Original article

In silico and in vitro combinatorial study in the fight against the multi-drug resistant uropathogen, *Pseudomonas aeruginosa*

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A R T I C L E I N F O

A B S T R A C T

Background: The emergence of drug-resistant pathogens has been found to increase mortality and the therapeutic cost of infections around the world. *Pseudomonas aeruginosa (P. aeruginosa),* one of the multi-drug resistant pathogens, causes nosocomial and urinary tract infections. The increasing concerns about its morbid effects and resultant mortality have provoked intensive studies on alternative therapeutic solutions to the challenge posed by multidrug-resistant pathogens. Therefore, this study combines in vitro and in silico models to investigate the antimicrobial potential of Azadirachta indica (A. Juss) and Phyllanthus amarus (P. amarus) (Schum and Thonn) leaves of ethanolic extract on multidrug-resistant pathogenic *P. aeruginosa*. **Methods:** Standard procedures were used for the preparation of extracts, phytochemical screening, minimum inhibitory concentration, minimum bactericidal concentration, fractional inhibitory concentration, mechanism of action and gas chromatography-mass spectrophotometer (GCMS). In silico study was carried out by molecular docking of the extracts with MexB-resistant protein encoding the 6IIA-resistant gene of *P. aeruginosa*. **Results:** Many of the ligands had high docking scores which indicate a strong potential for use as drugs to counter the resistant genes. Ligands like Oleanolic acid (-9.8kcal/mol), of P. amarus and Azadirachtol (-10.2kcal/mol) of A. indica, had the best docking scores. The sensitivity assay done using *P. aeruginosa* as a target revealed that both A. indica and P. amarus were able to effectively inhibit the uropathogen at 350mg/ml concentration and with the same zone of inhibition of 20.50±0.33. The minimum inhibitory concentration result revealed that A. indica (300mg/ml) is less effective when compared to P. amarus (200mg/ml). The minimum bactericidal result revealed in a similar fashion that P. amarus (300mg/ml) was more effective than A. indica (350mg/ml) because of the lesser concentration required. Fractional inhibitory concentration shows that the combination of A. indica and P. amarus gives an additive effect (0.83). Mechanism of action result showed that the release of Sodium content was more in P. amarus (17.3Mol/L) than A. indica (12.3Mol/L) and it was released the least in protein (1.0Mol/L- A. indica; 3.6Mol/L- P. amarus). **Conclusion:** The in-silico studies revealed that A. indica had the ligand with the best docking score and greatest potential, while the in-vitro investigation revealed that P. amarus had a higher antibacterial potency against the multidrug resistant uropathogen *P. aeruginosa* than A. indica.

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Introduction

The Pathogen, *Pseudomonas aeruginosa* causes several human infections, particularly in immunocompromised (susceptible) patients with burns or neutropenia [1]. *Pseudomonas* spp. is a prevalent variety of bacterium (germ) in the environment (soil and water) with *Pseudomonas aeruginosa* being the most common cause of infections in humans [2]. It is implicated in blood, lungs (pneumonia), nosocomial, and urinary tract infections. These bacteria are rapidly evolving new gene-based strategies to evade antibiotics meant to annihilate them. At the point where an antibiotic becomes inactive against a pathogen, such a pathogen has developed a resistance [2]. *P. aeruginosa* was found to resist resists numerous antimicrobials and dispersed predominantly in the environment, making infection management challenging. However, previous studies implied that environmental sources, notably water, may be important in the epidemiology of occasional *P. aeruginosa* infections.

Two traditional medicinal plants would pivotal in this study, to investigate their activities when tested against the clinically relevant uropathogen- *Pseudomonas aeruginosa*. The results of this study will contribute to our understanding of the potential of plant extracts as alternative therapeutic options for multidrug-resistant infections.

Materials and methods

Sample preparation

Fifty-nine *Pseudomonas aeruginosa* isolates were gotten (n=59) from the urine samples collected from the UTI patients, and these were, cultured at 37℃ by direct streaking onto agar plates and incubated for 18-24hrs in an incubated and observed, they were later sub-cultured and then stored in slants to preserve the cells and ensure their viability. When required for use, they were streaked directly into fresh agar plates and cultured at 37℃.

Collection of plant samples

Azadirachta indica and Phyllanthus amarus were obtained from areas within the Federal University of Technology, Akure, Ondo state, Nigeria, where the tree (dongoyaro tree) and the shrub (Phyllanthus amarus) of these herbs were found growing naturally. The plants were obtained and vetted in the herbarium of the department of crop, soil and pest management (CSP) of the Federal University of Technology, Akure.

Extraction process

After the leaves were gathered, they were allowed to dry and then ground into a powder. About 300 grammes of each pulverised leaf were immersed in each solvent. During the extraction procedure, ethanol was used as the extraction solvent.[20] After each solution had rested for a total of three days, it was filtered through a clean muslin fabric and then Whatman filter paper No. 1. Under vacuum conditions, the rotary evaporator was used to concentrate the filtrates. [3].

Isolation of test organism

Pure cultures of the test organisms used in antimicrobial research were obtained from urine samples of sick patients and cultivated at the Federal University of Technology, Akure's Postgraduate Laboratory of Medical Microbiology. The organisms were all cultivated on nutritional agar plates. The cultures were maintained alive by regularly subculturing them and keeping them at 4℃ before use. Macconkey agar, Urease agar, Blood agar, and Cystine Lactose Electrolyte Deficient Agar were also used to culture the isolate(s). *Pseudomonas aeruginosa*, a Gram-negative test bacterium, was isolated and preserved in slants.

Standard antibiotic sensitivity assay

The isolate (*Pseudomonas aeruginosa*) was first standardised using a spectrophotometer, and then it was injected onto agar plates using the spread plate technique. Following this, the antibiotic disk(s) were put on the agar and incubated at 37°C for 18-24 hours.

Antimicrobial susceptibility investigation of the plant extracts

Antimicrobial activity of both plant extracts were investigated using the standard methods reported by Ghosh and Jignasu [4,5].

Minimum inhibitory concentration of extracts

The MIC for both Azadirachta indica and Phyllanthus amarus was determined following the method of Ghosh [4].

Minimum bactericidal concentration of extracts

The MBC for both Azadirachta indica and Phyllanthus amarus was done according to the method of Zakaria [6].

Fractional inhibitory concentration (synergistic study)

The Fractional inhibitory concentration of the plant extracts was carried out by obtaining the MIC for A. Azadirachta indica and the MIC for P. Phyllanthus amarus, and the MIC for the combination of both extracts at the rate of 1:1. The result was then subjected to the FICindex formular:

$$\text{FIC}_{\text{index}} = \frac{\text{MIC } of\ antbiotic\ A\ in\ combination}{\text{MIC } of\ antibiotic\ A\ alone} + \frac{\text{MIC } of\ antibiotic\ B\ in\ combination}{\text{MIC } of\ antibiotic\ B\ alone}$$

When the FIC index is less than one then that refers to a synergistic effect. When the FIC index is equal to 1 then that refers to an additive effect, but when it is greater than 1, it has an antagonistic effect.

Mechanism of action

The test organism(s)' bacterium suspension was cultured in Mueller Hinton broth for 24 hours, while the plant extract concentration of the Minimum inhibitory concentration (MIC) was weighed, reconstituted with DMSO, and diluted according to the normal preparative process. In preparation for the Sodium, Potassium, and Protein measurement procedure(s), two millilitres of the produced bacteria suspension were mixed with two millilitres of the extract preparation in a tiny vial and centrifuged. A modified Maruna and Trinder colorimetric technique were used to estimate the salt content. Magnesium was prepared as Uranyl magnesium, and Sodium acetate salt was processed as uranyl acetate to precipitate sodium and proteins. Excess uranyl salts reacted with potassium ferrocyanide to produce a brownish tint. Photometrically, the colour intensity is measured at 530 nm (500 nm - 540 nm) and is inversely proportional to the content of sodium in the sample. The Turbidimetric technique is used to estimate potassium levels. Turbidity is proportional to potassium content and is measured photometrically at 578 nm (570 nm - 620 nm). For protein estimation, 1 ml of biuret reagent was pipetted into 10ltres of the sample, and the mixture was let to remain at room temperature for 10 minutes before testing the absorbance of both the reference and test samples against distilled water at 540 nm.

Protein target and ligand preparation

The crystallographic structure of *P. aeruginosa* resistant protein with ID 611A, a protein known to be responsible for the high-level resistance of *P. aeruginosa* to beta-lactam antibiotics such as penicillin, cephalosporins, and carbapenems was obtained at a minimal resolution from Protein Data Bank (https://www.rcsb.org) and the water molecules of these proteins were removed using BIOVIA Discovery Studio version 21.1. Chimera 1.16 was used to prepare these proteins for docking, while Discovery Studio version 21.1 and CASTp23 online tools were utilized to estimate the active site of each protein, which was then crosschecked against the target literature.

Active compound present in Azadirachta indica and Phyllanthus amarus from the GCMS analsysi was used as ligands, their structure was retrieved from PubChem. The 3D structures were obtained from the database in 3D SDF format and converted separately to PDBQT files using OpenBabel-3.1.0.

Molecular target

This was done using a computer application known as Swiss Target Prediction tool, it is a web-based tool for predicting the macromolecular target of a small bioactive molecule. It is based on the similarity principle, which states that two molecules with similar structures are likely to have comparable properties. In order to forecast the target of our hit compound, the canonical smile is entered and analysed in the search box.

Molecular docking

Using Autodock vina, the protease file for *Pseudomonas aeruginosa* resistant protein (611A) was created. The water molecule was eliminated, and polar hydrogen atoms and charges were introduced in its place. The file was then preserved for subsequent examination. The X, Y, and Z coordinates were determined using the grid box. AutoDock vina calculated the binding affinity using Lamarckian genetic algorithms. The conformations were ranked according to their energy [7]. The hydrogen bonds were viewed, and the structure was saved in pdbqt format before being converted to pdb format. PyMOL was utilized to visualize the 3D structure of the protease-ligand complex interaction, while Biovia Discovery Studio 2020 Client was used to visualize the 2D structure of the molecular interaction.

Results

Biochemical and morphological profile of *Pseudomonas aeruginosa*

Table 3 represents the morphological and biochemical profile of *Pseudomonas aeruginosa*, a bacteria isolated from urine samples. The bacteria are Gram-negative rod (cell shape) and appear opaque in the morphological test. In terms of biochemical tests, the bacterium is urease, indole,

and coagulase negative, while also catalase, citrate, oxidase and gas positive. The bacteria cannot ferment lactose, sucrose, glucose, galactose, fructose, and maltose, but it can utilize mannitol however this doesn't apply to citrate or starch. The bacteria appear rod in shape under the microscope but on plate it has a rough surface, irregular shape and an elevated edge on the media used.

Antibiotic susceptibility profile of *Pseudomonas aeruginosa* to conventional antibiotics

The table below provides information on the antibiotic susceptibility pattern of *Pseudomonas aeruginosa*, a multi-drug resistant type of bacteria, to conventional antibiotics. The susceptibility pattern is determined by measuring the zones of inhibition (ZOI), which represent the area of the bacterial growth inhibition around the antibiotic disk. The larger the ZOI, the more effective the antibiotic is against the bacteria. Ten different antibiotics were tested, and their ZOIs and susceptibility patterns are presented in the table. The results show that *Pseudomonas aeruginosa* is resistant to most of the antibiotics tested except one. The results show that among the antibiotics tested, pefloxacin was the most effective antibiotic in inhibiting the growth of *Pseudomonas aeruginosa*, with ZOIs of 29.83±1.01f. The bacteria were found to be susceptible (S) to these antibiotics. On the other hand, the bacteria showed resistance (R) to all the other antibiotics tested, including tarivid, gentamicin, augmentin, amoxacillin, sparfloxacin, streptomycin, chloramphenicol, ciprofloxacin, septrin, and erythromycin. Only peflloxacin was found to be effective against the bacteria.

Minimum inhibitory concentration, minimum bactericidal concentration and fractional inhibitory concentration

The table below provides the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and fractional inhibitory concentration (FIC) profile of Azadirachta indica and Phyllanthus amarus against multidrug-resistant uropathogens *Pseudomonas aeruginosa*. The study aimed to determine the concentration of the plant extracts required to inhibit the growth of the bacteria (MIC), kill the bacteria (MBC), and evaluate the potential synergy between the two extracts using FIC. The results showed that Azadirachta indica had higher MIC and MBC values compared to Phyllanthus amarus against *P. aeruginosa*, which were 300 to 200 mg/ml and 350 to 300 mg/ml,

respectively. This suggests that Phyllanthus amarus extracts has a higher potency in inhibiting the growth and killing of the bacteria compared to Azadirachta indica. Moreover, the study also evaluated the FIC of the extracts, which is an indicator of their potential synergy or antagonism. The FIC index of 0.83 suggests that the combination of Azadirachta indica and Phyllanthus amarus has a synergistic effect on the inhibition of *P. aeruginosa* growth. The interpretation of the results suggests that the combination of both Azadirachta indica and Phyllanthus amarus has a complimentary or synergistic inhibitory and bactericidal effects on *P. aeruginosa* at the tested concentrations.

Antimicrobial susceptibility profile of plant extract(s)

The table below shows the results of an antimicrobial susceptibility test comparing two plant extracts, Azadirachta indica and Phyllanthus amarus, against *Pseudomonas aeruginosa*, a multidrug-resistant uropathogen. The test was carried out using different concentrations of the plant extracts, and the results are expressed in millimeters (mm). The results show that both plant extracts exhibit antimicrobial activity against *P. aeruginosa*, with increasing activity observed at higher concentrations. At the lowest concentration tested (50 mg/ml), the Phyllanthus amarus extracts showed a much higher potency in antimicrobial activity against *P. aeruginosa* compared to A. indica which didn't show any zone of inhibition. At higher concentrations (100, 200, 300, and 350 mg/ml), both extracts showed increasing antimicrobial activity against the uropathogen with P. amarus proving to be more potent than A. indica with higher zones of inhibiton. Comparing the two plant extracts, P. amarus showed higher antimicrobial activity against *P. aeruginosa* than A. indica at all concentrations tested. At the highest concentration tested (350 mg/ml), P. amarus exhibited the highest antimicrobial activity, with a mean inhibition zone of 20.10±0.38 mm, while A. indica showed a mean inhibition zone of 18.10±0.15 mm.

Mechanism of action of Azadirachta indica and Phyllanthus amarus

Table 5 presents the results of testing for the mechanism of action of Azadirachta indica and Phyllanthus amarus using different parameters. The study aimed to investigate the extracts' potential mechanisms of action by measuring their effects on the levels of sodium, potassium, and protein in the

test organisms. The results show that Azadirachta indica and Phyllanthus amarus have different effects on the levels of sodium, potassium, and protein in the test organisms. Phyllanthus amarus extract has a higher concentration of sodium at 17.3 Mol/L compared to Azadirachta indica extract at 12.3 Mol/L. In contrast, Azadirachta indica extract has a higher concentration of potassium at 6.5 Mol/L compared to Phyllanthus amarus extract at 3.5 Mol/L.

Moreover, the study found that Phyllanthus amarus extract has a higher concentration of protein at 3.6 Mol/L compared to Azadirachta indica extract at 1.0 Mol/L. These differences in the effects of the extracts on sodium, potassium, and protein levels in the test organisms suggest that they may have different mechanisms of action. In conclusion, the results of this study suggest that Azadirachta indica and Phyllanthus amarus may have different mechanisms of action against the test organisms, as indicated by their differential effects on the levels of sodium, potassium, and protein. Further studies are needed to confirm these findings and elucidate the specific mechanisms of action of these extracts.

Molecular docking

The molecular docking result of **table (6)** shows that 18 different ligands present within Azadirachta indica were docked against the resistant protein, and their binding affinity was recorded as they were used to attack the 611A gene, encoded by the MexB resistant protein of *Pseudomonas aeruginosa*. The first docked ligand on this table with the best docking score is Azadirachtol with a binding affinity of -10.2kcal/mol while the last with the worst docking score is Nimbione with a docking score of -7.7kcal/mol, on the other hand, **table (2)** shows that 17 different ligands of Phyllanthus amarus were docked against the MexB resistant protein of *Pseudomonas aeruginosa*. The ligand having the best docking score from Phyllanthus amarus was oleanolic acid having a score of -9.8kcal/mol while the least is squalene having a docking score of -4.7kcal/mol. **Figure 2** shows the 2D and 3D images of the molecular binding structure of Azadirachtol (a ligand found in A. indica) and the MexB resistant protein. **Figure 3** shows the 2D and 3D images of the molecular binding structure of Oleanolic acid (a ligand found in P. amarus) and the MexB resistant protein.

Table 1. Molecular docking results of bioactive *A. indica* ligands against MexB Protein target (6IIA).

Ligand	Binding Affinity
Azadirachtol	-10.2
Quercitrin	-9.4
Azadirachtanin	-9.3
Epiazadiradione	-9.3
Epoxyazadiradione	-9.1
Meldenin	-9.1
Azadiradione	-9
Kaempferol	-8.9
Nimbocinolide	-8.8
Myricetin	-8.6
Beta-sitosterol	-8.5
Nelfinavir	-8.5
Nimbaflavone	-8.5
Nimbinene	-8
Salannolide	-7.8
Nimbione	-7.7

Table 2. Molecular docking results of bioactive compounds from *P. amarus* against MexB Protein target (6IIA)

Ligand	Binding Affinity
oleanolic acid	-9.8
Glochidiol	-9.1
Botulin	-9
phyllanthostatin A	-8.2
Phyllanthusiin E	-8.2
Phyllanthurinolactone	-8.1
Caryophyllene	-7.5
Hypophyllanthin	-7.4
Nirtetralin	-6.9
Phyltetralin	-6.6
Phyllanthin	-6
Mono(2-ethylhexyl) phthalate	-5.7
Hexadecanoic acid, methyl ester	-5.1
n-Hexadecanoic acid	-4.9
Squalene	-4.7

Table 3. Biochemical and morphological profile of *Pseudomonas aeruginosa* from urine sample

S/N	Biochemical test	Result	Morpholohical test	Result
1	Gram reaction	-	Opacity	Opaque
2	Urease	-		
3	Oxidase	+	Color on media	Pink growth on mannitol salt agar, creamy growth on nutrient agar.
4	Citrate	+		
5	Motility	+		
6	Indole	-	Media used	Blood Agar (BA), Macconkey Agar (MAC), Cystine Lactose Electrolyte Deficient Agar (CLED), Nutrient Agar (NA)
7	Catalase	+		
8	Coagulase	-		
9	Gas	+	Colony shape	Round
10	H$_2$S	-		
11	Mannitol	+	Elevation	Raised
12	Lactose	-		
13	Sucrose	-		
14	Glucose	-	Surface	Rough
15	Galactose	-		

16	Maltose	-		
17	Arabinose	-	Edge	Entire
18	Rhamnose	-		
19	Fructose	-		
20	Starch	-	Cell shape	Rod
21	Mannose	-		

Table 4. Antibiotic susceptibility pattern of *Staphylococcus epidermidis* to conventional antibiotics.

S/N	Type of antibiotics	Weight	Zones of inhibition (ZOI)	Susceptibility pattern
1	Septrin (SXT)	30µg	10.33±0.17[d]	R
2	Chloramphenicol (CN)	30µg	0.00±0.00[a]	R
3	Sparfloxacin (SP)	10µg	12.23±0.15[d]	R
4	Ciprofloxacin (CPX)	30µg	14.83±1.01[c]	R
5	Amoxacillin (AM)	30µg	12.00±0.00[d]	R
6	Augmentin (AU)	30µg	0.00±0.00[a]	R
7	Gentamycin (CN)	30µg	8.17±0.44[b]	R
8	Pefloxacin (PEF)	30µg	29.83±1.01[f]	S
9	Tarivid (OFX)	10µg	10.17±0.17[c]	R
10	Streptomycin (S)	30µg	15.00±0.58[f]	S

Table 5. Mechanism of action for Azadirachta indica and Phyllanthus amarus.

S/N	Parameters for testing for mechanism of action	Azadirachta indica	Phyllanthus amarus
1	Sodium (mol/l)	12.3	17.3
2	Potassium (mol/l)	6.5	3.5
3	Protein (mol/l)	1.0	3.6

Key: Molecule per litre (Mol/L)

Table 6. Antimicrobial susceptibility profile of *Azadirachta indica* and *Phyllanthus amarus* against multidrug drug resistance uropathogens *Pseudomonas aeruginosa*

S/N	Extract Concentration(mg/ml)	Azadirachta indica (mm)	Phyllanthus amarus (mm)
1	50	0.00±0.00[a]	12.00±0.58[b]
2	100	15.00±1.67[e]	14.60±0.58[c]
3	200	15.55±0.58[b]	17.00±0.67[e]
4	300	20.00±0.33[c]	18.80±0.67[c]
5	350	20.50±0.33[d]	20.50±0.33[c]

Key: 0–10=resistant, 11–13=intermediate,≥14=susceptible. Values are expressed in mean±SEM. Means of an extract without a common superscript letters difer P Mm= millimeter, mg/ml= milligram per milliliter

Figure 1. A- 2D molecular bonding structure of Azadirachtol and MexB resistant protein (611A) **B**-3D molecular bonding structure of Azadirachtol and MexB resistant protein (611A)
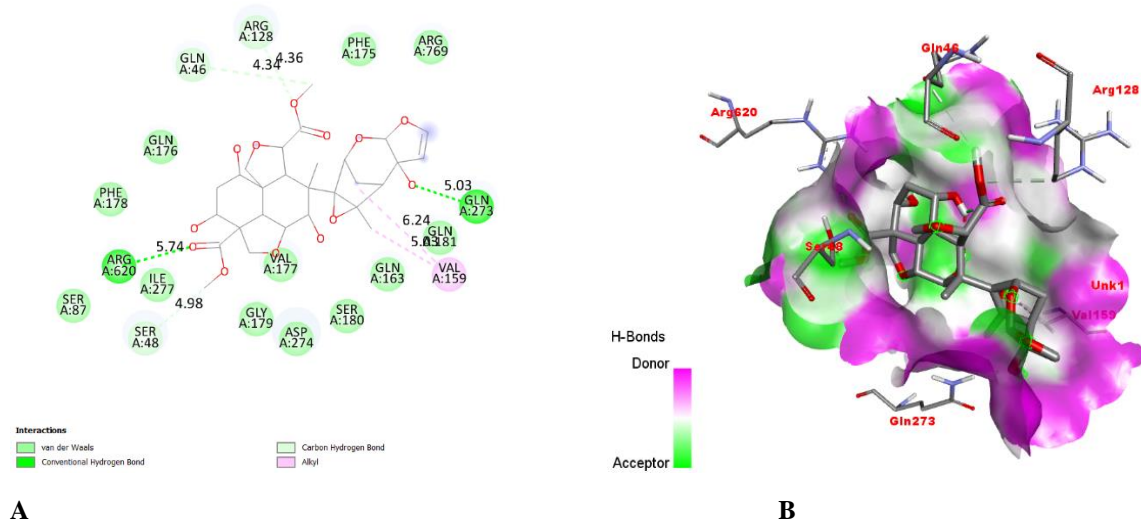

A   **B**

Figure 2. A- 2D molecular bonding structure of Oleanolic acid and MexB resistant protein (611A) B-3D molecular bonding structure of Oleanolic acid and MexB resistant protein (611A)
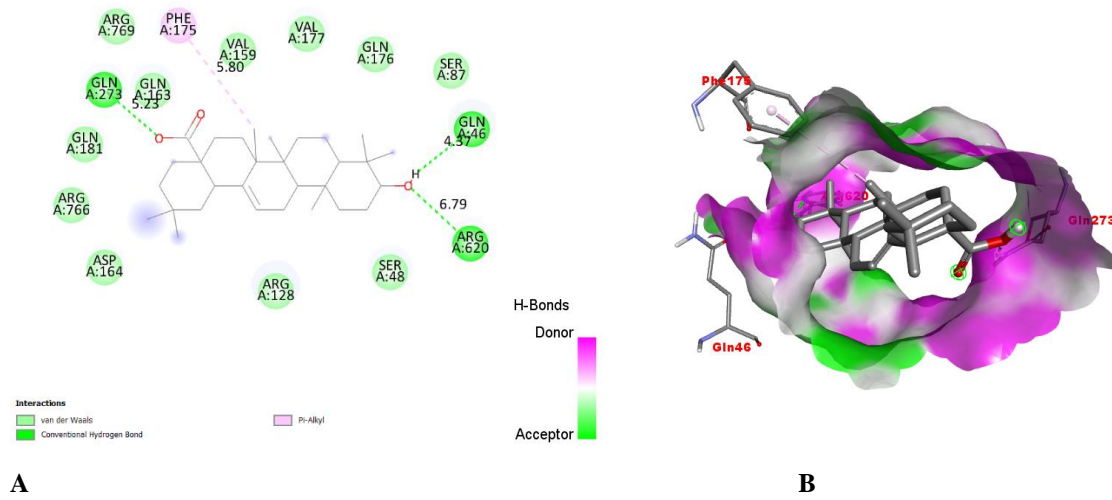

A   **B**

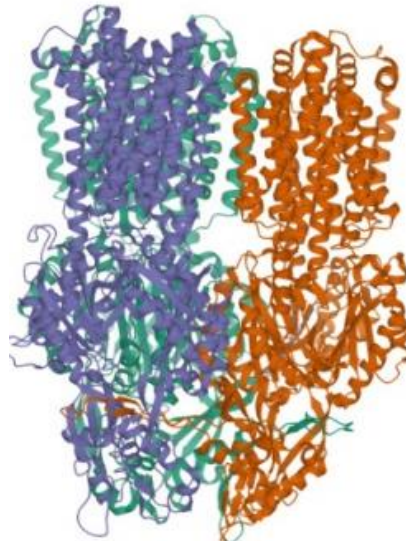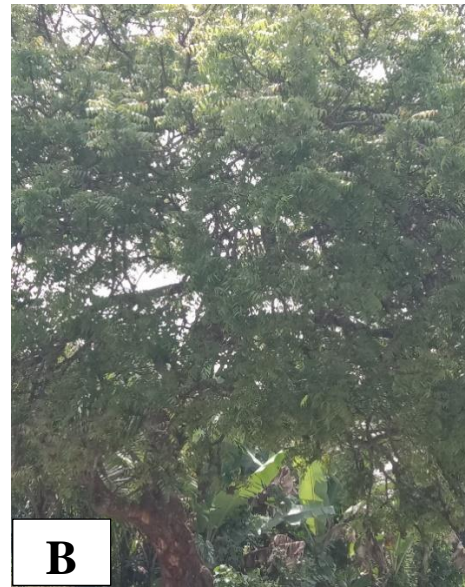**Figure 3. -**D structure of 611A target protein

Plate 1. (A) Phyllanthus amarus (B) Azadirachta indica (Dongoyaro tree)

Discussion

Pseudomonas aeruginosa is a gram-negative bacterium commonly associated with post operative wounds, but with recent research, more cases have linked *P. aeruginosa* [8] with Urinary tract infections [9]. As an opportunistic infectious agent, *Pseudomonas aeruginosa* can cause serious acute and long-term illnesses, especially in people who don't have strong immune systems [10]. Toun [11] noted that the spread of *P. aeruginosa* that is resistant to multiple drugs (MDR) has become a major problem in healthcare settings in developing countries. Certain authors [11,12] have found that these multidrug-resistant cases are linked to more deaths and higher costs due to longer hospital stays, the need for surgery, and longer treatment with drugs. Some studies [11] have shown that treating bacteria with subinhibitory concentrations (subMIC) of antibiotics can change their virulence factors, such as their ability to stick to surfaces, make biofilms, move around, and be sensitive to oxidative stress. This study is unique in many ways, and the ingenuity of the research was portrayed in scantiness of literature to back up some of the findings, The combination of A. indica and P. amarus revealed that they act in synergy with each other and this is portrayed by its Fractional inhibitory index result of 0.83. Another unique finding from this study revealed that P. amarus with a lesser MIC and MBC value of 200mg/ml and 300mg/ml respectively compared to the 300mg/ml and 350mg/ml of A. indica showed that P. amarus has a higher in-vitro antimicrobial effect, however

because of the uniqueness of the work, there aren't literatures that show a combination of both extracts. This result is further proven when the antimicrobial potency of both extracts were subjected to test on the uropathogen, the result showed that P. amarus with 20.10±0.38d Zone of inhibition has a greater effect on the uropathogen as compared to A. indica with 18.10± 0.15e Zone of inhibition. A study done by [12] showed that Doripenem was highly effective in inhibiting the uropathogen – *P. aeruginosa*, [13] which revealed low MIC values at which inhibition was accomplished, although an invitro study wasn't carried out on Doripenem, however, the high MIC values of the extract could be said to be due to the presence of impurities which if removed should give a much lower MIC scores. Also, all indication points to the fact that these plant extracts have a very significant potential to replace the conventional UTI antibiotics. This study through in-silico means, investigated the effect that Phyllanthus amarus and Azadirachta indica would have on the Mex B resistant protein of *Pseudomonas aeruginosa*. Findings from the in-silico study revealed that there were many important ligands within Azadirachta indica [14] and Phyllanthus amarus that were able to properly bind with the resistant protein from *Pseudomonas aeruginosa* and effectively inhibit or further kill their effect. Out of the 18 ligands of A. indica that were docked, about 14 of them were giving binding affinity scores lower than -8.0kcal/mol. In a molecular docking study carried out by Sharon [15] on A. indica, most of the not much lower than -8.0, compared to this study where

about 9 ligands had binding affinities ranging from -9.1kcal/mol and lower, indicating a very effective binding capability. These 9 include, Azadirachtol (-10.2kcal/mol), Quercitin (-9.4kcal/mol), Azadirachtanin (-9.3kcal/mol), Epiazadiradione (-9.3kcal/mol), Epoxyazadiradione (-9.1kcal/mol), Meldenin (-9.1kcal/mol), and Azadiradione (-9kcal/mol), findings from [16] also showed the rarity in Phyllanthus amarus having such effective binding ligands, however, the extremely outstanding binding affinity scores of Oleanolic acid (-9.8kcal/mol) coupled with those of Glochidiol (-9.1kcal/mol) and Botulin (-9kcal/mol) goes ahead to prove how effective a combination of A. indica and P amarus would be in tackling *Pseudomonas aeruginosa* multidrug resistance, especially the 611A resistance gene, also to make this even more interesting, when the results were compared to that of an FDA approved drug known as Doripenem with a binding affinity of -7.0, this gives a definite prove of the immense potential that these plants and their ligands carry, as they gave way higher docking scores than Doripenem, [17], although further experimentation still need to be carried out on these ligands to ensure their safety to humans and their consumability and also to check if they are extractable and to what extent. [24,25] To complement the in-silico studies, the Fractional inhibitory concentration has proven that the combination of both A. indica and P. amarus had a synergistic or complementing effect. Studies by [18] has proven the effectiveness of P. amarus against multidrug resistant pathogen in an invitro setting. The antimicrobial studies revealed that P. amarus with an inhibition zone of 20.10±0.38mm at 350mg/ml concentration) and a MIC of has a better effect on *Pseudomonas aeruginosa* as compared to A. indica and this is in contrast with the insilico study where-in A. indica seem to have the upperhand. [19] demonstrated in his study, the antimicrobial property contained within the neem tree was then revealed.

The reason for the above is that, the in-vitro study was designed in a way that all the biochemical compounds (ligands) inherent in the plant extracts were used at once as an antimicrobial agent to fight the uropathogen and in that study P. amarus had the upper hand, however in comparism, the in-silico study went deeper and analyzed the effect of each of the compounds present within the plant extracts and this revealed that A. indica had the compound with the best docking score, meaning that if the compounds were not separated and were band together, P. amarus would give a better output in inhibiting or killing the uropathogen but when each compound was analyzed as a single entity, the compound that had the highest docking score was from A. indica.

In conclusion

A. indica and P. amarus are a very interesting combination which could have far reaching advantages especially when combined and exposed to rigorous testing and experimentation, there is a great chance of a super drug being produced to combat the menace and influence of multidrug resistance. In other words, A. indica and P. amarus are could be the answer to multidrug resistance.

Conflict of interest

Authors declare no conflict of interest.

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