspot in the world, new species are still being discovered. Medicinal plants and their metabolites played a key role and continue to provide a vast resource for diverse applications in pharmacotherapy in modern medicine [24-26]. Scientific validation of unexplored medicinal plants is now critical due to the fast rate of habitat degradation.

The ethnobotanicals – the plants used by ethnic communities – constitute a rich and expansive source of plants for the discovery of new antipathogenic drugs. The Philippines hosts diverse ethno-linguistic groups with unique customs and cultures [27]. Their cultures and traditions are inextricably linked to their lands which they consider pure and sacred [28]. One of their notable customs is the use of ethnomedicinal plants that are usually found within their ancestral domains. These ethnic communities typically inhabit geographically isolated areas, and hence, most ethnobotanical species still have untapped pharmacological potential. Among these ethnic groups are the *Ilongots* and the *Ikalahans*. The *Ilongot* indigenous people comprise a major ethnic group in the Philippines. Their communities live mostly in the mountains of the provinces of Quirino, Aurora, and Nueva Vizcaya where plant biodiversity is high. There are five *Ilongot* subgroups, one of which is the *Eǵongots* who mostly reside in Aurora Province. The *Ilongot-Eǵongot* group has a deep knowledge of the vast resources of plants within their ancestral domain. Their practice of traditional medicine using these plants is handed down through generations which are still practiced in the modern era [29].

The *Ikalahans,* on the other hand, are indigenous people with ancestral domains in the north-east Philippines along the mountainous terrain of Cordillera and Caraballo which are mostly situated in altitudes of more than 3000 feet above sea level [30,31]. Their land is characterized by montane forests with a cool climate and heavy rainfall supporting high levels of biodiversity of approximately 1500 plant and animal species [30,32]. The *Ikalahans* are renowned for their environmentally sustainable 'indigenous knowledge practice systems' that are transferred, protected, and maintained through generations [30,32].

Parallel to the loss in biodiversity, indigenous knowledge of medicinal plants is also at risk of being lost due to modernization. There have been few ethnobotanical evaluations for scientific validation. Ethnobotanicals represents a contemporary collection of plants that have attracted attention as potential sources of antipathogenic compounds and demonstrates a promise of identifying potent, natural sources of QS inhibitors necessary for the development of safe, new antipathogenic therapies [33]. Several papers have reported the inhibition of QS pathways and virulence factors in bacteria and fungi using natural compounds [34-38]. Phytochemicals are natural QS inhibitors [39] and as such, bioactive molecules from ethnobotanicals can be promising alternatives to antibiotics. Ethnobotanicals offer a deep array of newly discovered bioactive molecules and are now contributing toward deeper, practical evidence-based approaches using traditional species. With the advancements in pharmacology, the development of several drugs can be attributed to discoveries in ethnobotany [40,41].

This review highlights the series of evaluations conducted to assess the potential of Philippine ethnobotanicals to control QS related virulence factors in MDR pathogens as well as the significant results of these evaluations. Two sets of Philippine ethnobotanicals of the following indigenous communities are included in this review: *Ikalahans* of Imugan, Nueva Vizcaya, Philippines, and the *Ilongot-Eǵongots* of Maria Aurora, Aurora, Philippines. The ethnobotanicals of the *Ikalahans* tested were *Ageratina adenophora, Alstonia scholaris, Ayapana triplinervis, Bidens pilosa, Cestrum nocturnum, Derris elliptica, Oreocnide trinervis, Pittosporum pentandrum, Sarcandra glabra,* and Lipang daga (no known scientific name) [Table 1]. Three solvents were used for crude extraction in this group of ethnobotanicals: ethanol, methanol, and n-hexane. The *Ilongot-Eǵongot* ethnobotanicals with local names and plant parts used in the evaluation were based on the survey by Balberona *et al.*, 2017 [29]: *Adenanthera intermedia*, *Ceiba pentandra*, *Cymbopogon winterianus*, *Dillenia philippinensis*, *Diplazium esculentum, Eleusine indica*, *Ficus* sp., *Hydrocotyle vulgaris*, *Hyptis suaveolens*, *Mikania micrantha*, *Premna odorata*, *Phyllanthus urinaria*, *Senna alata*, *Stachytarpeta jamaicensis*, *Urena lobata*, and Talahib (no known scientific name) [Table 1]. Only ethanolic extracts of these plants were evaluated.

This paper reviews the QS inhibition properties of the ethnobotanicals against the pathogenic bacteria *Pseudomonas aeruginosa* [42-47], *Staphylococcus aureus* [48-52], *Aeromonas hydrophila* [33], and *Streptococcus agalactiae* [53]. The *Ikalahan* ethnobotanicals were also tested against the QS reporter bacteria *Chromobacterium violaceum* [54,55]. Inhibition of biofilm formation by the *Ilongot-Eǵongot* ethnobotanicals against *Candida albicans* has been explored [56]. The test bacteria in the papers included in this review are drug-resistant bacteria that are responsible for a large number of infections in humans and constitute a serious threat to public health. *P. aeruginosa* and *S. aureus* form part of the ESKAPE pathogens which includes six MDR pathogens [57]. ESKAPE includes other bacterial pathogens such as *Enterococcus faecium, Klebsiella pneumoniae,*

Table 1: Ethnobotanicals from 2 Philippine ethnic communities evaluated for anti-quorum sensing activities.

Philippine Ethnobotanicals			
Ilongot-Egongot		***Ikalahan***	
Scientific name	**Local name**	**Scientific name**	**Local name**
Adenanthera intermedia	Kares	*Ageratina adenophora*	Panawel
Ceiba pentandra	Béték	*Ayapana triplinervis*	Pantallion
Cymbopogon winterianus	Taday	*Alstonia scholaris*	Palay
Dillenia philippinensis	Katmon	*Bidens pilosa*	Anwad
Diplazium esculentum	Pako-pako	*Cestrum nocturnum*	Dama de noche
Eleusine indica	Pag	*Derris elliptica*	Opay
Ficus sp.	Balete	*Oreocnide trinervis*	Lal-latan
Hydrocotyle vulgaris	Gotu kola	*Pittosporum pentandrum*	Lahwik
Hyptis suaveolens	Ambabangot	*Sarcandra glabra*	Hag-ob
Mikania micrantha	Ola-ola	Lipang daga (no known scientific name)	
Premna odorata	Asédaong		
Phyllanthus urinaria	Taltalikod		
Senna alata	Bensola		
Stachytarpeta jamaicensis	Luzviminda		
Urena lobata	Pukot		
Talahib (no known scientific name)			

Acinetobacter baumannii, and *Enterobacter* spp. This group of pathogens comprise the leading cause of life-threatening nosocomial infections globally [58] that employ a wide spectrum of mechanisms to evade actions of antimicrobials that eventually lead to antibiotic resistance [59] and has been put as a key priority for new therapy development [3]. *P. aeruginosa* and *S. aureus* are both opportunistic pathogens responsible for a number of hospital-acquired infections worldwide [60-62]. Virulence factors of both pathogens are largely regulated through QS which facilitates the production of these factors in a coordinated, cell-density-dependent manner. *P. aeruginosa* biofilms largely contribute to lung infections by adhering to mucin in the respiratory tract [50,63]. Aside from biofilms, it possesses an array of virulence factors such as the production of toxins essential for penetrating tissues [64,65]. Aside from disease progression and host colonization, the armory of virulence factors plays a major role in the bacteria's capability to adapt to environmental changes to rapidly evolve resistance to antibiotics [61]. QS regulates more than 10% of *P. aeruginosa* genes [66] that are primarily involved in virulence factor production, biofilm development, and other factors essential for colonization and disease progression such as the development of mechanisms for antibiotic resistance, motility and adjustment of pathways for metabolism [66-69]. *A. hydrophila* and *S. agalactiae* are significant fish pathogens where infections cause disease outbreaks and huge economic losses, particularly in intensive aquaculture productions where fish densities are high [70-74]. Both bacteria are also zoonotic agents [75-77] and have new emerging multidrug-resistant strains due to the heavy use of antibiotics in aquaculture. In *Candida* sp., surging antifungal resistance presents an emerging concern for the immediate development of control strategies [78,79]. *C. albicans* is one of the most prevalent nosocomial opportunistic commensals in humans [80] that is mainly linked to a number of infections such as candidiasis and bloodstream infections [81]. It possesses an array of strategies that are responsible for the emergence of its resistance to various classes of drugs with an alarming mortality rate of 40% in spite of antifungal treatments [82-84].

This review presents the QS inhibition activities of ethnobotanicals utilized by two major groups of ethnic communities in the Philippines [Table 1] in several virulence factors.

2. INHIBITION OF VIRULENCE FACTORS BY PHILIPPINE ETHNOBOTANICALS

2.1. Violacein Production

C. violaceum is a standard reporter bacterium often used to test quorum-sensing activities through the production of a purple-colored pigment violacein [85]. Violacein is a pigment required for swarming and formation of biofilm and is involved in the regulation of several virulence factors [86]. *C. violaceum* is also a significant human pathogen. Although not common, *C. violaceum* infections can cause high mortality rates [87,88] that are often due to multiorgan failure. *C. violaceum* infections are usually accompanied by pneumonia, severe sepsis, and septic shock [89].

Due to easy visualization, *C. violaceum* is routinely used to screen natural products for their QSI activities. Violacein is produced after the activation of QS through the mediation of acyl-homoserine lactone (AHL) signals [90]. Bacteria employ the AHL molecules as signals to regulate the expression of certain genes in response to the environment and population density that are recognized by specific receptors. Thus, through the visible inhibition of violacein production, it can be said that AHL signals are blocked. AHL signaling is not only required for violacein production in *C. violaceum*, but also critical for plant and animal diseases, specifically bacteria-caused infections. Blocking AHL signals can be tapped to control pathogen virulence [91].

The ethnobotanical extracts of the *Ikalahans* were evaluated for QSI by phenotypic visualization of violacein production reduction in *C. violaceum* through the standard disk diffusion assay. Two solvents – ethanol and methanol – were used for the extraction of plants. The ethanolic and methanolic extracts of S. *glabra, D. elliptica,*

A. adenophora, and *A. triplinervis* visibly showed consistent inhibition of the production of the purple pigment [54,55] [Table 2] as shown by the absence of growth around the disc. In addition, the absence of violacein production was also noted in bacterial cultures treated with the ethanol extracts of *C. nocturnum, A. scholaris* [54], and the methanolic extracts of *B. pilosa, O. trinervis, P. pentandrum* [55]. These extracts exhibited strong inhibitory effects as noted by the measured zones of inhibition around the discs.

2.2. Biofilm Formation

Biofilm formation is a QS-related process in which bacterial cells produce a complex matrix of extracellular polymeric substances that include polysaccharides and proteins that provide protection to the bacterial community and prevent or delay the penetration of antimicrobial agents into the cells [92-94]. Biofilms play a major role in the increased resistance of bacteria to antibiotics [95,96]. In fact, bacteria in biofilms tend to be more tolerant of antibiotics compared to those in the planktonic state [97]. Not only does it protect the bacterial community from antibiotics, but it also effectively blocks the host's immune cells [98] and provides protection against sudden changes in the environment and mechanical damage [99,100].

Biofilm production presents a serious health concern as it is related to most persistent chronic infections [101] and contributes to clinical complications. Biofilms play a major role in the development of AMR and are the centers of genetic transfer of mobile elements [102]. Another factor to consider is that mutation and horizontal gene transfer (HGT) are more frequent in bacteria with biofilms. Biofilms provide ideal conditions for HGT through conjugation that enables the transfer of AMR genes [103]. Biofilm-forming bacteria often use this self-produced biofilm to attach to surfaces that cannot be immediately controlled by antibiotics [104]. Aside from the clinical settings, the incidence of biofilm is common in the environment. Biofilms are commonly associated with food contamination [105-108] and biofouling in water treatment membranes [96,109]. Many different bacteria and fungi, as well as other microbes, can produce biofilm. Approximately 80% of infectious diseases are related to biofilms [110]. Controlling the formation of biofilm is a significant step in controlling pathogenesis and antimicrobial resistance.

P. aeruginosa biofilms are the major cause of chronic infections that are persistent [111,112]. *P. aeruginosa* biofilms, as with other biofilm-forming bacteria, play a significant role in resisting antibiotics and the host immune system through several mechanisms [57,65]. It is therefore important to increase the susceptibility of *P. aeruginosa* to antimicrobials by controlling biofilm development and attachment or by destroying it [112] to attenuate bacterial virulence and pathogenicity, and consequently, antibiotic resistance. A number of Philippine ethnobotanicals showed antibiofilm activity against *P. aeruginosa* [Table 3]. Santos *et al.* [47] tested the different plant parts of *Ilongot-Eǵongot* ethnobotanicals against a reference strain and one clinical isolate of *P. aeruginosa*. The crude extracts of *A. intermedia*, *D. esculentum, E. indica* leaves, *H. suaveolens* flowers, *H. vulgaris*, *M. micrantha*, and Talahib have biofilm inhibitory effects against *P. aeruginosa* clinical isolate. Against the reference strain *P. aeruginosa* PNCM 1335, a significant reduction in biofilm formation was observed in the crude extracts of *A. intermedia, C. pentandra, E. indica, D. esculentum, H. suaveolens, H. vulgaris, M. micrantha, S. jamaicensis., U. lobata,* and Talahib. These biofilm inhibition activities were confirmed through gene expression analyses of biofilm-linked genes (see discussion on the downregulation of QS-linked genes at the later part of the review). The *Ikalahan* methanolic crude extracts of *C. nocturnum, P. pentandrum*, and Lipang Daga (local name) also showed inhibition of biofilm formation in *P. aeruginosa* [43] [Table 2].

The *Ilongot-Eǵongot* ethnobotanicals were also evaluated against aquaculture pathogens *A. hydrophila and S. agalactiae* biofilm formation. A significant decrease in *A. hydrophila* biofilm formation was observed using the crude extracts of 13 *Ilongot-Eǵongot* ethnobotanicals namely C. *pentandra*, *C. winterianus*, bark and leaves of *D. philippinensis*, *D. esculentum*, roots and leaves of *E. indica*, *H. vulgaris*, *M. micrantha*, bark and leaves of *P. odorata*, *P. urinaria, S. jamaicensis*, and *U. lobata* [Table 3]. The plant extracts tested showed lower optical density (OD) values compared to the control (no extract) after performing the microtiter plate assay for biofilm

Table 2: Summary of QSI activities of the *Ikalahan* ethnobotanicals.

Ethnobotanicals	*Chromobacterium Violaceum*	*Pseudomonas aeruginosa*							*Staphylococcus aureus*						
	Disc diffusion assay	Pyocyanin production			Swarming motility			Biofilm formation	DNase			α- Hemolysin			Coagulase
Solvent	EE	ME	EE	ME	HEX	EE	ME	HEX	ME	EE	ME	HEX	EE	ME	HEX	EE
Bidens pilosa																
Cestrum nocturnum																
Sarcandra glabra																
Oreocnide Trinervis																
Pittosporum pentandrum																
Lipang-daga																
Derris elliptica																
Alstonia scholaris																
Ageratina adenophora																
Ayapana triplinervis																

Green: With QSI activity Red: No QSI activity Black With antibacterial activity CE: Crude Extract CE-AuNPs: Biologically synthesized gold nanoparticles. QSI: Quorum sensing inhibition

Table 3: Summary of QSI activities of the Philippine *Ilongot-Eǵongot* ethnobotanicals.

Ethnobotanicals	*Aeromonas hydrophila*		*Streptococcus agalactiae*		*Pseudomonas aeruginosa* clinical isolate		*Pseudomonas aeruginosa* reference strain		*Staphylococcus aureus* reference strain	MRSA		*Candida albicans*	
	Biofilm formation		Biofilm formation		Biofilm formation		Biofilm formation		Coagulase	Coagulase	DNase	Biofilm formation	
Solvent	CE	CE-AuNPs	CE	CE-AuNPs	CE	CE-AuNPs	CE	CE-AuNPs	CE	CE	CE	CE	CE-AuNPs
Hydrocotyle vulgaris													
Mikania micrantha leaves													
Dillenia philippinensis bark													
Dillenia philippinensis leaves													
Ceiba pentandra													
Cymbopogon winterianus													
Senna alata													
Urena lobata													
Premna odorata bark													
Premna odorata leaves													
Stachytarpeta jamaicensis leaves													
Eleusine indica roots													
Eleusine indica leaves													
Diplazium esculentum													
Phyllanthus urinaria													

Green: With QSI activity Red: No QSI activity Black With antibacterial activity CE: Crude Extract CE-AuNPs: Biologically synthesized gold nanoparticles. QSI: Quorum sensing inhibition

formation, [33]. In *S. agalactiae* biofilm formation, fourteen (14) *Ilongot-Eǵongot* ethnobotanical crude extracts, namely, *C. pentandra*, *C. winterianus*, *C. alata* leaves, bark and leaves of *D. philippinensis*, roots and leaves of *E. indica*, *D. esculentum*, *M. micrantha*, bark and leaves of *P. odorata*, *P. urinaria, S. jamaicensis*, and *U. lobata* [Table 3] showed a significant decrease in biofilm formation [53]. The genes related to biofilm formation were significantly downregulated as affected by the ethnobotanical extracts both in *A. hydrophila* and *S. agalactiae* (see later part of discussion).

The *Ilongot-Eǵongot* ethnobotanicals were also tested in *C. albicans* biofilm formation [Table 3]. The OD values of the *C. albicans* clinical isolate culture treated with extracts of *C. pentandra* leaf, *C. winterianus* leaf, *D. philippinensis* leaf, *D. esculentum,* roots and leaves of *E. indica*, *S. alata, H. vulgaris* leaf, *M. micrantha* leaf, *P. odorata* bark, *P. urinaria S. jamaicensis* leaf, and *U. lobata* leaf showed significantly lower OD values ranging from 0.062 to 0.083 mg/mL compared to the negative control (no extract) with a higher value of 0.19 mg/mL [56] showing inhibition of biofilm formation and were confirmed through gene expression analyses (see discussion on inhibition of quorum-sensing-linked genes using Philippine ethnobotanicals - Section 4.0).

2.3. Coagulase Formation

S. aureus is the only known human disease-causing bacteria that produce coagulase [113]. Coagulase is a virulence factor involved in establishing host infections with *S. aureus* [114,115]. *S. aureus* is a significant human pathogen with a remarkable genetic competence for antibiotic resistance [116]. Through adaptive mutations, resistant strains such as methicillin resistant *S. aureus* and vancomycin-resistant *S. aureus* have reached epidemic proportions globally [116-118]. The slow development of effective antibiotics and rapidly evolving resistance make *S. aureus* infections challenging to manage [119]. Hence, new therapeutic modalities with less potential for resistance must be developed immediately [120,121]. The Philippine ethnobotanicals were evaluated against coagulase formation in *S. aureus* through the tube coagulase assay using rabbit plasma. The tube coagulase assay is performed to measure the production of coagulase enzyme by *S. aureus* and uses rabbit plasma. Coagulase is an enzyme that causes the plasma to clot by converting fibrinogen to fibrin [122]. Clotting reaction after 4–24 h of incubation indicates QS activity. Three ethnobotanical crude extracts of the *Ilongot-Eǵongot* community showed inhibition of coagulation [37,50]. These are *D. philippinensis* bark, *E. indica* roots and *S. jamaicensis* leaves [Table 3]. Ethanol extracts of *Ikalahan* ethnobotanicals such as *A. triplinervis, A. adenophora, D. elliptica, O. trinervis and S. glabra* showed anti-coagulase activity against *S. aureus* [51] [Table 2].

2.4. α-Hemolysin

α-Hemolysin is a cytotoxic protein and a major QS-mediated virulence factor that plays a significant role in *S. aureus* pathogenesis [123-125]. *S. aureus* secretes hemolysins, one of which is the α-Hemolysin, which allows lysis of red blood cells. The α toxin is active against

a wide range of mammalian cells and implicated in a wide range of *S. aureus* diseases such as skin and soft-tissue infections [126], pneumonia [125], bacteremia and sepsis [127], lethal peritonitis [128], septic arthritis, brain abscess and corneal infections [123].

The α-Hemolysin assay was done using Blood Agar supplemented with the ethnobotanical crude extracts. 24-h culture of *S. aureus* was streaked onto the agar and incubated for no more than 24 h. The absence of hemolysis in plates indicates inhibition of α-Hemolysin production by the ethnobotanical extracts. Only the ethanol and n-hexane extracts of *Ikalahan* ethnobotanicals *A. scholaris, A. adenophora, A. triplinervis, B. pilosa, C. nocturnum, D. elliptica, O. trinervis, P. pentandrum, S. glabra,* and Lipang Daga inhibited the production of α-toxin in *S. aureus* [Table 2] [48,49]. This was observed as the absence of hemolysis in the blood agar plates cultured with the bacteria compared to the negative control (water) where hemolysis was evident. Methanolic extracts of these ethnobotanicals failed to inhibit the production of α-Hemolysin.

2.5. Deoxyribonuclease (DNase)

S. aureus produces an enzyme that is capable of breaking down DNA [129]. DNase is a virulence factor [130] that allows *S. aureus* to evade immune cells, particularly the neutrophils, from attacking them. Neutrophils are a critical part of the innate immune system and the primary line of protection against invading pathogenic bacteria [131]. Some bacteria have the capacity to circumvent destruction by neutrophils [115]. In the case of *S. aureus*, this is made possible by the production of DNase [132]. Avoiding destruction, this allows the bacteria to invade tissues and cause infections [133,134]. DNase has also been linked to *S. aureus*-related pus-forming illnesses. Aside from this, DNase is known to contribute to virulence factor production such as maturation of biofilm and is further involved in bacterial growth [135,136]. In some countries, phenotypic coagulase tests are usually conducted to confirm infections by *S. aureus* [137].

Deoxyribonuclease (DNase) assay is done through the addition of hydrochloric acid (HCl). DNase production can be observed through clear zones of depolymerized DNA around the bacterial colonies. The bacterial cultures treated with methanolic extracts of *D. elliptica* and *O. trinervis* [52] and the ethanolic extracts of *A. triplinervis, C. nocturnum,* and *O. trinervis* [48] inhibited the phenotypic expression of DNase [Table 2]. Clear zones surrounding the bacterial streak and colonies suggest the presence of DNase [138,139], hence, the QSI activity was noted by the absence of clearing around the bacterial colonies that are opaque and whitish due to polymerized DNA.

2.6. Swarming Motility

Swarming is a QS-related process where coordinated movements allow bacteria to spread across a surface [140]. Since it is QS-linked, bacterial density and growth medium are critical for this process [141]. In *P. aeruginosa*, swarming aids the bacterial community to cause infections by producing secretions that reduce surface tension in their environment [141,142]; it is a necessary step for bacterial migration and colonization as well as a major contributor for the production of biofilms. In the reviewed papers, only the *Ikalahan* ethnobotanical methanolic extracts (*A. adenophora, A. triplinervis, A. scholaris, B. pilosa, C. nocturnum, D. elliptica, O. trinervis, P. pentandrum, S. glabra,* and Lipang daga) significantly controlled swarming motility in *P. aeruginosa* [42] [Table 2].

2.7. Pyocyanin Production

Pyocyanin is a *P. aeruginosa* virulence factor that contributes to its pathogenesis by invasion and inhibition of several cells and cellular processes in humans such as those in respiratory epithelial cells and immune cells [143,144]. Only the ethanolic extracts of *Ikalahan* ethnobotanicals *O. trinervis, C. nocturnum,* and *A. triplinervis* showed significant inhibition of pyocyanin production in *P. aeruginosa* [44] [Table 2]. A number of *Ikalahan* ethnobotanical methanol extracts showed lower pyocyanin production (*B. pilosa, O. trinervis, A. triplinervis and D. elliptica*) [42] but were not significantly different from the negative control.

3. BIOLOGICALLY SYNTHESIZED GOLD NANOPARTICLES USING THE PHILIPPINE ETHNOBOTANICALS

Nanotechnology had gradually pushed drug design into new heights through novel approaches. Among the potential applications of nanotech-designed materials is the synthesis of nanoparticles to target multidrug resistance in bacteria. As mentioned earlier, MDR has become a global health concern. To address this, nanoparticles offer advantages in designing antimicrobial drugs through extremely reduced size, biocompatibility, and targeted drug delivery [145-147]. Among nanotechnology approaches, green synthesis of nanoparticles draws interest as it offers solutions to control diseases without the toxicity commonly associated with nanoparticles synthesized using the standard physical and chemical methods. Toxicity is avoided in nanoparticle green synthesis as it utilizes natural products as capping or reducing agents. The method also reduces the use of toxic chemicals, requires less energy, is cost-effective, relatively fast and easy to perform, and generally with less pollution, and thus, environment-friendly [148,149]. The exceptional properties of natural metabolites are tapped for the stable synthesis of nanoparticles [149]. While the research in biosynthesized nanoparticles is mostly still in the laboratory phase and their sustainability has to be established, these NPs show considerable potential applications in biomedical science and other industries [150].

Among the metallic nanoparticles, gold nanoparticles (AuNPs) have gained significant attention in drug development due to their unique properties. AuNPs are generally considered biocompatible, making them suitable for use in biological systems, particularly for applications in drug delivery [151]. It is an ideal carrier of antimicrobial agents as this is the least toxic metal [152] and does not cause adverse reaction with human body tissues and fluids. AuNPs are also relatively stable in the body due to its high reduction potential [153]. Moreover, the tunability of AuNPs allow for precise control of size and shape to cater to targeted drug delivery while minimizing side effects applications [154,155]. This property is crucial as their extremely reduced size allow them to penetrate cells easily and their surface can be modified to attach therapeutic agents. Furthermore, the Enhanced Permeability and Retention (EPR) of AuNPs allows them to accumulate in diseased tissues such as cancer tumors for extended period of time compared to normal tissues [156,157].

Combining the potential of nanoparticles for effective drug delivery in disrupting QS with the antimicrobial power of natural metabolites is a novel strategy to address multidrug resistance in microbes. On this premise, the *Ilongot-Egongot* ethnobotanicals were utilized to synthesize gold nanoparticles which were consequently evaluated

for anti-QS activities. The formation of the gold nanoparticles using the ethnobotanicals was confirmed through SPR peaks observed in ultraviolet-visible spectroscopy and the observation of the change in color to pink-red indicates a reduction in size. These were tested against *P. aeruginosa* reference strain and clinical isolate [33], *A. hydrophila* [33], *S. agalactiae* [53], and *C. albicans* [56] biofilm formation [Table 3]. The evaluated ethnobotanically synthesized NPs displayed efficiency in inhibiting biofilm formation in the test bacteria whereas treatments with biosynthesized NPs showed significantly lower biofilm formation compared to treatments with only crude extracts. Table 3 shows the comparison of the biosynthesized NPs (CE-AuNPs) with crude extracts (CEs). NPs synthesized using *C. pentandra, C. winterianus, D. philippinensis* bark, *E. indica* roots, *H. vulgaris, M. micrantha, P. urinaria, P. odorata,* and *S. alata* had displayed antibacterial activity in the pre-screening for QS assays showing a high level of action against *A. hydrophila* [33]. To continue to subsequent QS assays, the sub-MIC (minimum inhibitory concentration) has to be determined, of which these were not included in the reviewed studies. The same case can be observed for most biosynthesized NPs included in the evaluation against *S. agalactiae* [53]. A number of biosynthesized gold nanoparticles were also shown to be more efficient than crude extracts in inhibiting biofilm formation in both clinical isolates and reference strains of *P. aeruginosa*. In cases where both CE and CE-AuNPs displayed biofilm formation inhibition, several AuNP treatments had significantly higher biofilm formation inhibition as was the observation in *A. hydrophila, S. agalactiae,* and *C. albicans* [33,53,56].

Current control strategies for MDR in bacteria only include chemical-based drugs and antiseptic solutions [102] with reduced penetration in the cell or limited access because of biofilms. Nanoparticles constitute a novel option as antimicrobial drugs can be delivered more efficiently through their extremely reduced nanosize and increased surface area that consequently results in faster penetration of the cell membrane and other biological barriers. Integrated with active metabolites, its applications in the medical field is wide.

4. INHIBITION OF QUORUM-SENSING-LINKED GENES USING PHILIPPINE ETHNOBOTANICALS

QS is made possible by collective gene expression brought about by the secretion of signal molecules that are triggered once the density has reached a certain threshold. It is a complex signaling circuit with several linked pathways [8]. The LuxI/LuxR circuit regulates QS in gram negative bacteria. In *P. aeruginosa*, two major circuits that are homologues of LuxI/LuxR regulate QS: LasI/LasR and RhlI/RhlR. Both homologues, as is in LuxI/LuxR, produce homoserine lactones (HSL) as autoinducers. When HSLs reach a certain threshold level, their complex with LasR/RhlR activates the transcription of genes that leads to the expression of virulence factors [158]. The Las and Rhl systems, in conjunction with the respective HSLs, regulate up to 353 genes, accounting for approximately 6% of the *P. aeruginosa* genome [159]. When the threshold quorum concentration is reached, the QS molecules C4-HSL and 3-oxo-C12-HSL, which are synthesized by LasI and RhlI, respectively, are recognized by their associated receptors, LasR and RhlR. A cascade of gene expression of virulence genes follows after the activation of LasR, a regulator of pathogenicity in *P. aeruginosa* [160-162]. Subsequently, if lasR expression is blocked, regulation of other QS-linked genes, particularly those that play a role in biofilm formation, will be affected [163].

In Gram-positive bacteria, the signal molecule is in the form of peptides produced from precursors called autoinducing peptides (AIPs) [11]. Common to other QS systems, once the AIPs reaches a threshold concentration, a cascade of expression for the production of several virulence factors follow [121,164]. In *S. aureus,* the accessory gene regulator (*agr*) system produces this signal molecule and plays a significant role in the production of *S. aureus* virulence and pathogenesis [165]. The production extracellular toxins and enzymes by the *agr* system [121,166,167] is critical for *S. aureus* colonization and resistance to host immune response [168].

In *P. aeruginosa*, QS-linked genes were significantly downregulated in treatments in several *Ilongot-Eǵongot* ethnobotanical extracts as well as in biosynthesized gold nanoparticles [Table 3]. The significant decrease in biofilm formation by plants extracts and biosynthesized nanoparticles using *M. micrantha, A. intermedia,* and Talahib was confirmed through the downregulation of *lasR* [47] and also *rhlR* [46] validating the results in QSI biofilm quantification. Since *lasR* is a transcription regulator of several QS virulence factors e.g. biofilms, its downregulation may mean that the molecules in the ethnobotanical extracts could have blocked its transcription or a genetic pathway, leading to it that led to the decrease in biofilm production.

AhyR expression also showed downregulation in *A. hydrophila* as affected by the crude ethnobotanical extracts and the biosynthesized AuNPs [33] [Table 3]. *AhyR* is a *luxR* homologue in *A. hydrophila* responsible for HSL production and regulates pathogenicity [169,170]. As in other bacteria, blocking *AhyR* expression likewise results in the control of the QS cascade affecting the production of other virulence factors.

In *C. albicans*, the molecular expression of two biofilm-linked genes, *Bcr1* and *Hsp90,* showed significant downregulation as affected by both ethnobotanical crude extracts and the biosynthesized AuNPs [56] [Table 3]. Specifically, *Bcr1* expression was significantly downregulated in the treatments with decreased biofilm formation such as in *H. vulgaris*, *M. micrantha* leaf, *C. pentandra* leaf, *C. winterianus* leaf, *S. alata*, *U. lobata* leaf, *D. philippinensis* leaf, *P. odorata* bark, *S. jamaicensis* leaf, *E. indica* roots, *D. esculentum*, *E. indica* leaf, and *P. urinaria* leaf as compared to treatments without ethnobotanical extracts used. *Bcr1* is a transcription regulator. The polysaccharide matrix in *C. albicans* is regulated by the expression of *Bcr1*. The intricate biofilm and its complex polysaccharide matrix are therefore affected by *Bcr1* downregulation [171], which suggests that biofilm production will be suppressed or will not result in a robust extracellular matrix. *C. albicans* culture treated with CEs of *H. vulgaris, M. micrantha* leaf, *C. pentandra* leaf, *C. winterianus* leaf, *S. alata*, *U. lobata* leaf, *D. philippinensis* leaf, *P. odorata* bark, *S. jamaicensis* leaf, *E. indica* roots, *D. esculentum, E. indica* leaf, and *P. urinaria* showed significant downregulation of *Hsp90*. As a key regulator of biofilm development and drug resistance in *C. albicans* [172], compromised *Hsp90* regulation leads to weak biofilm formation [173]. Hence, targeting *Hsp90* can be tapped for *C. albicans* therapy and drug resistance.

Ilongot-Eǵongot ethnobotanicals *S. jamaicensis* leaves. *E. indica* roots and *D. philippinensis* bark significantly downregulated *agrA* confirming the inhibition of coagulase formation. A number of these ethnobotanicals also displayed downregulation of *agrA* in *S. agalactiae* [53].

5. FUTURE PERSPECTIVES

The papers included in this review have proven that Philippine ethnobotanicals possess QSI actions in virulence factors in bacteria such as violacein formation [54,55], a- Hemolysin [44,45], swarming motility [42], DNase [48], pyocyanin production [42,44], coagulase [50,51], and biofilm formation [33,43,46,47,53,56], showing the immense prospects of tapping these plants for anti-virulence drug design. Aside from QSI activities, these plants have shown potent biological activities against gout [174], cancer cell lines [175], diabetes [176,177], inflammation and pain [178], and also possesses antioxidant properties [179]. These results present opportunities for further evaluations to exploit the potential of Philippine ethnobotanicals. All these plants have been used for centuries in traditional folk medicine to treat infections and diseases that are being practiced today.

Not much research on QSI activities has been reported on Philippine ethnobotanicals. Research targeting bacterial QS using ethnobotanicals is largely unexplored at present; hence, this offers an additional area of research on plants with pharmacological potential leading toward drug development. The Philippine ethnobotanicals showed considerable potential as sources of QS inhibitory compounds for new therapeutic directions in preventing pathogenicity without the threat of the development of bacterial resistance, hence, determining these molecules offer a new direction on this research. In addition, while laboratory-based assays indicate the huge promise of QSI agents, *in vivo* research on animal models needs to be conducted to substantiate *in vitro* results.

6. CONCLUSIONS

Ethnobotanicals have not been fully tapped for applications on QS-based drug development. The applications of ethnobotanicals and QS inhibition are timely and provide a wide range of long-term solutions to human and animal health. All Philippine ethnobotanicals from the two ethnic communities included in this review showed inhibition on one or more virulence factors highlighting their activities against QS and emphasizes their potential for QS-based drug development.

QS inhibition is a practical option in controlling pathogenesis that avoids antibiotic resistance. QS inhibition only targets the production of virulence, without affecting bacterial growth. This limits the exposure of bacteria to selective pressure and the evolution of resistant mutants, hence, seen to be a practical alternative to antibiotic management. In recent years, intensive research on QS mechanisms has presented the potential to control resistance. Integrating the bioactive molecules from ethnobotanicals with inhibiting QS provides a novel solution to microbial multidrug resistance. With the World Health Organization's announcement in 2014 of the beginning of the post-antibiotic era, interest is now geared toward exploring new therapies that target the production of virulence factors instead of directly killing bacteria.

Although much research has been explored in targeting QS as a strategy to control bacterial pathogenesis, still, several issues have to be resolved to fully harness the potential of QSI as a novel approach in the fight against microbial infection and resistance. These limitations include evaluations on its efficacy *in vivo*, interactions with the host physiology, reactions with other compounds and long-term effectiveness. As with the majority of antibiotics, targeting QS may also impact not only pathogenic bacteria but also beneficial microbes. Also, the high specificity and complexity of QS systems makes it challenging to develop a broad-spectrum antimicrobial drug that can serve as universal QS inhibitor which can target multiple pathogens. Resolving these challenges is critical for the advancement of QS-based drugs. Albeit, the field of QS-based drug development is dynamic, and constantly delving on the mechanisms involved to optimize the potential applications and overcome limitations and challenges.

7. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. FUNDING

This article received no funding from any institutions or agencies.

9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

All data analyzed in this article are available from the author on reasonable request.

12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

13. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

1. Centers for Disease Control and Prevention. U.S. Department of Health and Human Services; 2021. Available from: https://cdc.gov [Last accessed on 2023 Jun 24].
2. O'Neill J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. The Review on Antimicrobial Resistance. United Kingdom: Government of the United Kingdom; 2016. Available from: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf [Last accessed on 2023 Jun 26].
3. World Health Organization. WHO Outlines 40 Research Priorities on Antimicrobial Resistance; 2023. Available from: https://www.who.int/news/item/22-06-2023-who-outlines-40-research-priorities-on-antimicrobial-resistance [Last accessed on 2023 Jul 01].

4. Miller MB, Bassler BL. Quorum sensing in bacteria. Annu Rev Microbiol 2001;55:165-99.
5. Geske GD, O'Neill JC, Blackwell HE. Expanding dialogues: From natural autoinducers to non-natural analogues that modulate quorum sensing in Gram-negative bacteria. Chem Soc Rev 2008;37:1432-47.
6. Whitehead NA, Barnard AM, Slater H, Simpson NJ, Salmond GP. Quorum-sensing in Gram-negative bacteria. FEMS Microbiol Rev 2001;25:365-404.
7. Papenfort K, Bassler BL. Quorum sensing signal-response systems in Gram-negative bacteria. Nat Rev Microbiol 2016;14:576-88.
8. LaSarre B, Federle MJ. Exploiting quorum sensing to confuse bacterial pathogens. Microbiol Mol Biol Rev 2013;77:73-111.
9. Fuqua C, Parsek MR, Greenberg EP. Regulation of gene expression by cell-to-cell communication: Acyl-homoserine lactone quorum sensing. Annu Rev Genet 2001;35:439-68.
10. Antunes LC, Ferreira RB, Buckner MM, Finlay BB. Quorum sensing in bacterial virulence. Microbiology (Reading) 2010;156:2271-82.
11. Rutherford ST, Bassler BL. Bacterial quorum sensing: Its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med 2012;2:a012427.
12. Castillo-Juárez I, Maeda T, Mandujano-Tinoco EA, Tomás M, Pérez-Eretza B, García-Contreras SJ, *et al.* Role of quorum sensing in bacterial infections. World J Clin Cases 2015;3:575-98.
13. Zhao X, Yu Z, Ding T. Quorum-sensing regulation of antimicrobial resistance in bacteria. Microorganisms 2020;8:425.
14. Naga NG, El-Badan DE, Ghanem KM, Shaaban MI. It is the time for quorum sensing inhibition as alternative strategy of antimicrobial therapy. Cell Commun Signal 2023;21:133.
15. Bhardwaj AK, Vinothkumar K, Rajpara N. Bacterial quorum sensing inhibitors: Attractive alternatives for control of infectious pathogens showing multiple drug resistance. Recent Pat Antiinfect Drug Discov 2013;8:68-83.
16. Chen X, Zhang L, Zhang M, Liu H, Lu P, Lin K. Quorum sensing inhibitors: A patent review (2014-2018). Expert Opin Ther Pat 2018;28:849-65.
17. Ahmed E, Arshad M, Saboor A, Qureshi R, Mustafa G, Sadiq S, *et al*. Ethnobotanical appraisal and medicinal use of plants in Patriata, New Murree, evidence from Pakistan. J Ethnobiol Ethnomed 2013;9:13.
18. Siraj J. Ethnobotany. Medicinal Plants. London: IntechOpen; 2022.
19. Iwu MM. Ethnobotanical approach to pharmaceutical drug discovery: Strengths and limitations. In: Wootton JC, editor. Advances in Phytomedicine. Vol. 1., Ch. 25. Netherlands: Elsevier; 2002. p. 309-20.
20. Rasmussen TB, Givskov M. Quorum sensing inhibitors: A bargain of effects. Microbiology (Reading) 2006;152:895-904.
21. Teplitski M, Robinson JB, Bauer WD. Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density dependent behaviors in associated bacteria. Mol Plant Microbe Interact 2000;13:637-48.
22. Convention on Biological Diversity. UN Environment Programme. Available from: https://www.cbd.int [Last accessed on 2023 Jul 28].
23. Philippine Clearing House Mechanism. Endemism and Uniqueness of Philippine Biodiversity; 2023. Available from: https://www.philchm.ph/status-of-philippine-biodiversity-2/endemism-2 [Last accessed on 2023 Jul 8].
24. Giang PM, Otsuka H. New compounds and potential candidates for drug discovery from medicinal plants of Vietnam. Chem Pharm Bull 2018;66:493-505.
25. Atanasov AG, Zotchev SB, Dirsch VM, International Natural Product Sciences Taskforce, Supuran CT. Natural products in drug discovery: Advances and opportunities. Nat Rev Drug Discov 2021;20:200-16.
26. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: Current approaches and prospects. Nucleus (Calcutta) 2022;65:399-411.
27. United Nations Development Programme (UNDP). Fast Facts: Indigenous Peoples in the Philippines; 2013. Available from: https://www.undp.org/philippines/publications/fast-facts-indigenous-peoples-philippines [Last accessed on 2023 Jun 17].
28. National Commission on Indigenous Peoples (NCIP); 2021. Available from: https://ncip.gov.ph [Last accessed on 2023 Jun 17].
29. Balberona AN, Noveno JJ, Angeles MG, Santos RI, Cachin E, Judan Cruz KG. Ethnomedicinal plants utilized by the Ilongot Egongot community of Bayanihan, Maria Aurora, Aurora, Philippines. Int J Agric Technol 2018;14:145-59.
30. Indigenous Peoples' and Community Conserved Areas and Territories (ICCAs). The Philippines: The Ikalahan Community of Imugan, Santa Fe, Nueva Vizacaya, Northern Luzon: Threats and Responses; 2013. Available from: https://www.iccaconsortium.org/2013/08/15/the-ikalahan-community-of-imugan-santa-fe-nueva-vizacaya-northern-luzon-philippines-threats-and-responses [Last accessed on 2023 Jun 17].
31. Ethnic Groups Philippines; 2021. Available from: https://www.ethnicgroupsphilippines.com
32. Pedregosa, SB. Threats to ICCAs and Community Responses- The Ikalahan Experience. The ICCA Consortium: Promoting the Appropriate Recognition and Support of Indigenous Peoples' and Community Conserved Areas and Territories; 2012. Available from: https://www.iccaconsortium.org/wpcontent/uploads/2015/08/grassroot-philippines-ikalahan-2012-en.pdf [Last accessed on 2023 Jun 17].
33. Fernando SI, Judan Cruz KG. Ethnobotanical biosynthesis of gold nanoparticles and its down regulation of quorum sensing-linked AhyR gene in *Aeromonas hydrophila*. SN Appl Sci 2020;2:570.
34. Bouyahya A, Dakka N, Et-Touys A, Abrini J, Bakri Y. Medicinal plant products targeting quorum sensing for combating bacterial infections. Asian Pac J Trop Med 2017;10:729-43.
35. Asfour HZ. Anti-quorum sensing natural compounds. J Microsc Ultrastruct 2018;6:1-10.
36. Deryabin D, Galadzhieva A, Kosyan D, Duskaev G. Plant-derived inhibitors of AHL-mediated quorum sensing in bacteria: Modes of action. Int J Mol Sci 2019;20:5588.
37. Lima EM, Winans SC, Pinto UM. Quorum sensing interference by phenolic compounds - A matter of bacterial misunderstanding. Heliyon 2023;9:e17657.
38. Naga NG, Zaki AA, El-Badan DE, Rateb HS, Ghanem KM, Shaaban MI. Inhibition of *Pseudomonas aeruginosa* quorum sensing by methyl gallate from *Mangifera indica*. Sci Rep 2023;13:17942.
39. Nazzaro F, Fratianni F, Coppola R. Quorum sensing and phytochemicals. Int J Mol Sci 2013;14:12607-19.
40. Okogun JI. Drug discovery through ethnobotany in Nigeria: Some results. In: Wootton JC, editor. Advances in Phytomedicine. Vol. 1., Ch. 12. Netherlands: Elsevier; 2002. p. 145-54.
41. Qureshi R, Ghazanfar SA, Obied H, Vasileva V, Tariq MA. Ethnobotany: A living science for alleviating human suffering. Evid Based Complement Alternat Med 2016;2016:9641692.
42. Barrogo KN, Jacinto WR, Judan Cruz KJ. Quorum sensing inhibition activities of Philippine ethnobotanicals against virulence factors in *Pseudomonas aeruginosa*. Int J Biosci 2018;13:173-82.
43. Judan Cruz KG, Gatchalian JJ, Jacinto WR. Philippine ethnobotanicals inhibit quorum sensing-controlled biofilm formation in *Pseudomonas aeruginosa*. Int J Biol Pharm Allied Sci 2018;7:527-37.
44. Limos GB, Judan Cruz KG, Jacinto WR. Quorum sensing inhibition bioactivities of Philippine ethnobotanicals against *Pseudomonas aeruginosa*. Int J Pure Appl BioSci 2018;6:47-56.
45. Padilla KG, Jacinto WR, Judan Cruz KG. Philippine ethnobotanicals

inhibit quorum sensing-mediated swarming motility in *Pseudomonas aeruginosa*. Adv BioRes 2018;9:7-13.
46. Velasco AT, Fernando SI, Judan Cruz KG. lasR/rhlR expression linked to quorum sensing-mediated biofilm formation in *Pseudomonas aeruginosa* using gold nanoparticles synthesized with ethnobotanical extracts. BioNanoScience 2020;10:876-84.
47. Santos RI, Jacinto WR, Judan Cruz KG. Philippine ethnobotanicals downregulate lasR expression linked to quorum sensing- mediated biofilm formation in *Pseudomonas aeruginosa*. J Microbiol Biotech Food Sci 2021;10:592-7.
48. Limos GB, Jacinto WR, Judan Cruz KG. Philippine ethnobotanicals inhibit virulence factors in *Staphylococcus aureus*. Int J Biosci 2018;13:178-87.
49. Padilla KG, Jacinto WR, Judan Cruz KG. Quorum sensing-mediated α- hemolysin inhibition of Philippine ethnobotanicals in *Staphylococcus aureus*. Int J Biol Pharm Allied Sci 2018;7:1537-50.
50. Salamanca GT, Fernando SI, Judan Cruz KG. The agrA expression linked to quorum sensing-mediated coagulase formation using Philippine *Ilongot* ethnobotanicals against *Staphylococcus aureus*. Int J Biosci 2019;15:33-41.
51. Vias JG, Jacinto WR, Judan Cruz KG. Philippine ethnobotanicals inhibit formation of coagulase in *Staphylococcus aureus*. Adv BioRes 2018;9:1-6.
52. Barrogo KN, Jacinto WR, Judan Cruz KG. Quorum sensing inhibition activities of Philippine ethnobotanicals against virulence factors in *Staphylococcus aureus*. Int J Agric Technol 2021;17:1305-16.
53. Fernando SI, Judan Cruz KG, Watanabe K. Quorum sensing- linked agrA expression by ethno-synthesized gold nanoparticles in Tilapia *Streptococcus agalactiae* biofilm formation. BioNanoScience 2020;10:696-704.
54. Jimenez JJ. Detection of Quorum Sensing Inhibition Potential of Ethnobotanical Extracts from the Imugan Ancestral Domain through Bioreporter Bacterial Strain *Chromobacterium violaceum* and *Pseudomonas aeruginosa*. An Undergraduate Thesis of the Department of Biological Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines; 2016.
55. Gatchalian JJ. Detection of Quorum Sensing Inhibition in *Chromobacterium violaceum* and *Pseudomonas aeruginosa* using the Methanol Extracts of Ethnbotonanicals from the Imugan Ancestral Domain. Bachelor of Science in Biology. Department of Biological Sciences, Central Luzon State University. Science City of Munoz, Nueva Ecija, Philippines; 2016.
56. Judan Cruz KG, Alfonso ED, Fernando SI, Watanabe K. *Candida albicans* biofilm inhibition by ethnobotanicals and ethnobotanically-synthesized gold nanoparticles. Front Microbiol 2021;12:665113.
57. Ciofu O, Tolker-Nielsen T. Tolerance and resistance of *Pseudomonas aeruginosa* biofilms to antimicrobial agents-how *P. aeruginosa* can escape antibiotics. Front Microbiol 2019;10:913.
58. Rice LB. Progress and challenges in implementing the research on ESKAPE pathogens. Infect Control Hosp Epidemiol 2010;31 Suppl 1:S7-10.
59. Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. Biomed Res Int 2016;2016:2475067.
60. Nathwani D, Raman G, Sulham K, Gavaghan M, Menon V. Clinical and economic consequences of hospital-acquired resistant and multidrug-resistant *Pseudomonas aeruginosa* infections: A systematic review and meta-analysis. Antimicrob Resist Infect Control 2014;3:32.
61. Ruffin M, Brochiero E. Repair process impairment by *Pseudomonas aeruginosa* in epithelial tissues: Major features and potential therapeutic avenues. Front Cell Infect Microbiol 2019;9:182.
62. Poolman JT, Anderson AS. *Escherichia coli* and *Staphylococcus aureus*: Leading bacterial pathogens of healthcare associated infections and bacteremia in older-age populations. Expert Rev Vaccines 2018;17:607-18.
63. Landry LM, An D, Hupp JT, Singh PK, Parsek MR. Mucin-*Pseudomonas aeruginosa* interactions promote biofilm formation and antibiotic resistance. Mol Microbiol 2006;59:142-51.
64. Strateva T, Mitov I. Contribution of an arsenal of virulence factors to pathogenesis of *Pseudomonas aeruginosa* infections. Ann Microbiol 2011;61:717-32.
65. Wilson MG, Pandey S. *Pseudomonas aeruginosa*. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557831 [Last accessed on 2023 Aug 8].
66. Moradali MF, Ghods S, Rehm BH. *Pseudomonas aeruginosa* lifestyle: A paradigm for adaptation, survival, and persistence. Front Cell Infect Microbiol 2017;7:39.
67. Venturi V. Regulation of quorum sensing in *Pseudomonas*. FEMS Microbiol Rev 2006;30:274-91.
68. Williams P, Cámara M. Quorum sensing and environmental adaptation in *Pseudomonas aeruginosa*: A tale of regulatory networks and multifunctional signal molecules. Curr Opin Microbiol 2009;12:182-91.
69. Barr HL, Halliday N, Cámara M, Barrett DA, Williams P, Forrester DL, *et al. Pseudomonas aeruginosa* quorum sensing molecules correlate with clinical status in cystic fibrosis. Eur Respir J 2015;46:1046-54.
70. Musa N, Wei LS, Musa N, Hamdan RH, Leong LK, Wee W, *et al*. Streptococcosis in red hybrid tilapia (*Oreochromis niloticus*) commercial farms in Malaysia. Aquac Res 2009;40:630-2.
71. Zmysłowska I, Korzekwa K, Szarek J. *Aeromonas hydrophila* in fish aquaculture. J Comp Pathol 2009;141:313.
72. Al-Harbi AH. Phenotypic and genotypic characterization of *Streptococcus agalactiae* isolated from hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). Aquaculture 2016;464:515-20.
73. Vijayakumar S, Vaseeharan B, Malaikozhundan B, Gobi N, Ravichandran S, Karthi S, *et al*. A novel antimicrobial therapy for the control of *Aeromonas hydrophila* infection in aquaculture using marine polysaccharide coated gold nanoparticle. Microb Pathog 2017;110:140-51.
74. Mzula A, Wambura PN, Mdegela RH, Shirima GM. Current State of modern biotechnological-based *Aeromonas hydrophila* vaccines for aquaculture: A systematic review. Biomed Res Int 2019;2019:3768948.
75. Phuoc NN, Linh NT, Crestani C, Zadoks RN. Effect of strain and environmental conditions on the virulence of *Streptococcus agalactiae* (Group B *Streptococcus*; GBS) in red tilapia (*Oreochromis* sp.). Aquaculture 2021;534:736256.
76. Alazab A, Sadat A, Younis G. Prevalence, antimicrobial susceptibility, and genotyping of *Streptococcus agalactiae* in Tilapia fish (*Oreochromis niloticus*) in Egypt. J Adv Vet Anim Res 2022;9:95-103.
77. Wang C, Shi S, Wei M, Luo Y. Characterization of a novel broad-spectrum endolysin PlyD4 encoded by a highly conserved prophage found in *Aeromonas hydrophila* ST251 strains. Appl Microbiol Biotechnol 2022;106:699-711.
78. Wani MY, Ahmad A, Aqlan FM, Al-Bogami AS. Azole based acetohydrazide derivatives of cinnamaldehyde target and kill *Candida albicans* by causing cellular apoptosis. ACS Med Chem Lett 2020;11:566-74.
79. Dhasarathan P, AlSalhi MS, Devanesan S, Subbiah J, Ranjitsingh AJ, Binsalah M, *et al*. Drug resistance in *Candida albicans* isolates and related changes in the structural domain of Mdr1 protein. J Infect Public Health 2021;14:1848-53.
80. Lee Y, Puumala E, Robbins N, Cowen LE. Antifungal drug resistance: Molecular mechanisms in *Candida albicans* and beyond. Chem Rev 2021;121:3390-411.
81. Costa-de-Oliveira S, Rodrigues AG. *Candida albicans* antifungal

resistance and tolerance in bloodstream infections: The triad yeast-host-antifungal. Microorganisms 2020;8:154.
82. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004;39:309-17.
83. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: Human fungal infections. Sci Transl Med 2012;4:165rv13.
84. Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. Twenty years of the SENTRY antifungal surveillance program: Results for *Candida* species from 1997–2016. Open Forum Infect Dis 2019;6:S7-94.
85. Kothari V, Sharma S, Padia D. Recent research advances on *Chromobacterium violaceum*. Asian Pac J Trop Med 2017;10:744-52.
86. Singh VK, Mishra A, Jha B. Anti-quorum sensing and anti-biofilm activity of *Delftia tsuruhatensis* extract by attenuating the quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa*. Front Cell Infect Microbiol 2017;7:337.
87. Castro-Gomes T, Cardoso MS, DaRocha WD, Laibida LA, Nascimento AM, Zuccherato LW, *et al.* Identification of secreted virulence factors of *Chromobacterium violaceum*. J Microbiol 2014;52:350-3.
88. Muñoz-Cazares N, García-Contreras R, Soto-Hernández M, Martínez-Vázquez M, Castillo-Juárez I. Natural products with quorum quenching-independent antivirulence properties. In: Atta-ur-Rahman, editor. Studies in Natural Products Chemistry. Vol. 57. Netherlands: Elsevier; 2018. p. 327-51.
89. Sharmin S, Jahan AA, Kamal SM, Sarker P. Fatal infection caused by *Chromobacterium violaceum*: A case report from a tertiary care hospital in Bangladesh. Case Rep Infect Dis 2019;2019:6219295.
90. Wang Y, Ikawa A, Okaue S, Taniguchi S, Osaka I, Yoshimoto A, *et al.* Quorum sensing signaling molecules involved in the production of violacein by *Pseudoalteromonas*. Biosci Biotechnol Biochem 2008;72:1958-61.
91. Parsek MR, Greenberg EP. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: A signaling mechanism involved in associations with higher organisms. Proc Natl Acad Sci U S A 2000;97:8789-93.
92. Donlan RM. Biofilms: Microbial life on surfaces. Emerg Infect Dis 2002;8:881-90.
93. Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: An emerging battleground in microbial communities. Antimicrob Resist Infect Control 2019;8:76.
94. Srinivasan R, Santhakumari S, Poonguzhali P, Geetha M, Dyavaiah M, Xiangmin L. Bacterial biofilm inhibition: A focused review on recent therapeutic strategies for combating the biofilm mediated infections. Front Microbiol 2021;12:676458.
95. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 2010;35:322-32.
96. Morohoshi T, Shiono T, Takidouchi K, Kato M, Kato N, Kato J, *et al*. Inhibition of quorum sensing in *Serratia marcescens* AS-1 by synthetic analogs of N-acylhomoserine lactone. Appl Environ Microbiol 2007;73:6339-44.
97. Lewis K. Riddle of biofilm resistance. Antimicrob Agents Chemother 2001;45:999-1007.
98. Chen L, Wen YM. The role of bacterial biofilm in persistent infections and control strategies. Int J Oral Sci 2011;3:66-73.
99. Yin W, Wang Y, Liu L, He J. Biofilms: The microbial "protective clothing" in extreme environments. Int J Mol Sci 2019;20:3423.
100. Flemming HC, van Hullebusch ED, Neu TR, Nielsen PH, Seviour T, Stoodley P, *et al*. The biofilm matrix: multitasking in a shared space. Nat Rev Microbiol 2023;21:70-86.
101. Borges A, Abreu AC, Dias C, Saavedra MJ, Borges F, Simões M. New perspectives on the use of phytochemicals as an emergent strategy to control bacterial infections including biofilms. Molecules 2016;21:877.
102. Sahli C, Moya SE, Lomas JS, Gravier-Pelletier C, Briandet R, Hémadi M. Recent advances in nanotechnology for eradicating bacterial biofilm. Theranostics 2022;12:2383-405.
103. Liu W, Huang Y, Zhang H, Liu Z, Huan Q, Xiao X, *et al*. Factors and mechanisms influencing conjugation *in vivo* in the gastrointestinal tract environment: A review. Int J Mol Sci 2023;24:5919.
104. Reichling J. Anti-biofilm and virulence factor-reducing activities of essential oils and oil components as a possible option for bacterial infection control. Planta Med 2020;86:520-37.
105. Galié S, García-Gutiérrez C, Miguélez EM, Villar CJ, Lombó F. Biofilms in the food industry: Health aspects and control methods. Front Microbiol 2018;9:898.
106. Bai X, Nakatsu CH, Bhunia AK. Bacterial biofilms and their implications in pathogenesis and food safety. Foods 2021;10:2117.
107. Zhu T, Yang C, Bao X, Chen F, Guo X. Strategies for controlling biofilm formation in food industry. Grain Oil Sci Technol 2022;5:179-86.
108. Liu X, Yao H, Zhao X, Ge C. Biofilm formation and control of foodborne pathogenic bacteria. Molecules 2023;28:2432.
109. Luo J, Lv P, Zhang J, Fane AG, McDougald D, Rice SA. Succession of biofilm communities responsible for biofouling of membrane bio-reactors (MBRs). PLoS One 2017;12:e0179855.
110. Nourbakhsh F, Nasrollahzadeh MS, Tajani AS, Soheili V, Hadizadeh F. Bacterial biofilms and their resistance mechanisms: A brief look at treatment with natural agents. Folia Microbiol (Praha) 2022;67:535-54.
111. Ghafoor A, Hay ID, Rehm BH. Role of exopolysaccharides in *Pseudomonas aeruginosa* biofilm formation and architecture. Appl Environ Microbiol 2011;77:5238-46.
112. Thi MT, Wibowo D, Rehm BH. *Pseudomonas aeruginosa* biofilms. Int J Mol Sci 2020;21:8671.
113. Sharma N, Sharma N, Sharma S, Sharma P, Devi B. Identification, morphological, biochemical, and genetic characterization of microorganisms. In: Bhatt AK, Bhatia RK, Bhalla TC, editors. Basic Biotechniques for Bioprocess and Bioentrepreneurship. Ch. 3. Netherlands: Elsevier; 2023. p. 47-84.
114. Ko YP, Kang M, Ganesh VK, Ravirajan D, Li B, Höök M. Coagulase and Efb of *Staphylococcus aureus* have a common fibrinogen binding motif. mBio 2016;7:e01885-15.
115. McAdow M, Missiakas DM, Schneewind O. *Staphylococcus aureus* secretes coagulase and von Willebrand factor binding protein to modify the coagulation cascade and establish host infections. J Innate Immun 2012;4:141-8.
116. Hiramatsu K, Katayama Y, Matsuo M, Sasaki T, Morimoto Y, Sekiguchi A, *et al*. Multi-drug-resistant *Staphylococcus aureus* and future chemotherapy. J Infect Chemother 2014;20:593-601.
117. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol 2009;7:629-41.
118. Loomba PS, Taneja J, Mishra B. Methicillin and vancomycin resistant *S. aureus* in hospitalized patients. J Glob Infect Dis 2010;2:275-83.
119. Taylor TA, Unakal CG. *Staphylococcus aureus*. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441868 [Last accessed on 2023 Jul 22].
120. Totsika M. Benefits and challenges of antivirulence antimicrobials at the dawn of the post-antibiotic era. Drug Deliv Lett 2016;6:30-7.
121. Mahdally NH, George RF, Kashef MT, Al-Ghobashy M, Murad FE, Attia AS. Staquorsin: A novel *Staphylococcus aureus* Agr-mediated quorum sensing inhibitor impairing virulence *in vivo* without notable resistance development. Front Microbiol 2021;12:700494.

122. Kasprowicz A, Białecka A, Białecka J. Diagnostics: Routine identification on standard and chromogenic media, and advanced automated methods. In: Savini V, editor. Pet-To-Man Travelling Staphylococci. Ch. 15. Netherlands: Elsevier; 2018. p. 185-98.
123. Burnside K, Lembo A, de Los Reyes M, Iliuk A, Binhtran NT, Connelly JE, *et al.* Regulation of hemolysin expression and virulence of *Staphylococcus aureus* by a serine/threonine kinase and phosphatase. PLoS One 2010;5:e11071.
124. Vandenesch F, Lina G, Henry T. *Staphylococcus aureus* hemolysins, bi-component leukocidins, and cytolytic peptides: A redundant arsenal of membrane-damaging virulence factors? Front Cell Infect Microbiol 2012;2:12.
125. Kebaier C, Chamberland RR, Allen IC, Gao X, Broglie PM, Hall JD, *et al*. *Staphylococcus aureus* α-hemolysin mediates virulence in a murine model of severe pneumonia through activation of the NLRP3 inflammasome. J Infect Dis 2012;205:807-17.
126. Sampedro GR, DeDent AC, Becker RE, Berube BJ, Gebhardt MJ, Cao H, *et al*. Targeting *Staphylococcus aureus* α-toxin as a novel approach to reduce severity of recurrent skin and soft-tissue infections. J Infect Dis 2014;210:1012-8.
127. Thomer L, Schneewind O, Missiakas D. Pathogenesis of *Staphylococcus aureus* bloodstream infections. Annu Rev Pathol 2016;11:343-64.
128. Adhikari RP, Thompson CD, Aman MJ, Lee JC. Protective efficacy of a novel alpha hemolysin subunit vaccine (AT62) against *Staphylococcus aureus* skin and soft tissue infections. Vaccine 2016;34:6402-7.
129. Uwaezuoke JC, Aririatu LE. A survey of antibiotic resistant *Staphylococcus aureus* strains from clinical sources in Owerri. J Appl Sci Environ Manag 2004;8:67-9.
130. Berends ET, Horswill AR, Haste NM, Monestier M, Nizet V, von Köckritz-Blickwede M. Nuclease expression by *Staphylococcus aureus* facilitates escape from neutrophil extracellular traps. J Innate Immun 2010;2:576-86.
131. Kobayashi SD, Malachowa N, DeLeo FR. Neutrophils and bacterial immune evasion. J Innate Immun 2018;10:432-41.
132. Sumby P, Barbian KD, Gardner DJ, Whitney AR, Welty DM, Long RD, *et al.* Extracellular deoxyribonuclease made by group A *Streptococcus* assists pathogenesis by enhancing evasion of the innate immune response. Proc Natl Acad Sci U S A 2005;102:1679-84.
133. Gordon RJ, Lowy FD. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis 2008;46 Suppl 5:S350-9.
134. Zarringhalam M, Zaringhalam J, Shadnoush M, Safaeyan F, Tekieh E. Inhibitory effect of black and red pepper and thyme extracts and essential oils on enterohemorrhagic *Escherichia coli* and DNase activity of *Staphylococcus aureus*. Iran J Pharm Res 2013;12:363-9.
135. Mann EE, Rice KC, Boles BR, Endres JL, Ranjit D, Chandramohan L, *et al.* Modulation of eDNA release and degradation affects *Staphylococcus aureus* biofilm maturation. PLoS One 2009;4:e5822.
136. Haas B, Bonifait L, Vaillancourt K, Charette SJ, Gottschalk M, Grenier D. Characterization of DNase activity and gene in *Streptococcus suis* and evidence for a role as virulence factor. BMC Res Notes 2014;7:424.
137. Kateete DP, Kimani CN, Katabazi FA, Okeng A, Okee MS, Nanteza A, *et al*. Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Ann Clin Microbiol Antimicrob 2010;13:9-23.
138. Beatty BR, Farnsworth RJ, Lund AJ, Lyon RH, Ward GE. Medium to culture and differentiate coagulase-positive and -negative staphylococci from bovine milk. J Food Prot 1985;48:1019-21.
139. Varada VV, Panneerselvam D, Pushpadass HA, Mallapa RH, Ram C, Kumar S. *In vitro* safety assessment of electrohydrodynamically encapsulated *Lactiplantibacillus plantarum* CRD7 and *Lacticaseibacillus rhamnosus* CRD11 for probiotics use. Curr Res Food Sci 2023;6:100507.
140. Köhler T, Curty LK, Barja F, van Delden C, Pechère JC. Swarming of *Pseudomonas aeruginosa* is dependent on cell-to-cell signaling and requires flagella and pili. J Bacteriol 2000;182:5990-6.
141. Tremblay J, Richardson AP, Lépine F, Déziel E. Self-produced extracellular stimuli modulate the *Pseudomonas aeruginosa* swarming motility behaviour. Environ Microbiol 2007;9:2622-30.
142. Caiazza NC, Merritt JH, Brothers KM, O'Toole GA. Inverse regulation of biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. J Bacteriol 2007;189:3603-12.
143. Hall S, McDermott C, Anoopkumar-Dukie S, McFarland AJ, Forbes A, Perkins AV, *et al*. Cellular effects of pyocyanin, a secreted virulence factor of *Pseudomonas aeruginosa*. Toxins (Basel) 2016;8:236.
144. Noto MJ, Burns WJ, Beavers WN, Skaar EP. Mechanisms of pyocyanin toxicity and genetic determinants of resistance in *Staphylococcus aureus*. J Bacteriol 2017;199:e00221-17.
145. Zaidi S, Misba L, Khan AU. Nano-therapeutics: A revolution in infection control in post antibiotic era. Nanomedicine 2017;13:2281-301.
146. Xie M, Xu Y, Huang J, Li Y, Wang L, Yang L, *et al.* Going even smaller: Engineering sub-5 nm nanoparticles for improved delivery, biocompatibility, and functionality. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2020;12:e1644.
147. Ranjha MM, Shafique B, Rehman A, Mehmood A, Ali A, Zahra SM, *et al.* Biocompatible nanomaterials in food science, technology, and nutrient drug delivery: Recent developments and applications. Front Nutr 2022;8:778155.
148. Kshtriya V, Koshti B, Gour N. Green synthesized nanoparticles: Classification, synthesis, characterization, and applications. In: Verma SK, Das AK, editors. Comprehensive Analytical Chemistry. Vol. 94., Ch. 4. Netherlands: Elsevier; 2021. p. 173-222.
149. Ying S, Guan Z, Ofoegbu PC, Clubb P, Rico C, He F, *et al*. Green synthesis of nanoparticles: Current developments and limitations. Environ Technol Innov 2022;26:102336.
150. Ahmed SF, Mofijur M, Rafa N, Chowdhury AT, Chowdhury S, Nahrin M, *et al*. Green approaches in synthesising nanomaterials for environmental nanobioremediation: Technological advancements, applications, benefits and challenges. Environ Res 2022;204:111967.
151. Kadhim RJ, Karsh EH, Taqi ZJ, Jabir MS. Biocompatibility of gold nanoparticles: *In-vitro* and *in-vivo* study. Mater Today Proc 2021;42:3041-5.
152. Botteon CE, Silva LB, Ccana-Ccapatinta GV, Silva TS, Ambrosio SR, Veneziani RC, *et al*. Biosynthesis and characterization of gold nanoparticles using Brazilian red propolis and evaluation of its antimicrobial and anticancer activities. Sci Rep 2021;11:1974.
153. Kang MS, Lee SY, Kim KS, Han DW. State of the art biocompatible gold nanoparticles for cancer theragnosis. Pharmaceutics 2020;12:701.
154. Sánchez A, Mejía SP, Orozco J. Recent advances in polymeric nanoparticle-encapsulated drugs against intracellular infections. Molecules 2020;25:3760.
155. Yu W, Liu R, Zhou Y, Gao H. Size-tunable strategies for a tumor targeted drug delivery system. ACS Cent Sci 2020;6:100-16.
156. Lee J, Chatterjee DK, Lee MH, Krishnan S. Gold nanoparticles in breast cancer treatment: Promise and potential pitfalls. Cancer Lett 2014;347:46-53.
157. Xu L, Xu M, Sun X, Feliu N, Feng L, Parak WJ, *et al*. Quantitative comparison of gold nanoparticle delivery via the enhanced permeation and retention (EPR) effect and mesenchymal stem cell (MSC)-based targeting. ACS Nano 2023;17:2039-52.

158. O'Loughlin CT, Miller LC, Siryaporn A, Drescher K, Semmelhack MF, Bassler BL. A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation. Proc Natl Acad Sci U S A 2013;110:17981-6.
159. Tay SB, Yew WS. Development of quorum-based anti-virulence therapeutics targeting Gram-negative bacterial pathogens. Int J Mol Sci 2013;14:16570-99.
160. Adonizio A, Kong KF, Mathee K. Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* by South Florida plant extracts. Antimicrob Agents Chemother 2008;52:198-203.
161. Gambello MJ, Kaye S, Iglewski BH. LasR of *Pseudomonas aeruginosa* is a transcriptional activator of the alkaline protease gene (apr) and an enhancer of exotoxin A expression. Infect Immun 1993;61:1180-4.
162. Pearson JP, Pesci EC, Iglewski BH. Roles of *Pseudomonas aeruginosa* las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. J Bacteriol 1997;179:5756-67.
163. De Kievit TR, Gillis R, Marx S, Brown C, Iglewski BH. Quorum-sensing genes in *Pseudomonas aeruginosa* biofilms: Their role and expression patterns. Appl Environ Microbiol 2001;67:1865-73.
164. Boles BR, Horswill AR. Agr-mediated dispersal of *Staphylococcus aureus* biofilms. PLoS Pathog 2008;4:e1000052.
165. Yarwood JM, Bartels DJ, Volper EM, Greenberg EP. Quorum sensing in *Staphylococcus aureus* biofilms. J Bacteriol 2004;186:1838-50.
166. Li S, Huang H, Rao X, Chen W, Wang Z, Hu X. Phenol-soluble modulins: Novel virulence-associated peptides of staphylococci. Future Microbiol 2014;9:203-16.
167. Tuchscherr L, Loffler B. *Staphylococcus aureus* dynamically adapts global regulators and virulence factor expression in the course from acute to chronic infection. Curr Genet 2016;62:15-7.
168. Le KY, Otto M. Quorum-sensing regulation in staphylococci-an overview. Front Microbiol 2015;6:1174.
169. Kirke DF, Swift S, Lynch MJ, Williams P. The *Aeromonas hydrophila* LuxR homologue AhyR regulates the N-acyl homoserine lactone synthase, AhyI positively and negatively in a growth phase-dependent manner. FEMS Microbiol Lett 2004;241:109-17.
170. Bi X, Liu YJ, Lu CP. Contribution of AhyR to virulence of *Aeromonas hydrophila* J-1. Res Vet Sci 2007;83:150-6.
171. Douglas LJ. *Candida* biofilms and their role in infection. Trends Microbiol 2003;11:30-6.
172. Robbins N, Cowen LE. Roles of Hsp90 in *Candida albicans* morphogenesis and virulence. Curr Opin Microbiol 2023;75:102351.
173. Robbins N, Uppuluri P, Nett J, Rajendran R, Ramage G, Lopez-Ribot JL, *et al*. Hsp90 governs dispersion and drug resistance of fungal biofilm. PLoS Pathog 2011;7:e1002257.
174. Jose KJ, David ES, Cruz KJ. 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; 2015.
175. Pooten EE, Oyong GG, Judan Cruz KG. Philippine ethnobotanicals show anti-proliferative and cytotoxic activities in human breast cancer cells (MCF-7). Int J Biosci 2018;13:239-51.
176. Alfonso FR, David ES, Cruz KJ. 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; 2015.
177. Angeles MG, Divina CC, Judan Cruz KG. *Dillenia philippinensis*, an Ilongot ethnobotanical of Aurora, Philippines shows antidiabetic activity. Int J Biol Pharm Allied Sci 2018;7:1374-83.
178. Judan Cruz KG, Gabriel CM, Abella EA. Biological activities of Philippine ethnotoxic plants. Int J Biol Pharm Allied Sci 2018;7:709-18.
179. Divina RB, David ES, Cruz KJ. 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, Philippines; 2015.

How to cite this article:
Cruz KGJ. Quorum sensing inhibition activities of Philippine ethnobotanicals against multidrug-resistant pathogens. J App Biol Biotech. 2024;12(4):1-13. DOI: 10.7324/JABB.2024.164825