PLANTS PHENOLICS AS POSSIBLE *PSEUDOMONAS AERUGINOSA* RESISTANCE INHIBITORS

ABSTRACT

*Pseudomonas aeruginosa*virulence has for long been a serious medical, economic and social problem. It is responsible for numerous nosocomial infections like pneumonia, urinary tract infections, surgical site infections and some of community-acquired infections such as otitis, ulcerative keratitis and soft tissue infections. Its ability of adhering to various kinds of surfaces, such as hospital and surgical materials (thus: implicated in causing nosocomial infections) is one of the many reasons why *P. aeruginosa*is of utmost medical and economic importance. This bacterium has an extensive adaptive capability to different kinds of physical surfaces and conditions. *P. aeruginosa* has high capability for the formation of resistant biofilms and the regulation of efflux pumps, thus; these two contributes highly towards an elevated resistance to numerous antibiotics. Several antibiotic resistance genes are responsible for *P. aeroginosa*drug resistance virulence. Plant phenolicshave the ability to bind to protein and non-protein domains leading to modification or inhibition protein–protein/co-factor interactions. These are a diverse group of aromatic secondary metabolites involved in plant defense. *P. aeruginosa*resistance genes mechanisms and evasion tactics can be affected and neutralized by ethno-plant secondary metabolites especially Phenolics, in several different ways due to the nature of its inter-molecular interactions. Ethno-plant phenolics could really provide an alternative natural remedy for the management and neutralization of *P. aeruginosa*Multi-drug resistance Genes.

Key Words: Phenolic, Bacteria, Genes, Efflux Pumps, Resistance

INTRODUCTION

Ethno-medicinal Plants are potential source of medical compounds and agents, which are traditionally used to treat many diseases, especially infectious disease including diarrhea, fever and cold [1]. “With increasing number of bacterial strains resistant to various antibiotics, many attempts to use the antimicrobial potential of plants have been done” [1]. “On the other hand, emergence of resistant strains among gram negative bacillus and positive coccis such as of genus *Pseudomonas, Klebsiella, Enterobacter, Staphylococcus* and *Enterococcus* has been quite a serious problem, causing numerous detrimental effects in treating infections caused by these

bacteria" [2]. "Infections due to *P. aeruginosa*are normally difficult to extinguish, because of their elevated intrinsic resistance as well as their capacity to acquire resistance to different antibiotics" [3]. "*P. aeruginosa*produces various mechanisms of resistance to antibiotics such as broad-spectrum b-lactamases, metallo-b-lactamases and alteration of protein binders of penicillin. *P. aeruginosa* is usually responsible for numerous nosocomial outbreaks in tertiary healthcare centers" [4]. "The pathogenesis of *P. aeruginosa* is as a result of the production of an arsenal of virulence factors classified into cell-associated and secreted" [5]. "This includes exotoxin A (exoA) that play a main role in tissue lysis and bacterial invasion. Hemolysin phospholipase H (plcH), also acts in destroying lipids and lecithin contributing to tissue invasion. *P. aeruginosa* also produces exoenzyme S (exoS), a cytotoxin responsible for damage to many types of host cells and elastase B (lasB) that play an important role during the acute infection" [6]. Gomes *et al.* [7] reported that plants phyto-metabolites like; alkaloids, steroids, tannins, flavonoids and terpenoids, are active against bacterial resistance genes, virulent proteins and snake venom enzymes.

An Overview on *P. aeroginosa*

P. aeruginosa is a gram-negative bacillus bacteria, normally found almost everywhere in both aquatic and terrestrial environments. One of the reason that make this bacterium very important, is its ability of adhering to various kinds of surfaces, including hospital and surgical materials (thus: implicated in causing nosocomial infections). This bacterial has an extensive adaptive capability to different kinds of physical surfaces and conditions [8].

"*P. aeruginosa* is an aerobic rod-shaped bacterium, belonging to the Pseudomonadaceae bacterial family and a member of γ-proteobacteria. It is however one of the subtypes among a group of 12 members. The pathogen is a free-living organism in diverse planktonic form environment. This bacterium is a non-fermenting Gram-negative bacilli and an impotant causative agent of opportunistic infections in immunocompromised patients" [9].

This bacterium can simply be identified on the basis of its gram morphology,monoflagellation, inability to ferment lactose, fruity odour (grape like), a positive oxidase test (reaction) and its ability to grown at 452^{o}C which helps in distinguishing it from other Pseudomonas species. *P. aeroginosa*usually grows well around a temperature range of 25^{o}C - 37^{o}C [10].

_P. aeroginosa_Identification

Biochemical Identification of *P. aeroginosa* involves all the standard protocols used in isolation, evaluation and characterization of the bacterium. These protocols includes characteristic features of the bacterium, its microbial, metabolic and chemical processes [9]. Most of these can be reviewed as follows [11]:

Table 1 : Standard protocols use in isolation, evaluation and characterization of the bacterium

S/N	**Characteristic (variable) Test**	***P. aeroginosa***
1	Gram Staining	Negative
2	Shape (Cocci/Diplococci/Rods)	Rods
3	Motility (Motile / Non-Motile)	Motile (Unipolar)
4	Capsule (Capsulated/Non-Capsulated)	Non-Capsulated
5	Spore (Sporing/Non-Sporing)	Non-Sporing

6	Flagella (Flagellated/Non-Flagellated)	Single Flagella
7	Catalase	Positive (+ve)
8	Oxidase	Positive (+ve)
9	Methyl Red	Negative (-ve)
10	VPOF (Oxidative/Fermentative)	Negative (-ve)
11	OxidativeIndole	Negative (-ve)
12	Urease	Negative (-ve)
13	Nitrate Reduction	Positive (+ve)
14	Citrate	Positive (+ve)
15	H_2S	Negative (-ve)
16	Gas	Positive (+ve)- From Nitrate
17	Gelatin Hydrolysis	Positive (+ve)
18	Cetrimide Test	Positive (+ve)
19	Pigment	Positive (+ve)
20	Glucose	Negative (-ve)
21	Inulin	Negative (-ve)
22	Lactose	Negative (-ve)
23	Maltose	Negative (-ve)
24	Mannitol	Positive (+ve)
25	Sorbitol	Negative (-ve)
26	Sucrose	Negative (-ve)
27	Arginine dehydrolase	Positive (+ve)
28	Lipase	Positive (+ve)

29	Lysine	Negative (-ve)
30	Ornithine decarboxylase	Negative (-ve)

Pathogenesis of *P. aeroginosa*

*"P. aeruginosa*virulence has for long been a serious medical, economic and social problem.It is responsible for numerous nosocomial infections like pneumonia, urinary tract infections, surgical site infections, and some of community-acquired infections such as otitis, ulcerative keratitis and soft tissue infections. It is also implicated in respiratory, burn and wound infections" [12]. Keratitis cause by *P. aeruginosa* are normally related to contact lens wear, but other risk factors for keratitis in non-contact lens wearers include ocular trauma, ocular surgery and prior ocular surface disease (also known as a major agent/cause of opportunistic infections).

*"P. aeroginosa*virulence is normally as a result of its production of an arsenal of virulence factors classified into cell-associated and secreted agents. Tissue lysis and invasion by this bacterium is normally as a result one of its toxins known as the exotoxin A (*exoA*). While lipids destruction is cause by the presence of the hemolysinprotein, phospholipase H (*plcH*). It however produces an exoenzyme S (*exoS*), which is actually a cytotoxin, normally responsible for damage to many types of host cells. Elastase B (*lasB*) is another cytotoxin that play important role during acute infection by this bacterium" [13].

***P. aeroginosa*Drug Resistance**

“These are the kind bacteria that are naturally resistant to a number of antibiotics, due to their possession of some specific resistance genes. Some of these resistance genes includes;catB that conferschloramphenicol resistance.The regulation of efflux pumps also contributes towards an elevated resistance to antibiotics by these bacteria (e.g. expression MexAB-OprMefflux pump contributes towards intrinsic resistance for broad spectrum of antibiotics), MexXY-OprMhowever is involved in the adaptive resistance to aminoglycosides.Additionally *P. aeruginosa*often acquired transferrable virulent determinants, such as those associated with transposons and integrons. This pathogenesis, however varies according to the area and condition where the strains have been isolated or growing on”[11].

An Overview on *P.aeruginosa* and Biofilm

“Biofilms **(**complex aggregation of microbes)are usually formedwhen different kinds of microorganismsadhere to anabiotic or biotic surface surrounded by a polysaccharide-matrix, functioningas a protective barrierfor the organisms (against either microbialattack or aggressions of the external or internalenvironment).*P. aeruginosa*formed biofilms,normally exhibits a quite a higher antimicrobial resistance when compared to the planktonic form and assists in the evasion of the host immune response, especially under stress or attack” [14]. Organisms inside biofilm tends to have a different kind of genomic phenotype, in gene transcription, metabolism and growth (thus: giving these bacteria a higher rate of resistance to numerous kinds of antibiotics [15].

***P. aeuriginosa*Resistance throughEfflux Pumpin Biofilms**

Most multi-drug resistance by *P. aeroginosa*is as a result of the activity of efflux pump systems. That is to say its expression of the main mechanisms responsible for antimicrobial resistance in biofilms. This is particularly important in non-enzymatic mechanism of resistance to many antibiotics like β-lactams. These pumps aids in the external transport of several kinds of detrimental molecules, such as; detergents, biocides, and antibiotics[16].

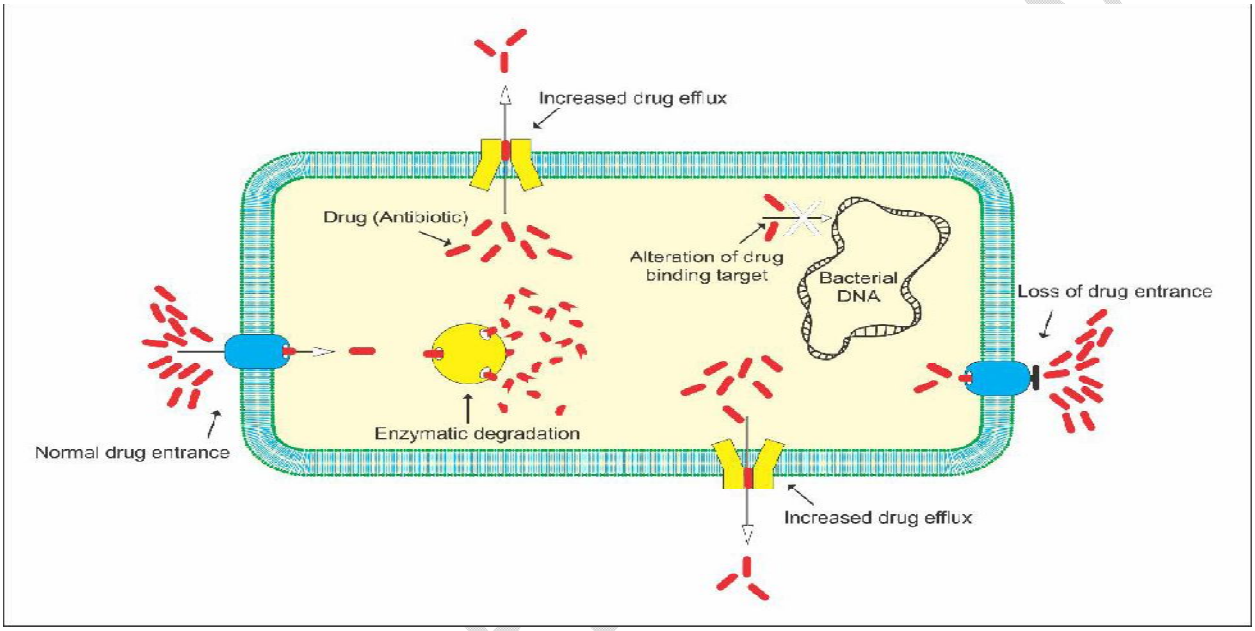

Fig.1: *P. aeruginosa* Resistance through Efflux Pump in Biofilms (Schematic representation of drug-Efflux pump system)[17]

"The efflux systems are classified into six super families: major facilitator superfamily (MFS), ATP-binding cassette (ABC), small multidrug resistance (SMR), resistance splitting division (RND), multidrug and toxic compound extrusion and drug metabolite transporter (DMT). The most clinically relevant efflux systems in Gram-negative bacteria are those of the RND family. Several of them are expressed by *P. aeruginosa*. Among them, MexA-MexB-OprM, MexC-MexD-OprJ, MexEMexF-OprN and MexX-MexY-OprM are significant determinants for resistance to various drugs" [18].

Pseudomonas aeroginosa Resistance Mechanisms

*Pseudomonas aeruginosa*produces various kinds of mechanisms for resistance to different types of antibiotics such as; broad-spectrum b-lactamases, metallo-b-lactamases and alteration of protein binders of penicillin. Its virulence has for long been a serious medical, economic and social problem [4]. It is responsible for a number of nosocomial and community-acquired infections such as otitis, ulcerative keratitis, pneumonia, urinary tract infections, surgical site infections and so many kinds of diseases and infections [19]. Its ability of adhering to various kinds of surfaces, such as hospital and surgical materials is one of the many reasons why *P. aeruginosa*is of utmost medical and economic importance. This bacterium however has an extensive adaptive capability to different kinds of physical and biochemical conditions [20]. The pathogenesis of *P. aeruginosa* is as a result of the production of an arsenal of virulence factors classified into cell-associated and secreted [21]. "These includes exotoxin A (exoA) that play a major role in tissue lysis and bacterial invasion. Hemolysin phospholipase H (plcH), also acts in destroying lipids and lecithin contributing to tissue invasion. *P. aeruginosa* also produces exoenzyme S (exoS), a cytotoxin responsible for damage to many types of host cells and elastase B (lasB) that plays an important role during the acute infection" [6].

This bacterium has a high capability for the: Formation of resistant biofilms and regulation of bacterial efflux pumps. These attributes contributes highly to an elevated resistance to numerous bacterial antibiotics. Several antibiotic virulence and resistance genes are responsible for *P. aeroginosa*drug resistance and virulence [19]. Some of the most important *P. aeroginosa*antibiotic resistance and virulence genes includes:

<u>Virulent Genes</u>

a. toxA gene: ------- exotoxin A

b. lasA and lasB genes: ------ elastolytic proteases

c. lasR gene: ------ encodes a transcriptional activator, the LasR protein

The LasR protein however serves as a transcriptional activator of aprA,lasA and lasB virulence genes [22].

Resistance Genes

i. blaCTX-M

ii. **blaOxa23**

iii. ampC genes [22]

"The blaCTX-M, **blaOxa23** and ampC genes are the main antibiotic-resistance genes that induce resistance patterns to cefotaxime, amoxicillin and tetracycline, highlighting *P. aeruginosa* strains potential public health concern"[22]."Alteration of target sites, active efflux of drugs and enzymatic degradations are the ways used by the pathogenic bacteria, especially *P. aeroginosa,* to develop intrinsic resistance to numerous antibiotics" [23].

Medicinal Plants Phenolicsas Potential Inhibitor of *P. Aeroginosa*Resistance Proteins

*"Ficussycomorus, Cymbopogoncitratus*and*Balanitesaegyptiaca*are used traditionally in treating different type of infections and illness, including treatment of snake bites, numerous bacterial/fungal infections, jaundice, chest pains, dysentery, cool, coughs/throat infections,

elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmic, pneumonia jaundice, intestinal worm infection, wounds, malaria, syphilis, epilepsy, dysentery, constipation, hemorrhoid, stomach aches, asthma and fever and vascular disorders" [25]. These plant phytometabolites are proven responsible for these pathogenic bacterial neutralization [1]. "Alteration of target sites, active efflux of drugs and enzymatic degradations are the ways used by the pathogenic bacteria, especially *P. aeroginosa,*to develop intrinsic resistance to numerous antibiotics. This has led to an increased interest in medicinal plants as one of the only ways forward and makes researching into plants phyto-metabolites no longer a luxury but a necessity" [24].

Phenolics are hydroxyl group (OH) containing class of phytochemicals which are attributed with numerous antimicrobial activity [25].Phenolicshave the ability to bind to protein domains leading to modification or inhibition protein–protein interactions. These are a diverse group of aromatic secondary metabolites involved in plant defense. They consist of flavonoids, quinones, tannins, and coumarins.

Flavonoids

These are phenolic structures that are normally found in common edible plant parts such as: Vegetables, seeds, fruits and nuts. Plant cells carrying out photosynthesis are usually the store house of fourteen kinds of known flavonoids [35]. Many researchers have make known the antimicrobial potentials of flavonoids against numerous kinds of bacterialand fungal pathogens [26]."Flavonoids usually acts out microbial cell membranes by increasing its permeability and disruptingits interaction with membrane proteins present on bacterial cell wall. Flavonoids are

known to possess antioxidant, anti-inflammatory and antitumor activity [36]. Various plants that have been studied for their antimicrobial activity against *P. aeruginosa* contain flavonoids in higher quantity" [27].

Tannins

Tannins are polymeric phenolic substances, which usually found in almost all plant parts, especially photosynthetic plants. Tannins like flavonoids have also been studied and proven to possess antimicrobial activities. Tannins acts by inactivating cell envelope, transport proteins and microbial adhesins [28]."From a chemical point of view it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers" [38]. "It might be stated tannins are a heterogeneous group of high molecular weight polyphenolic compounds with protein capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, homicellulose, pectin)" [39]. Tannins are divided into four groups on the basis of their structures:

i) Gallotannins
ii) Ellagitannins
iii) complex tannin
iv) condensed tannins [38]

"Several health benefits have been recognized for the intake of tannins and some epidemiological associations with the decreased frequency of chronic diseases and zoonotic anti venom activity" [37]. "Tannins are also use in dye stuff industry as cosmetics for cationic dyes (tannin dyes) and also in the production of ink (iron gallate ink). In the food industry, tannins are

used to clarify wine, bears, and fruit juices. Other industrial uses of tannin include textile dyes, as antioxidant in the fruit juice, bear, wine industries and as coagulants in rubber production" [40].

Prospectsof Plant Phenolics Against Multi-drug Resistance *P. Aeruginosa*

Plant extracts and phytochemicals used, possess both known and unknown antimicrobial activities, which could be of great importance in therapeutic treatments, especially when dealing with resistant or mutant pathogens. The structural diversity of phenolic phytochemicals is what makes them so important when trying to neutralize pathogenic genes and determinants [27].

Medicinal Plants are potential source of medical compounds and agents, which are traditionally used in the treatment and management of diseases and infections [1]. These plants has been confirmed to have so many forms of antimicrobial activities, against different kinds of pathogenic microorganisms [29]. Plants phyto-metabolites have been proven responsible for these antimicrobial activities [30]. Phenolics are hydroxyl group (OH) containing class of phytochemicals which are attributed with a number of antimicrobial activity [25]. They have the ability to bind to protein domains leading to the modification or inhibition of protein–protein interactions [30]. Phenolics usually aid plants in defense against virulent pathogenic attack. They consist of flavonoids, quinones, tannins and Coumarins [31]. Flavonoids occur as aglycones, glycosides and methylated derivatives.Many researchers have made known the antimicrobial potentials of flavonoids against numerous kinds of bacterial and fungal pathogens [26]. Flavonoids usually acts on microbial cell membranes by increasing its permeability and disrupting its interaction with membrane proteins present on bacterial cell wall [32].

Various plants that have been revealed to have antimicrobial activity against *P. aeruginosa* contain flavonoids in higher quantity [27]. Meanwhile tannins are structurally complex phenolic compounds.Tannins like flavonoids have also been studied and proven to possess antimicrobial activities. Tannins usually act by inactivating cell envelope, transport proteins and microbial adhesins 28.

P. aeruginosa resistance mechanisms can be affected by the activities of plant secondary metabolites especially flavonoids and tannins, in several different ways due to the nature of their detrimental activity against pathogenic bacterial cells [34].

These series of modulations includes;

- The disruption of membrane function and structure (including the efflux system),
- Interruption of DNA/RNA synthesis and function
- Interference with intermediary metabolism
- Induction of coagulation of cytoplasmic constituents and
- Interruption of normal cell communication (quorum sensing) [35]

Which are all the most prominent mechanisms employed by *P. aeruginosa*in evasion, disruption and clearance of antibiotics [36].

Conclusion

Ethno-plant phenolics could provide an alternative natural remedy for the management and neutralization of *P. aeruginosa*Multi-drug resistance Genes.

Recommendation

i. To evaluate and determine the anti-biogram profile of different strains of *P. aeruginosa* clinical and environmental isolates, in different parts of Nigeria
ii. To determine the presence and effects of *P. aeruginosa* virulent and resistance genes such as; blaTEM, blaOxa23, ampC, lasA, lasB and toxA genes
iii. Determination of the antibacterial activity of the crude phenolics fractions on multi-drug resistance *P. aeruginosa*isolates
iv. To evaluate and determine the effects plants phenolic fractions from on some of the most resistant *P. aeruginosa* isolates in Nigeria.

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