<u>*Review Article*</u>

PLANTS PHENOLICS AS POTENTIAL INHIBITORS OF *PSEUDOMONAS AERUGINOSA* RESISTANCE

UNDER PEER REVIEW

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]. By increasing number of bacterial strains resistant to various antibiotics, many attempts have been doneby using the antimicrobial potential of plants. On the other hand, emergence of resistant strains among gram negative bacillus and positive coccissuch as genus of*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.

UNDER PEER REVIEW

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 reasons 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 important 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 grow at 452°C which helps in distinguishing it from other Pseudomonas species. *P. aeroginosa*usually grows well around a temperature range of 25°C - 37°C [10].

UNDER PEER REVIEW

***P. aeroginosa*Identification**

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

UNDER PEER REVIEW

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 wear 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 caused by the presence of the hemolysinprotein, phospholipase H (*plcH*). However it 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 plays an important role during acute infection by this bacterium [13].

*P. aeroginosa*Drug Resistance

UNDER PEER REVIEW

These are one of the bacteria that are naturally resistant to a number of antibiotics, due to their possession of some specific resistance genes. Some of these resistance genes include;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 virulentdeterminants such as those associated with transposons and integrons. However, this pathogenesis 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 biofilmsnormallyexhibits higherantimicrobial resistance when compared to the planktonic form and assists in the evasion of the host immune response under stress or attack [14]. Organisms inside biofilm tendto have different kinds of genomic phenotype: in gene transcription, metabolism and growth (thus: giving these bacteria a higher rate of resistance to numerous kinds of antibiotics [15].

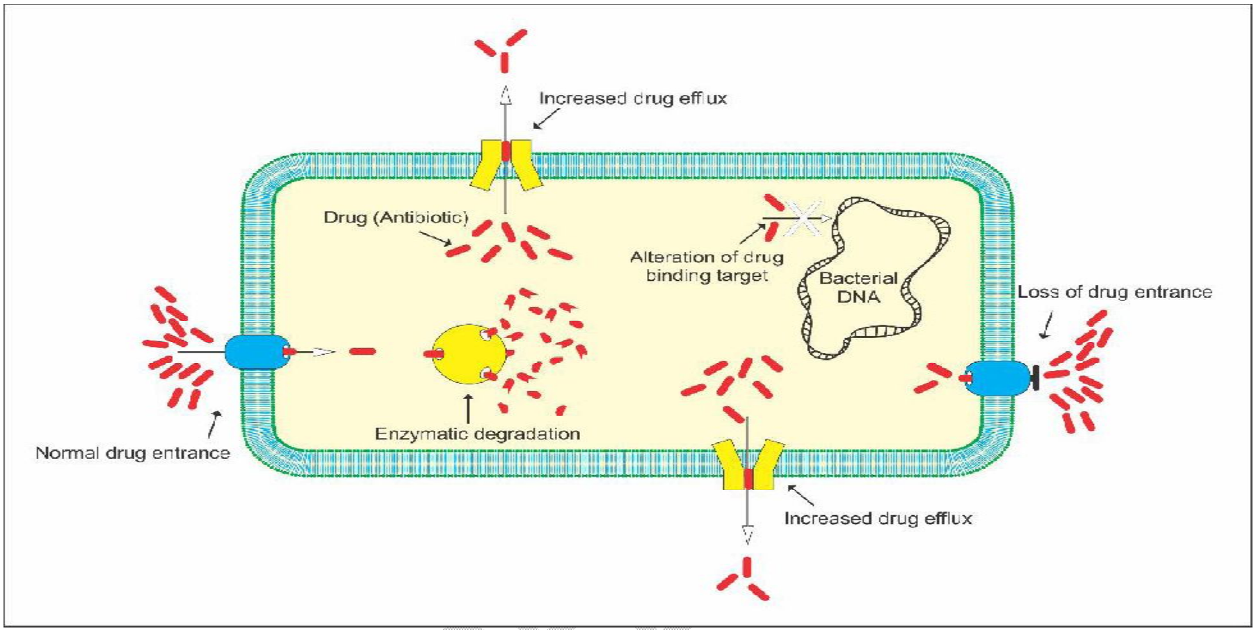
*P. aeuriginosa*Resistance throughEfflux Pumpin Biofilms

Most multi-drug resistance by *P. aeroginosa*is a resulting of the activity of efflux pump systems. That expresses the main mechanisms responsible forthe antimicrobial resistance in biofilms. This

UNDER PEER REVIEW

is particularly important in non-enzymatic mechanism of resistance for 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. aeuriginosa* 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**

Pseudomonas aeruginosa produces various kinds of mechanisms for resistance to different types of antibiotics such as; broad-spectrum b-lactamases, metallic-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 to adhere 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 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 include exotoxin A (exoA) that plays 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 theformation of resistant biofilms and regulation of bacterial efflux pumps. These attributes highly contributes 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:

Virulent Genes

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

UNDER PEER REVIEW

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

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

The LasRprotein 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].

UNDER PEER REVIEW

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

*Ficussycomorus, Cymbopogoncitratus*and*Balanitesaegyptiaca*are used traditionally in treating different types 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. These phyto-metabolites plants are responsible for thispathogenic bacterial neutralization [1]. Alteration of target sites, active efflux of drugs and enzymatic degradations are ways used by the pathogenic bacteria, especially *P. aeroginosa*,to develop intrinsic resistance to numerous antibiotics. This has led to interesting increases 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 activities[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.

UNDER PEER REVIEW

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. Many researchershave known the antimicrobial potentials of flavonoids against numerous kinds of bacterialand fungal pathogens [26].Flavonoids usually acton microbial cell membranes by increasing its permeability and disruptingits interaction with membrane proteins presented on bacterial cell wall. Flavonoids are known to posseantioxidant, anti-inflammatory and antitumor activities. 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 adhesions [28].

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

Plant extracts and phytochemicals arepossessing 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 sources of medical compounds and agents, which are traditionally used in treatment and management of diseases and infections [1]. These plants have been confirmed to have so many forms of antimicrobial activities against different kinds of pathogenic microorganisms [29]. Plants phyto-metabolites proven the responsibility for antimicrobial activity [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 known the antimicrobial potentials of flavonoids against numerous kinds of bacterial and fungal pathogens [26]. Flavonoids usually act

UNDER PEER REVIEW

onmicrobial cell membranes by increasing its permeability and disrupting its interaction with membrane proteins presented 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 adhesions[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 [19].

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) [33]

Which are all the most prominent mechanisms employed by *P. aeruginosa*in evasion, disruption and clearance of antibiotics [34].

UNDER PEER REVIEW

Conclusion

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

Recommendation

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

UNDER PEER REVIEW

REFERENCE

1. Hassan A. U., A. H. Madu, U. O. Ozojiofor, A. H. Galadanci, I. B. Mato and R. Jafaru (2021)a, Antimicrobial Activities of *Cymbopogoncitratus* and *Ximenia Americana* Leaf Extracts Against Some Selected Bacterial and Yeast Clinical Isolates. *Asian Journal of Biochemistry, Genetics and Molecular Biology*; 9(1): 1-10, DOI: 10.9734/AJBGMB/2021/v9i130204

2. Wogenie B. (2008), *Effects on increasing levels of energy and protein supplementation on feed intake, body weight change and carcass composition of Blackhead Somali sheep fed on grass hay*. M.Sc. Thesis Presented to School of Graduate Studies, Haramaya University, Ethiopia.
3. Breidenstein EBM, De la Fuente-Nuñez C, Hancock REW (2011).*Pseudomonas aeruginosa: all roads lead to resistance.Trends in Microbiology* A/I press Cambridge; 19:419-426.
4. Tan TT. (2008) "Future" threat of gram-negative resistance in Singapore. *Ann Academy Medical Singapore*; 37:884-890.

5. Hassan AU, R. Jafaru, I. B. Mato, E. Kereakede, A. H. Galadanci, A. H. Madu, I. A. Oyong, K. Onuh and U. O. (2021), Ozojiofor, Isolation and Biochemical Characterization of Cellulase Produced by Bacterial Isolates from Sugarcane Waste Soil. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, 9(2): 28-35, DOI: 10.9734/AJBGMB/2021/v9i230213.

6. Elmouaden C, Amin L., Latifa E., Mohammed Bakkali and Mohammed Abid (2019), Virulence genes and antibiotic resistance of Pseudomonas aeruginosa isolated from patients in the Northwestern of Morocco. *Journal of Infectious 13(1)*:892-898
7. Gomes A, Das R, Sarkhel S, Mishara R, Mukherjee S and Bhattacharya S (2010), Herbs and herbal constituents active against snake bite. *Indian Journal of Experimental Biology*; 48:865–878.
8. Habibi, A.; Honarmand, R. (2015), Profile of Virulence Factors in the Multi-Drug Resistant Pseudomonas aeruginosa Strains of Human Urinary Tract Infections (UTI). *Iran. Red Crescent Med. J*, 17, e26095.
9. Cotar, A.I.; Chifiriuc, M.C.; Dinu, S.; Bucur, M.; Iordache, C.; Banu, O.; Dracea, O.; Larion, C. and Lazar, V. (2010), Screening of molecular virulence markers in Staphylococcus aureus and Pseudomonas aeruginosa strains isolated from clinical infections. *Int. J. Mol. Sci*., 11, 5273–5291.
10. Pachori, P.; Gothalwal, R. and Gandhi, P. (2019), Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. *Genes Dis*., 6, 109–119
11. Galdino, A.C.M.; Branquinha, M.H.; Santos, A.L. and Viganor, L. (2017), Pseudomonas aeruginosa and its arsenal of proteases: Weapons to battle the host. In Pathophysiological Aspects of Proteases; Chakraborti, S., Dhalla, N., Eds.; Springer: Singapore; pp. 381–397.
12. Heidary, Z.; Bandani, E.; Eftekhary, M. and Jafari, A.A. (2016), Virulence genes profile of multidrug resistant Pseudomonas aeruginosa isolated from Iranian children with UTIs. *Acta Med. Iran*., 54, 201–210.
13. Yousefi-Avarvand, A.; Khashei, R.; Ebrahim-Saraie, H.S.; Emami, A.; Zomorodian, K. and Motamedifar, M. (2015), The frequency of exotoxin A and exoenzymes S and U genes among clinical isolates of Pseudomonas aeruginosa in Shiraz, *Iran. Int. J. Mol. Cell. Med*., 4, 167–173.

14. Abba Umar H., (2015). Review: '*Phytochemical screening of plant extracts*'**,** Academia.edu/s/b79da63595 Online scholar's library (www.academia.edu/researchcenter).

UNDER PEER REVIEW

15. Elmaraghy, N., Abbadi, S., Elhadidi, G., Hashem, A. and Yousef, A. (2019), Virulence genes in Pseudomonas aeruginosa strains isolated at Suez Canal University Hospitals with respect to the site of infection and antimicrobial resistance. *Int. J. Clin. Microbiol. Biochem. Technol.*, 2, 8–19.
16. Ertugrul, B.M.; Oryasin, E.; Lipsky, B.A.; Willke, A. and Bozdogan, B. (2017), Virulence genes fliC, toxA and phzS are common among Pseudomonas aeruginosa isolates from diabetic foot infections. *Infect. Dis*., 50, 273–279.
17. Djordjevic ZM, Folic MM and Jankovic SM (2017), Correlation between cefepime utilization and Pseudomonas aeruginosa resistance rate to beta lactams and carbapenems in patients with healthcare associated infections. *Journal of Global Antimicrobial Resistance*; 13:60-64
18. Nitz, F., de Melo, B.O., da Silva, L.C.N., de Souza Monteiro, A. Marques, S.G. *et al.*, (2021), Molecular Detection of Drug-Resistance Genes of blaOXA-23-blaOXA-51 and mcr-1 in Clinical Isolates of *Pseudomonas aeruginosa*. *Microorganisms*, 9, 786.
19. Ouyang, J., Sun, F., Feng, W., Sun, Y., Qiu, X., Xiong, L., et al. (2016). Quercetin Is an Effective Inhibitor of Quorum Sensing, Biofilm Formation and Virulence Factors in *Pseudomonas aeruginosa*. *J. Appl. Microbiol*. 120, 966–974. doi:10.1111/jam.13073
20. Koulenti, D., Fragkou, P. C., and Tsiodras, S. (2020). Editorial for Special Issue "Multidrug-Resistant Pathogens". *Microorganisms* 8, 1383.
21. Sarwat T, Rashid M, Rastogi V and Chander Y (2015) Original research article a comparative study of antibiogram of Pseudomonas aeruginosa in hospital and community acquired infections. *International Journal of Curriculum Microbiology Application Science* 1: 286-291.
22. Saak, C. C., Dinh, C. B., and Dutton, R. J. (2020). Experimental Approaches to Tracking mobile Genetic Elements in Microbial Communities. *FEMS Microbiol*. Rev. 44, 606–630. doi:10.1093/femsre/fuaa025
23. Vatan, A., Saltoglu, N., Yemisen, M., Balkan, I. I., Surme, S., Demiray, T., et al. (2018). Association between Biofilm and Multi/extensive Drug Resistance in Diabetic Foot Infection. *Int. J. Clin. Pract*. 72, e13060. doi:10.1111/ijcp.13060
24. Al-Dahmoshi, H.O.; Al-Khafaji, N.S.; Jeyad, A.A.; Shareef, H.K. and Al-Jebori, R.F. (2018), Molecular detection of some virulence traits among Pseudomonas aeruginosa isolates, Hilla-Iraq. *Biomed. Pharmacol. J.*, 11, 835–842.
25. Mirghani ME, Liyana Y and Parveen J (2012). Bioactivity analysis of lemon grass essential oil. *International food research Journal;* 19(2):569-575.
26. Nostro A. (2006), *Activity of plant extracts and plant-derived compounds against drug-resistant microorganisms.* In: Ahmad I., Aqil F., Owais M., editors. Modern phytomedicine: turning medicinal plants into drugs. Wiley-VCH Verlag GmbH & Co. KGaA; Weinheim, Germany.
27. Bielecki P, Puchałka J, Wos-Oxley ML, Loessner H, Glik J, et al. (2011), In-Vivo Expression Profiling of Pseudomonas aeruginosa Infections Reveals Niche-Specific and Strain-Independent Transcriptional Programs. *PLoS ONE,* 6(9): e24235. doi:10.1371/journal.pone.0024235.
28. Birdi T.J., Brijesh S. and Daswani P.G. (2014), Bactericidal effect of selected antidiarrhoeal medicinal plants on intracellular heat-stable enterotoxin–producing Escherichia coli. *Indian J Pharm Sci.*; 79:229–235.

UNDER PEER REVIEW

29. Zahara, K., Panda, S. K., Swain, S. S., and Luyten, W. (2020). Metabolic Diversity and Therapeutic Potential of HolarrhenaPubescens: An Important Ethnomedicinal Plant. *Biomolecules* 10, 1341. doi:10.3390/biom10091341
30. Mohammed, M. J., Anand, U., Altemimi, A. B., Tripathi, V., Guo, Y., and Pratap-Singh, A. (2021). Phenolic Composition, Antioxidant Capacity and Antibacterial Activity of White Wormwood (Artemisia Herba-alba*). Plants 10*, 164. doi:10.3390/plants10010164
31. Hassan AU, M. S. Makeri, U. O. Ozojiofor and A. J. Alhassan (2020), Effects of Ficussycomorus Phenolic Extracts on the Activity of Hyaluronidase and Phospholipase A_2 Enzymes of Echisocellatus Venom. *Asian Journal of Biotechnology and Genetic Engineering*; 3(4): 24-33.
32. Naik S., Mohanty S., Padhi A., Pati R. and Sonawane A. (2014), Evaluation of antibacterial and cytotoxic activity of Artemisia nilagirica and Murrayakoenigii leaf extracts against mycobacteria and macrophages. *BMC Complement Altern Med.*; 14:87.
33. Anand, U., Nandy, S., Mundhra, A., Das, N., Pandey, D. K., and Dey, A. (2020). A Review on Antimicrobial Botanicals, Phytochemicals and Natural Resistance Modifying Agents from Apocynaceae Family: Possible Therapeutic Approaches against Multidrug Resistance in Pathogenic Microorganisms. *Drug Resist.* Updates 51, 100695. doi:10.1016/j.drup.2020.100695
34. Koh C.L., Sam C.K., Yin W.F., Tan L.Y., Krishnan T. and Chong Y.M. (2013), Plant-derived natural products as sources of anti-quorum sensing compounds. *Sensors*; 13:6217–6228

UNDER PEER REVIEW