

## Evaluation of Medicinal Plants Extract against Biofilm Formation in *Pseudomonas aeruginosa*

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### Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

### Article Information

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### ABSTRACT

The antibacterial and biofilm inhibitory activity of leaf extracts of *Ocimum gratissimum*, *Moringa oleifera*, *Hibiscus sabdariffa*, *Azadirachta indica* and seed extract of *Garcinia kola* against clinical isolates of *Pseudomonas aeruginosa* were evaluated using the agar diffusion and crystal violet assay methods. The ethanol extracts of *O. gratissimum* and *H. sabdariffa* exhibited significant antibacterial activity against the *P. aeruginosa* with diameter zones of inhibition of 25 mm and 30mm with minimum inhibitory concentrations (MIC) of 0.16 mg/ml and 0.18 mg/ml respectively. While the ethanol extracts of *A. indica*, *M. oleifera* and *G. kola* showed no antibacterial effect against the test organism. However, all five plants extract inhibited the formation of biofilm with optical density (OD) values reduction from 0.168 to 0.160 for *O. gratissimum*, 0.170 to 0.151 for *H. sabdariffa*, 0.140 to 0.138 for *A. indica*, 0.145 to 0.137 for *M. oleifera* and 0.135 to 0.130 for *G. kola*. Out of five plants extract tested, *H. sabdariffa* exhibited the best growth and biofilm inhibition activity of *P. aeruginosa*. The results of this study indicate that various concentrations of the plants extract particularly *H. sabdariffa*, may provide an alternative to control biofilm-related infections caused by *P. aeruginosa*.

**Keywords:** Biofilm; optical density; antibacterial; inhibitory; plant extract.

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## 1. INTRODUCTION

Nosocomial infection or otherwise called Hospital-acquired infections are infections that develops within 3 days of a hospital admission, mainly caused by high antibiotic resistant strains of bacteria [1]. These organisms are mostly found on hospital equipment surfaces, sinks and other moist areas of the environment [1]. Several organisms have been implicated in causing nosocomial infection in different geographical area but of particular importance and prominence is *Pseudomonas* spp. [2].

*Pseudomonas aeruginosa* is an opportunistic pathogen seen mainly as the source of nosocomial infections, including infections associated with artificial implants, contact lenses, urinary catheter tubes [3,4]. It is a major cause of opportunistic infections especially among immunocompromised individuals and responsible for high morbidity and mortality rates in hospital patients [5]. It is found in mostly in soil, plants and animal tissues, water and water treatment systems and in other places with low nutrient levels over a wide temperature range [6]. Of particular significance of this organism is that it occurs as a biofilm in nature attached and swimming in several water bodies and wet surfaces [7].

A biofilm is a community of cells attached to either a biotic or abiotic surface enclosed in a complex exopolymeric substance (EPS) [8]. Biofilm protects the bacteria from the immune system, antibiotics and other adverse environmental factors thus enabling the microorganisms to survive in hostile environmental conditions [9]. Biofilm formation is a key virulence factor for the organism's ability to cause infection. It helps the organism to resist the effects of antibiotics and thus making the management and treatment of nosocomial infections problematic and difficult [8]. This also increases hospital stays and cost of medical care for affected patients. There is therefore an increasing need to seek alternative antimicrobial agents that present novel or unexplored properties to efficiently control and manage these infectious diseases [10]. Approaches targeted at the inhibition of biofilm formation in bacterial pathogens are becoming increasingly looked at as a possible solution [11]. Medicinal plants have proven to be an effective and readily available alternative source of antimicrobial and antibiofilm medicine. Thus, this study is aimed at evaluating the antibacterial and biofilm inhibitory activity of

leaf extracts of *Ocimum gratissimum*, *Moringa oleifera*, *Hibiscus sabdariffa*, *Azadirachta indica* and seed extract of *Garcinia kola* against clinical isolates of *Pseudomonas aeruginosa*.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

The test organism *Pseudomonas aeruginosa* used for this research was obtained from the Federal Medical Centre (FMC) Owerri, Imo State, Nigeria. While the plant materials were obtained in Owerri, Imo State Nigeria and were authenticated by the Taxonomist in Botany department of Imo State University Owerri, Nigeria.

### 2.2 Preparation of Plant Extracts

The plant materials viz., leaf extracts of *Ocimum gratissimum*, *Moringa oleifera*, *Hibiscus sabdariffa*, *Azadirachta indica* and seed extract of *Garcinia kola* were air dried and grounded using a sterile mortar and pestle and used for extraction with ethanol solvent. For the extraction, 100grams of the grounded plant materials were soaked in 500ml of ethanol in a conical flask for 24hours. After which the resulting solution was filtered using a filter paper and the residue separated. The solvent content of the filtrate was evaporated completely by heating leaving a dried extract.

### 2.3 Antibacterial Assay of Plant Extracts by Agar Well Diffusion Method

Well Diffusion method was used to test the antibacterial activities of the extracts [12]. The ethanol extract was tested for antibacterial activity using agar diffusion on Mueller Hinton Agar. The media was sterilized by autoclaving at 121°C at 15 lbs pressure for 15 minutes. The molten agar was allowed to cool to 45°C and then 20ml of Mueller Hinton agar was poured aseptically into Petri plates. The agar was allowed to set and harden. Using a sterile swab, lawn of the test organism was spread onto the Mueller Hinton agar plates. The wells were punctured in the centre by using a sterile cork borer. Then the wells were filled with the extracts. The plates were incubated at 37°C for 24 hrs. After incubation the plates were observed for the zone of inhibition. The zones were measured using zone measuring scale. The tests were done in triplicates.

## 2.4 Biofilm Inhibition Activity

Biofilm formation assay was carried out by the modified method of crystal violet staining assay in a glass test tube [13]. Tryptic soy broth was prepared and 30  $\mu$ l of the broth was transferred sterile test tube. The broth was then sterilized by autoclaving at 121°C at 15 lbs pressure for 15 minutes. The test tube was then inoculated with 3  $\mu$ l of overnight grown culture of *P. aeruginosa* strain. The Ethanol extracts was added into each tube. The tubes were then incubated at 37°C for 18 hours in a slanted manner. After incubation the supernatant (non adherent cells) was carefully decanted without disturbing the adhering cells. The tube containing biofilm were washed with saline (0.85% NaCl). Then 30 $\mu$ l saline was added and mixed well to separate the cells adhered to surface and to remove loosely attached cells. 1% Crystal violet solution was added to observe for the adhered cells. The optical density was measured at 550 nm using a spectrophotometer.

## 2.5 Data Analysis

The mean and standard deviation of the measurements were used to present the findings of the study. The difference in mean values was analyzed using t test with GraphPad Prism.

## 3. RESULTS AND DISCUSSION

The antibacterial activity of the ethanol extracts of the plant materials against *P. aeruginosa* was

investigated and the results presented in Table 1. From the results obtained, the extracts of *Azadirachta indica* and *Garcinia kola* recorded no zone of inhibition which indicates that the extracts had no antibacterial activity against the test organism. While, extracts of *Ocimum gratissimum* and *Hibiscus sabdariffa* recorded diameter zones of inhibition of 25mm and 30mm respectively (Table 1).

The reason for the inactivity of *Azadirachta indica* and *Garcinia kola* extracts may be that the concentrations used were below effective levels or that the organism was inherently resistant to the plant extracts. This however, contradicts previous reports stating that these plants extract possessed antibacterial activity against *Pseudomonas* [14]. On the other hand, extracts of *Ocimum gratissimum* and *Hibiscus sabdariffa* demonstrated good antibacterial activity against *Pseudomonas* with diameter zones of inhibition of 25 mm and 30 mm respectively. This report corroborates the findings of other previous researchers who have stated the antibacterial effect of extracts of *Ocimum* and *Hibiscus* against *Pseudomonas* spp. respectively [15,16]. However, there are other contradictory reports stating the inactivity of *H. sabdariffa* against *Pseudomonas* and other Gram-negative bacteria [17]. These varying results could be attributed to difference in extract concentrations, extraction methods, difference in the composition of active metabolites, strain of *Pseudomonas* used and experimental design [15,18,19].

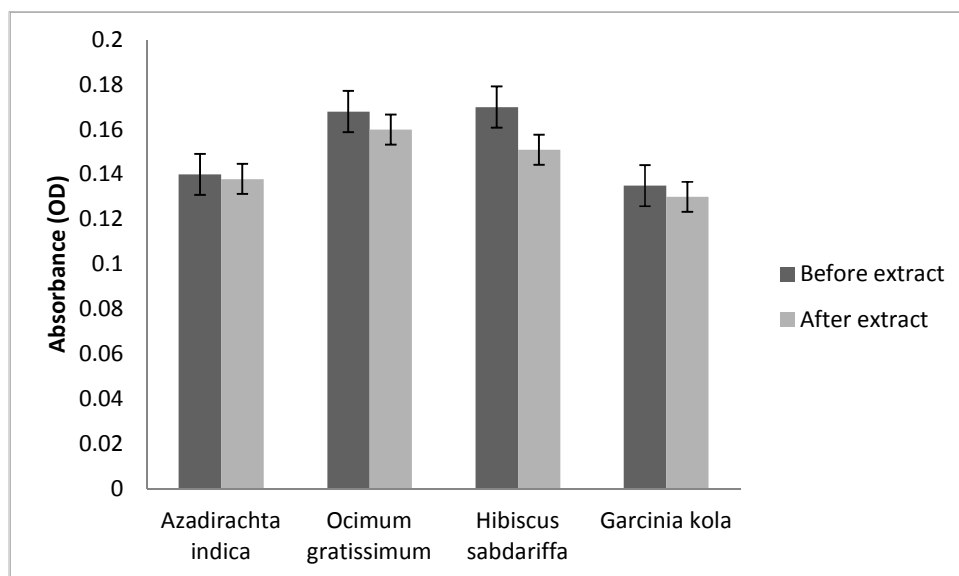


Fig. 1. Biofilm inhibition activity of plant extracts against *Pseudomonas* at 490 nm

**Table 1. Antibacterial activity of plants extract against *Pseudomonas aeruginosa***

Plant extract	Diameter zone of inhibition (mm)	MIC (mg/ml)
<i>Azadirachta indica</i>	Nil	ND
<i>Ocimum gratissimum</i>	25	0.16
<i>Hibiscus sabdariffa</i>	30	0.18
<i>Garcinia kola</i>	Nil	ND
<i>Moringa oleifera</i>	Nil	ND

ND = Not Determined

**Table 2. Biofilm inhibition activities (Optical Density) of the plant extracts against *P. aeruginosa***

Well no.	Extract used	OD before addition of extract	OD after addition of extract
1	<i>Azadirachta indica</i>	0.140	0.138
2	<i>Ocimum gratissimum</i>	0.168	0.160
3	<i>Hibiscus sabdariffa</i>	0.170	0.151
4	<i>Garcinia kola</i>	0.135	0.130
5	<i>Moringa oleifera</i>	0.145	0.137
	Mean	0.15160	0.14320
	SD	0.01629	0.01207
	SEM	0.00728	0.00540
	N	5	5
	P value	0.0431	

The difference is considered to be statistically significant at 95% confidence level

For the biofilm inhibition activity, however, all five plants extract inhibited the formation of biofilm with optical density (OD) values reduction from 0.168 to 0.160 for *O. gratissimum*, 0.170 to 0.151 for *H. sabdariffa*, 0.140 to 0.138 for *A. indica*, 0.145 to 0.137 for *M. oleifera* and 0.135 to 0.130 for *G. kola* (Table 2). From the results, it was observed that all four plants extract demonstrated antibiofilm activity against the test organism with the most activity shown by *H. sabdariffa* (Fig. 1). Worthy of note is the fact that both *A. indica* and *G. kola* which did not possess any antibacterial effect against *Pseudomonas* were able to inhibit the formation of biofilm in the organism. This suggests that these plants extracts might have quorum quenching compound which inhibit biofilm formation.

#### 4. CONCLUSIONS

*Pseudomonas aeruginosa* is and still remains a major problem in hospital related infections and its high levels of antibiotic resistance. To circumvent this, researchers has looked the way of biofilm because it is key to its virulence and its inhibition will enhance the action of antibiotics and the immune system in tackling the organism. For this reason we have looked at plant derived antibiofilm agents and have found that from our study, *H. sabdariffa* and *O. gratissimum* both are

strong antibacterial agents and biofilm inhibitors. Thus, these plants extracts could be incorporated into antibiotic treatment regimens for the treatment of nosocomial infections.

#### COMPETING INTERESTS

Author has declared that no competing interests exist.

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