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# Evaluation of the effect of some medicinal plants against quorum sensing regulated virulence factors in *Staphylococcus aureus*

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**Abstract**
The effect of some commonly used medicinal plants in Southeast Nigeria namely *Carica papaya*, *Psidium guajava* and *Citrus sinensis* against quorum sensing regulated virulence factor such as twitching motility, proteolytic activity and cell adhesion in *Staphylococcus aureus* was evaluated in this study using spectrophotometric methods. Result showed that *Carica papaya* and *Psidium guajava* plant extract revealed the best inhibition of *Staphylococcus aureus* for twitching motility. *Psidium guajava* plant extract showed greater inhibition on cell adhesion while the inhibition of proteolytic activity was lowest in *Carica papaya* and highest in *Psidium guajava*. Overall, the plant extract showed variable effects with *Psidium guajava* plant extract showing the best effect against quorum sensing regulated virulence factors in *Staphylococcus aureus*. This study shows the potential use of these plant extracts in the treatment of microbial infections by inhibiting bacterial virulent factors and its associated antibiotics resistance capabilities.

**Keywords:** Anti-qourom sensing, bacterial infections, *Staphylococcus aureus*, plant extracts

## 1. Introduction
It has become evident that bacterial function and growth within a population is a fundamental aspect of bacterial survival and atypical life style of microorganisms (Davey, 2000) [24]. This typical lifestyle of survival has been highlighted in more recent studies (George and Muir, 2007; Clatworthy *et al.*, 2007; Rasko *et al.*, 2010; Kobayashi *et al.*, 2011; Daly *et al.*, 2015) [5, 4, 16, 13, 25]. Therefore, some organisms are resistant to all approved antibiotics, because the environmental antibiotics pressure activates the evolutionary mechanisms that select for resistant strains (Ali *et al.*, 2018) [1]. Bacterial populations coordinate communal behavior through a process of cell-to-cell signaling mediated by diffusible signal molecules (Schauder and Bassler, 2001) [18]. This process, termed quorum sensing (QS). Quorum sensing is a recently discovered chemical communication system that enhances survival of bacteria, as a group allowing resident bacteria to assume specialized roles vital for intra- and inter bacterial gene regulation, and for keeping bacterial colonies intact (Chan *et al.*, 2011) [2]. These involve several processes, such as specific signaling molecules that bind to and activate receptors that transduce the quorum-sensing signal into intracellular second messenger responses, in a similar fashion to ligand-receptor interaction (Stanley *et al.*, 2018) [21]. This similarity opens a novel alternative that should be looked for in combating drug resistance by the infectious bacteria, with inhibitor drugs that could be designed using current standard pharmacologic principles (Kumar *et al.*, 2016) [14]. Therefore, quorum-sensing inhibition offers new hope in combating resistant bacteria with inhibitor drugs that might have novel mechanisms of action, and could, therefore, be more effective against antibiotic resistant strains of bacteria (Fuqua *et al.*, 2001; Rasko and Sperandio, 2010) [26, 16].
In the past few years, inhibition of QS has become an intense area of research because of its applications in medicine, industry, and biotechnology (Okhee *et al.*, 2018) [15]. In the quest for QS inhibitors, studies have demonstrated that many eukaryotes, particularly plants, and even bacteria themselves produce anti-QS substances (Kalia, 2013) [11]. Ajoene from garlic, catechin from *Combretum albiflorum*, and iberin from horseradish specifically inhibit QS in

reporter strains (Vandeputte *et al.*, 2010) [22]. As an adaptive evolution, many plant species produce metabolites that can control the growth of microbes and have traditionally been used to treat human diseases, particularly microbial infections (Hayek *et al.*, 2013) [6]. Traditional medicine is increasingly been recognized as an accepted alternate regimen to orthodox health-care system (Vasavi *et al.*, 2016) [23]. This work focuses on evaluating selected medicinal plant extract against expression of quorum sensing regulated virulence factors in *Staphylococcus aureus*.

## 2. Methodology

### 2.1. Collection of plant materials

Leaves of *Carica papaya* (PawPaw), *Psidium guajava* (Guava), and *Citrus* × *sinensis* (Orange) were collected from Owerri, Imo state, Nigeria and used in the study.

### 2.2. Preparation of Plant Extract

The plant extract were prepared according to method described by (Ibe, 2017) [9]. The collected plant material will be air-dried under shade at room temperature, finely ground into powder using domestic mixture and will be stored in airtight labeled plastic sampling bags for further studies.

### 2.3. Extraction of plant samples

The grounded plant samples were extracted using three solvents i.e cold water, hot water and ethanol.

#### 2.3.1. Cold water

10 g of the grounded plant samples were soaked in 100 ml cold distilled water for 72 hours with occasional agitation. The extract of each plant were filtered using (Whatman No.1) filter paper.

#### 2.3.2 Hot water

10 g of the grounded plant samples were soaked in 100 ml hot distilled water for 72 hours with occasional agitation. The extract of each plant were filtered using (Whatman No.1) filter paper.

#### 2.3.3 Ethanol extracts

Extraction was carried out by the modified method of Hussaini and Mahasneh. The plant material were extracted at room temperature with ethanol 95% (100 mL/10 g of plant material). The extract of each plant was filtered using (Whatman No.1) filter paper and evaporated under vacuum at 40 °C using a rotary vacuum evaporator, the concentrated extract thus obtained was collected in screw cap vial and was used for further studies.

### 2.4. Test Organism and growth condition

Isolate of the test organism i.e *Staphylococcus aureus* was obtained from the department of microbiology, Federal Medical Centre, Owerri. The isolate was propagated on nutrient agar plates and maintained on the plates at 4 °C. The isolates were sub-cultured in nutrient agar at 37 °C for 24 hours prior to further studies.

### 2.5 Quorum sensing mediated virulence factors

The following quorum sensing mediated virulence factors such as twitching motility, Cell adhesion and proteolytic activity of the test organism were conducted.

#### 2.5.1. Twitching Motility

Twitching Motility was determined according to methods described by Karthick and Vivek (2016) [12]. 500 µl of respective plant extracts and 250 µl of bacterial inoculums was prepared in LB (Lysogeny broth) broth and were mixed in sterile eppendorf tube, kept in room temperature for 30 minutes. The respective plant extracts treated cultures were stab inoculated through LB agar plates. The plates were incubated at 32 °C for 24 hours. Bacterial grew at the interface between the plastic surface and the agar, which is indicative of twitching motility. To visualize the bacterial growth on the plastic surface, the agar was removed and the plate was stained with a 1% solution of crystal violet. Twitching motility was determined by measuring the diameter of the stained growth.

#### 2.5.2 Cell Adhesion

Cell adhesion was studied by using 96 well flat bottom micro well plate was previously coated with bovine serum albumin (BSA). Wells was coated with 150 µl of freshly prepared 1.0% BSA, incubated at 30 °C for 30 minutes. After the incubation period, wells was washed thrice with sterile phosphate buffered saline (PBS). Fifty microlitre of bacterial inocula thus prepared was transferred to the well followed by the addition of 50 µl of the respective plant extracts. Seeded microtitre plate was incubated at 37 °C for 24 hours. Cells were allowed to adhere and the non-adhered cells were washed 5 times with PBS at room temperature. Adhered cells were detected by adding 50 µl of 0.1% crystal violet per well, incubated at room temperature for 30 minutes. Wells was washed with sterile distilled water to remove excess stain. 10 µl of ethanol was added to fix the adhered cells. 50 µl of 0.2% Triton X was then be added to the wells for lyse of cells and the absorbance was read at 570nm.

#### 2.5.3 Proteolytic activity

Proteolytic activity was carried out by modified method of Karthick and Vivek (2016) [2]. Crude enzyme preparation 0.1 ml of tryptic soy broth bacterial culture was inoculated into 100 ml of protease production media (Yeast extract 5 mg/l, Peptone-10 mg/l, Glucose-10 mg/l, Caesin-15 mg/L) supplemented with 200 µl of respective plant extracts. Flasks were incubated at 37 °C for 48 hours. Broth was centrifuged after the incubation period at 10,000 rpm for 10 minutes; the collected supernatant was used as the source of protease enzyme.

### 2.4 Statistical analysis

The One-Way analysis of variance (ANOVA) was employed in determining if there is significance in the difference that exists between test samples and also with control sample.

## 3. Result

### 3.1. Effects on twitching motility

The results of extracts on twitching motility of *Staphylococcus aureus* is presented in Figure 1.

### 3.2. Effects on cell adhesion

Cell adhesion initiates the biofilm formation and pathogenicity in the host. The distribution for the plant extracts is presented in Figure 2.

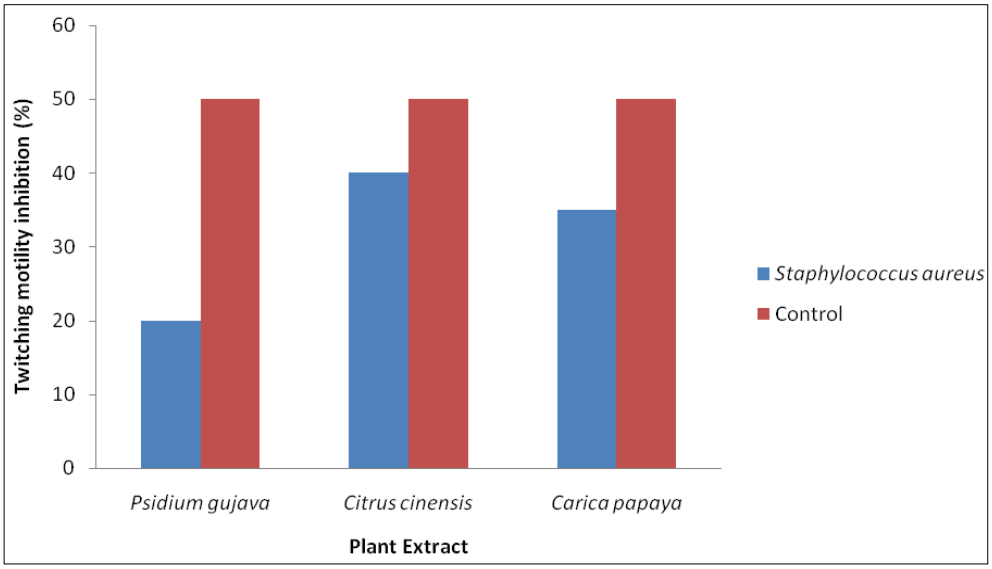


**Fig 1:** Effect (cm) of plant extract against twitching motility of *Staphylococcus aureus*

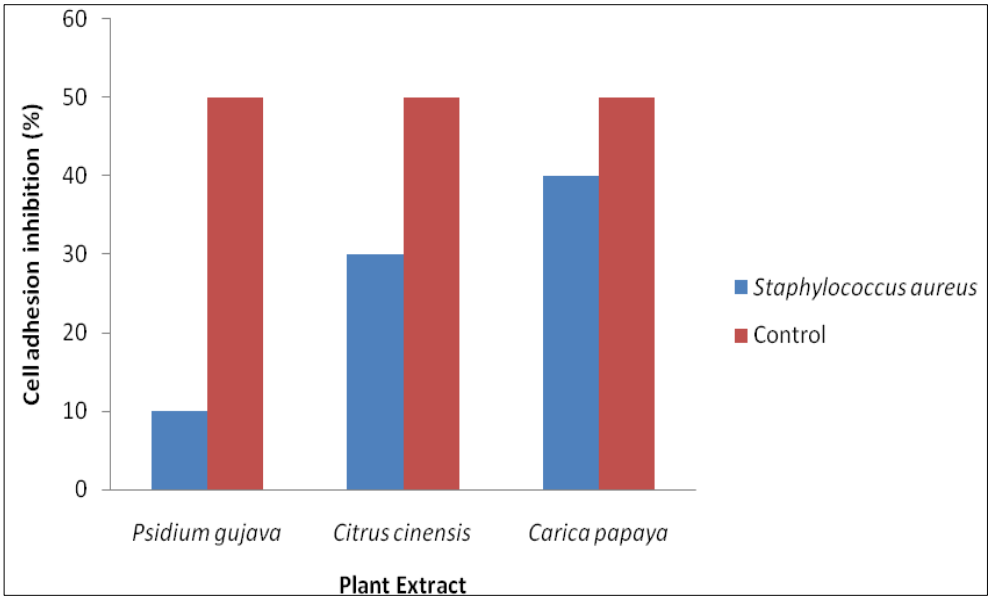


**Fig 2:** Inhibitory effect (%) of plant extract on cell adhesion of *Staphylococcus aureus*

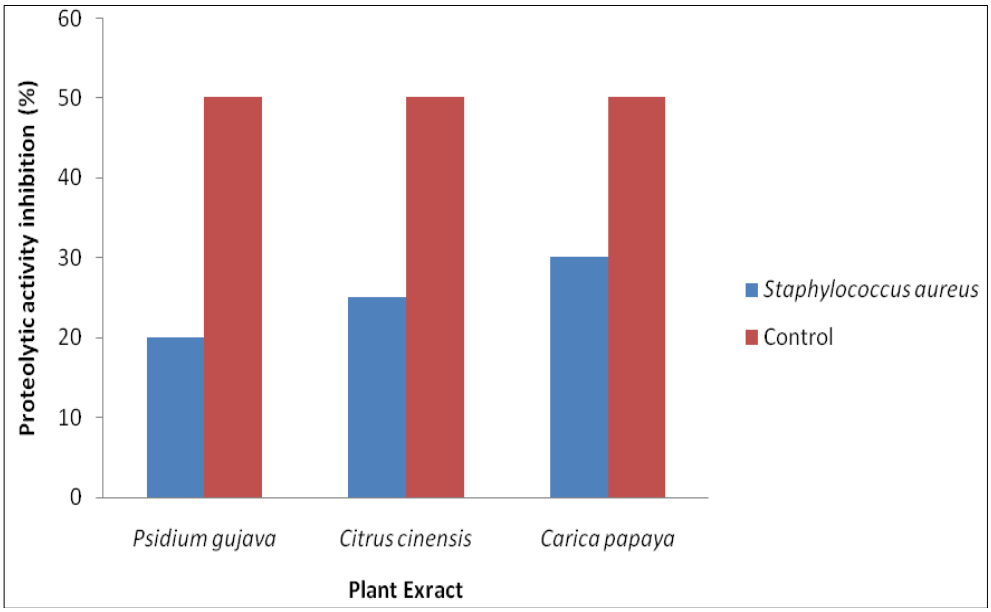


**Fig 3:** Inhibitory effect (%) of plant extract on proteolytic activity of *Staphylococcus aureus*

### 3.3. Effects on proteolytic activity
The distribution for the plant extracts against proteolytic activity is presented in Figure 3.

## 4. Discussion
The results of extracts on twitching motility of *Staphylococcus aureus* are displayed in Figure 1. Twitching motility is associated with high cell densities and cell to cell communication, which are the hallmarks of quorum-sensing systems in bacteria. Recently, quorum sensing has been shown to be involved in initiating and controlling motility. Diameter of the stained growth (radius) of tested bacterial strain treated with the plant extracts was found to be less that of control. *Psidium guajava and Carica papaya* leave extracts, showed the best inhibition of twitching motility in *Staphylococcus aureus*. The comparative plot for the effects of the plant extract is presented in Figure 1. Among the plant extracts tested, *Psidium guajava* and *Carica papaya* extract revealed inhibition with 20 cm and 35 cm of stained growth of *Staphylococcus aureus*. In a related study, Karthick and Vivek (2016) [12] obtained similar results for *Aegle marmelos* (22 cm) and *Cynodon dactylon* (38 cm).
Cell adhesion initiates the biofilm formation and pathogenicity in the host. The results for the tested plant extracts on cell adhesion in figure 2. *Psidium guajava* extracts, showed the highest inhibition of cell adhesion in *Staphylococcus aureus*.
Total proteolytic activity of *Staphylococcus aureus* treated with plant extracts were determined by measuring the reduction of azocasein as the substrate by the crude protease present in the supernatant. The result for the tested plant extracts on proteolytic activity is presented in figure 3. *Psidium guajava* plant extracts, showed the best inhibition for *Staphylococcus aureus*.

## 5. Conclusion
The continuous emergence of multidrug-resistant bacteria caused increased need of anti-pathogenic and anti-infective strategy to combat bacterial infections. Natural products provide alternative medicine for treating emerging bacterial infections without leading to antibiotic resistance. *Psidium guajava* and *Carica papaya* plant extract revealed the best inhibition of *Staphylococcus aureus* for twitching motility. *Psidium guajava* plant extract showed greater inhibition on cell adhesion while the inhibition of proteolytic activity was lowest in *Carica papaya* and highest in *Psidium guajava*. Overall, the plant extract showed variable effect with *Psidium guajava* plant extract showing the best effect against quorum sensing regulated virulence factors in *Staphylococcus aureus*. This study shows the potential use of these plant extracts in the treatment of microbial infections by inhibiting bacterial virulent factors and its associated antibiotics resistance capabilities. It is recommended for medicinal plant extracts such as *Psidium guajava* to be used as an alternative to existing drugs which will reduce the occurrence of resistance of antibiotics in target organisms

## 6. Conflict of interest
The authors declared that there is no conflict of interest regarding the publication of this manuscript.

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