Research Article

iMedPub Journals
www.imedpub.com

Archives of Clinical Microbiology
ISSN 1989-8436

2020
Vol.11 No. 4:1000117

DOI: 10.36648/1989-8436.11.4.117

Molecular Typing of 16S rRNA of *Azadirachta indica* and *Ocimum gratissimum* Resistant *Pseudomonas spp.* from Sabe in Oke-Ogun, Nigeria

Adeoti OM[1,2,5*], Olaleye OM[1], Adeoye KA[1,4]. Adesina DA[3], Olaoye OJ[1] and Adeoye BA[6]

[1]Department of Science Laboratory Technology, The Oke-Ogun Polytechnic, Oke-Ogun, Saki Oyo State, Nigeria

[2]Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

[3]Department of Microbiology and Botany, University of Ibadan, Ibadan, Nigeria

[4]Department of Zoology, Parasitology Unit, University of Ibadan, Ibadan, Nigeria

[5]Department of Zoology, Cellular Parasitology Unit, University of Ibadan, Ibadan, Nigeria

[6]Leeds City University, Ibadan, Nigeria

[*]**Corresponding author**: Adeoti OM, The Oke-Ogun Polytechnic, Saki Oyo State Nigeria, Department of Science Laboratory Technology, Oke-Ogun, Nigeria**,** Tel: +08067285175; E-mail: txy23m@yahoo.com

Received date: July 09, 2020**; Accepted date:** July **23**, 2020**; Published date:** August 01, 2020

Citation: Adeoti OM, Olaleye OM, Adeoye KA, Adesina DA, Olaoye OJ, et al. (2020) Molecular Typing of 16S rRNA of *Azadiracht indica* and *Ocimum gratissimum* Resistant *Pseudomonas spp.* from Sabe in Oke-Ogun, Nigeria. Arch Clin Microbiol Vol. 11 No. 4:117

Abstract

The aim of this study was to present the molecular typing of bacteria which were resistant to extract of the leaves of *Ocimum gratissimum* and *Azadirachta indica* and to investigate whether there was any relationship between non-sensitivity to antibiotics and the extract of plants of four isolates: *Salmonella typhii, Escherichia coli, Pseudomonas aeruginosa and Pseudomonas monteilii*. Four solvents used for the extraction of the active ingredients of the plants were Ethanol, N-Hexane, Water, Ethanol and Water combined in five different concentrations.

The four microorganisms listed above were isolated from raw milk, Borehole water and soil. The isolates were variously subjected to conventional morphological, biochemical tests and subsequently molecular screening (Polymerase Chain Reaction (PCR) and Sanger sequencing method). *Pseudomonas aeruginosa* (KF530797) and *Pseudomonas monteilii* (KJ676707) identified were subjected to BLAST using pairwise alignment technique from NCBI database. The two plant extracts had earlier been documented as resistant to *Escherichia coli* and *Salmonella spp.* which were earlier isolated from milk and water, respectively.

There was no zone of inhibition as observed in all concentrations of plant extract considered except a noticeable clear zone of inhibition against synthetic antibiotics in the order of Ofloxacin and Gentamycin. Although suspected gene markers from the two-soil isolate were suspected to be amplified around 1400 bp. The 16S rRNA sequences of *Pseudomonas aeruginosa* (94.43%) was somewhat more variable than *Pseudomonas monteilii* (99.93%) indicating a pairwise alignment of Pseudomonas monteilii to be more conserved than *P. aeruginosa*.

Keywords: Pseudomonas monteilii; BLAST; Antimicrobial; Pairwise-alignment; Gene Markers

Abbreviations:

BLAST: Basic Local Alignment Sequence Tools

Introduction

The ingenuity of chemists has produced many new potent antimicrobial agents, but resistant bacterial strains have consistently emerged. Many authors have reported that plants contain a largeform of bioactive constituents [1,2]. Neemmay be a well-known plant that originated from Bharat and its neighbouring countries as way back as thousands of years ago [3]. It is thought to be one amongst the foremost helpful medicinal plants with a large spectrum of biological activity. Neem tree derived its name from 'Arishtha' that is translated as 'reliever of sickness' and therefore it is considered as 'Sarbaroganibarini' in Bharat. The primary bitter compound isolated from Melia Azadirachta oil, with overa hundred thirty-five compounds are isolated from completely differentcomponents of neem; these aremultifariouslyinvolved as antimicrobials against some organisms: *Eubacteriummutans* and *S. faecalis.* Oil from *Melia Azadirachta* leaves, seeds and bark possesses a large spectrum of bactericide action against microorganisms, as well asMycobacteriumand antibiotic resistant strains. This plant manufacturea largeform of

secondary metabolites thatare used either directly as precursors or as lead compounds within the pharmaceutical business and it is expected that plant extracts showing target sites are active against drug resistant microbial pathogens [4,5]. Ocimum gratissimum is a scented ligneous plant with lime-inexperienced leaves [6]. Ocimum gratissimum is employed through geographical region as anti-protozoal infection. The crushed leaf juice is employedwithin the treatment of convulsion, abdomen pain and redness. Oil from the leaves are found to possess antiseptics, bactericide, and antifungal activities [2]. The plant is highly consumed by the Igbos as a foliaceous vegetables and therefore theorganic process importance of this plant centre on its utility as a seasoning because of its aromatic flavour. It isconjointlyemployed by the Igbos within the treatment of the baby's wire. Pseudomonas is Gram-negative ubiquitous microorganism with extreme metabolic versatility [7]. *P.aeruginosa* is known to have colonized several environmental niches and is able to persist and grow under intense poor-nutrient and inhospitable habitat [8]

Although the 16S rRNA gene is the basic tool of the current bacterial classification system, it is known that closely related species of bacteria cannot be differentiated based on this gene. Therefore, over the past 10 years, other gene sequences have been used as phylogenetic molecular markers in taxonomic studies [9-11], such as atpD, gyrB, rpoB, recA, and rpoD Mulet and collaborators have demonstrated that the analysis of the sequences of four housekeeping genes (16S rRNA, gyrB, rpoB, and rpoD) in all known species of the genus clarified the phylogeny and greatly facilitated the identification [12,13]. The multilocus sequence analysis (MLSA) approach based on the sequence analysis of the four housekeeping genes has proven reliable for species delineation and strain identification in Pseudomonas [14].

Justification

The use 16S rRNA gene sequences as housekeeping molecular markers cannot be over emphasized varying from evolutionary and taxonomic importance. Bacterial 16S rRNA genes contain nine hypervariable regions (V1-V9) that indicates considerable sequence diversity among different microorganisms. The present study presented another opportunity to explore 16S rRNA analysis of antimicrobial resistance to local herbs vis-a-viz their phylogenetic relatedness of two strains of Pseudomonas isolated in the rhizosphere of the two herbal plants.

Materials and Methods

Collection of plants samples

The plant leaves of both *Ocimum gratissimum*(scent leaf) and *Azadirachta indica* were collected from a farm settlement at Sabe community in ATISBO Local Government Area of Oyo State in Southwest region of Nigeria.The two plantswere sent for identification in the Department of Biological sciences, University of Ibadan, Oyo State. The residual moisture was evaporated at room temperature thereafter the fresh leaves samples could air dry completely for two weeks at a room temperature before used them for this study **(Figure 1)**.

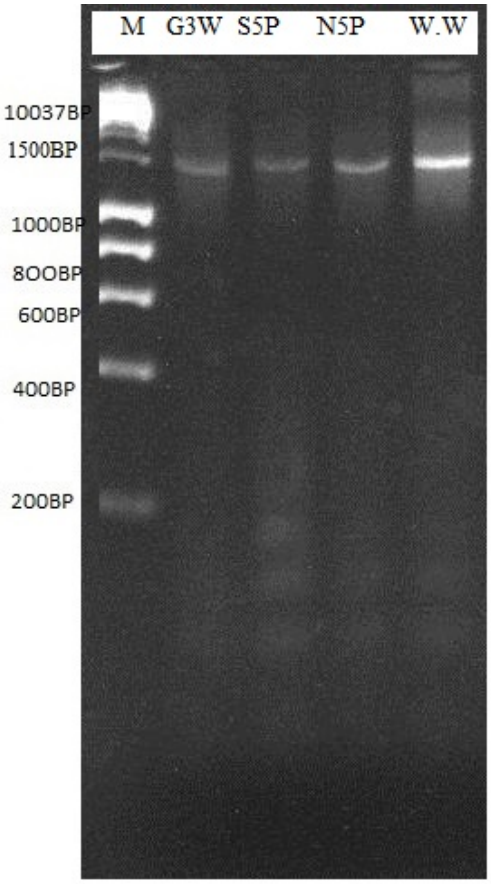

Figure 1: Gel electrophoresis purification of Polymerase Chain Reaction amplicon's on representatives' bacteria isolate from soil (S5P and N5P) using Pseudomonas aeruginosa and Pseudomonas monteilii.

Sample preparations and extraction

The air-dried plant sample was grinded using pestle and mortar into a powdered form and was thoroughly sieved through 2 mm sized mesh sieve and was later stored in plastic containers.

Biochemical analysis

Gram staining procedures: Using wire loop to inoculate the bacterial colony by preparing a smear which was prepared and fixed on clean glass slide the stained with crystal violet for 30 seconds before the smear was washed with distilled water. Gram's Iodine was added thereafter for 10 seconds then the smear was washed with tap water and decolorized with 95% Acetone alcohol and Safranin which is a secondary dye was finally added to the smear for 30 seconds in tap water. This

This article is available from: 10.36648/1989-8436.11.4.117

was air dried and mounted to be observed under oil immersion objective 100X.

Oxidase test

The reagent was prepared according to manufacturer's instruction. A 2-3 drops of freshly prepared oxidase reagent was placed on to the filter paper contained in a Petri dish. Using a wooden stick some of the growth of test organism grown on non-selective agar (i.e. nutrient agar) was smear-flooded onto the treated filter paper. Those bacteria which gave the positive result by changing the filter paper into deep blue purple colour within 10 second were reported as oxidase positive modification of the [15,16].

Identification of isolates

Isolates from primary cultures was incubated at ± 37°C-44°C) were aseptically subcultured on to fresh Agar media (EMB, MacConkey agar, Simon's Citrate, Centrimid, SSA and Nutrient agar) in at least five successive generations. The resultant pure isolates were subcultured into already prepared slant bottles for the purpose of identification and characterization. At this level, cultural characteristics, and appropriate biochemical identification such as oxidase, catalase, urease, indole, and citrate were used to determine the production and utilization of some various enzymes **(Table 1)**.

Table 1: The mean of *Azadirachta indica* and *Ocimum gratissimum* of Organism used *Pseudomonas aeruginosa*.

Conc. (µM)	Ethanol	N-Hexane	Ethanol + aqueous	Aqueous
25	9.67 ± 10.00	10.00 ± 11.00	8.33 ± 11.67	10.00 ± 11.33
20	8.33 ± 8.33	8.67 ± 9.33	7.67 ± 6.67	8.33 ± 9.33
15	7.67 ± 7.33	8.00 ± 7.00	5.00 ± 7.00	5.00 ± 7.00
10	5.00 ± 6.00	4.33 ± 6.00	4.00 ± 5.00	4.00 ± 5.00
5	3.33 ± 4.00	3.67 ± 4.33	3.33 ± 3.67	3.33 ± 4.33

Isolation of the plants Rhizosphere microorganisms

The soil sample was serially diluted in distilled water under the aseptic conditions under laminar air flow chamber. Serial dilutions were made up into the ratio of 10^{-3} and 10^{-5} with 0.1 ml of each dilution poured on different sterile Petri dish containing antibacterial agent (50 µg/ml Streptomycin) [17].

Table 2: The mean of *Azadirachta indica* and *Ocimum gratissimum* of Organism used *Pseudomonas monteilii*.

Conc. (µM)	Ethanol	N-Hexane	Ethanol + aqueous	Aqueous
25	10.00 ± 10.33	11.67 ± 11.67	8.33 ± 10.33	11.67 ± 11.00
20	9.33 ± 10.00	8.33 ± 10.00	6.67 ± 9.33	8.00 ± 9.67
15	8.00 ± 9.00	7.00 ± 9.00	6.33 ± 9.00	7.00 ± 8.00
10	7.33 ± 8.33	5.00 ± 6.67	5.67 ± 7.67	5.00 ± 6.33
5	5.67 ± 6.67	3.67 ± 5.00	4.33 ± 5.67	4.33 ± 4.67

Nutrient Agar (NA) was sterilized in the autoclave at 121°C for 15 minutes. It could cool for 5 minutes and then poured on the Petri dishes which was initially filled with 0.1ml of d diluted samples. Morphologically, different bacteria colonies were separated by inoculating each colony on the different selective media such as Eosin Methylene Blue Agar (EMB), MacConkey Agar, Centrimid, and Salmonella Shigella Agar to maintain the pure culture **(Table 2)**.

Molecular Characterization

DNA extraction

Fungal/Bacterial DNA Miniprep. (Manufactured by Zymo research cat number: D6005)2 millilitres of bacterial cells broth were added to a ZR Bashing TM Lysis Tube. Then 750 µl Lysis Solution to the tube. And then it was secured in a bead fitted with 2 ml tube holder assembly and was processed at maximum speed for maximum of 5 minutes. The ZR Bashing Bead TM Lysis Tube was centrifuged in a micro-centrifuge at >10,000 xg for 1 minute and then transferred up to 400 µl supernatant to a Zymo-SpinTM IV Spin Filter (orange top) in a Collection Tube and also centrifuged at 7,000 xg for 1 minute. Add 1,200 µl of Fungal/Bacterial DNA Binding Buffer to the filtrate in the Collection Tube and Transfer 800 µl of the mixture to a Zymo-SpinTM IIC Column in a Collection Tube before centrifuged at 10,000 xg for 1 minute. The flow through from the Collection Tube was discarded and repeated again as from above, Add 200 µl DNA Pre-Wash Buffer to the Zymo-Spin TM IIC Column in new Collection Tube and centrifuged at 10,000 xg for 1 minute then add another 500 µl Fungal/Bacterial DNA Wash Buffer to the Zymo-SpinTM IIC Column and centrifuged at 10,000 xg for 1 minute then transferred the Zymo-SpinTM IIC Column to a clean 1.5 ml microcentrifuge tube and add 100 µl (35 µl minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 xg for 30 seconds to elute the DNA **(Table 3)**.

Table 3: Antibiotics Sensitivity profile of *Pseudomonas aeruginosa*.

Antibiotics (µg)	10^{-3}	10^{-5}	10^{-7}	10^{-9}
Ceffazidine (CAZ)	R	R	R	R
Cefuroxine (CRX)	R	R	R	R
Gentamicin (GEN)	13(I)	14(I)	12(S)	17(S)
Ceftriazone (CTR)	R	R	R	21(S)
Erythromycin (ERY)	R	R	R	R
Cloxacilin (CAC)	R	R	R	R
Ofloxacin (OFL)	23(S)	20(S)	23(S)	29(S)
Augmentin (AUG)	R	R	R	R
Resistance (R) Ranges from (0-11), Susceptible (S)<15, = Intermediate (12-14).				

Gel Electrophoresis for DNA and PCR analysis for band visualization

Measure 1 g and 2 g of agarose (for DNA) and PCR respectively. Agarose powder was mixed with 100 mL 1 × TAE in a microwavable flask and microwaved for 3 minutes until the agarose was completely dissolved (but do not over boil the solution, as some of the buffer will evaporate) and thus alter the final percentage of agarose in the gel then wait till the agarose solution cool down to about 50°C (about when you can comfortably keep your hand on the flask) for about 5 minutes.

Table 4: Antibiotics Sensitivity profile of *Pseudomonas monteilii.*

Antibiotics (µg)	10^{-3}	10^{-5}	10^{-7}	10^{-9}
Ceffazidine(CAZ)	R	R	R	R
Cefuroxine(CRX)	R	R	R	R
Gentamicin (GEN)	13(S)	15(S)	10(I)	12(S)
Ceftriazone (CTR)	R	R	R	21(S)
Erythromycin (ERY)	R	R	R	R
Cloxacilin (CAC)	R	R	R	R
Ofloxacin (OFL)	25(S)	14(S)	22(S)	23(S)
Augmentin (AUG)	R	R	R	R
Resistance (R) Ranges from (0-11), Susceptible (S)<15 = Intermediateranges from (12-14).				

Then add 10 µL Ethidium Bromide was added to visualize the DNA stained. EtBR2 binds to the DNA and allowed to visualize the DNA under ultraviolet (UV) light. The agarose was poured into a gel tray with the well comb in place and was placed into newly poured gel at 4°C for 10^{-15} minutes **(Table 4)**.

Loading samples and running an agarose gel

Add loading buffer to each of the DNA samples or PCR products once it is solidified, place the agarose gel into the gel box (electrophoresis unit). Then gel box was filled with 1 x TAE (or TBE) until the gel was completely covered. Carefully, load a molecular weight ladder into the first lane of the gel before loading the samples into the additional wells of the gel to run the gel at 80-150 V for about 1-1.5 hours then turn OFF the power button. The electrodes from the power source was disconnected immediately, and then carefully removed the gel from the gel box to visualize DNA fragments or PCR product under UV trans-illuminator.

PCR mix components

The PCR mix was made up of 12.5 µL of Taq. 2X Master Mix from New England Bio labs (M0270) containing 1 µL each of 10 µM forward and reverse primer; 2 µL of DNA template and then made up with 8.5 µL Nuclease free water. The Primer Sequences used for Bacterial identification 27F: AGAGTTTGATCMTGGCTCAG1525R: AAGGAGGTGWTCCARCCGCA. The initial denaturation at 94°C for 5 minutes was followed by 36 cycles of denaturation at 94°C for 30 seconds, at annealing temperature of 55°C for 30 seconds while elongation was at 72°C for 45 sec. Followed by a final elongation step at 72°C for 7 minutes and hold temperature at 10°C forever.

16S rRNA sequencing techniques

DNA sequencing method was performed by using Sanger sequencing techniques for confirmative detection of pathogenic strains of *Pseudomonas spp.* based on 16SrRNA gene. The purified 16srRNA gene in PCR product samples was sent to Gen Lab for onward transmission according to the protocol described by [18].

BLAST

The resulting genomic sequences were assembled and submitted in GenBank-NCBI then multiple sequence pairwise alignment search tool for phylogenetic tree construction and phylogenetic analysis by using MEGA 5.2 software.

Statistical analysis

Statistical analysis was performed using the software Statistical Package for Social Sciences (SPSS) The disc diffusion values of different concentrations of *Azadirachta indica* and *Ocimum gratissimum* leaves extract, positive against all the bacteria were entered in the SPSS software for statistical analysis. Descriptive statistics was retrieved, and data were analysed using one-way analysis of variance (ANOVA), while Tukey post-hoc test was used for comparison within the group and with different groups at the statistical significance level set at $P<0.05$.

Sample code: NSP

Identified organism: Pseudomonas aeruginosa strain P1

% Pairwise identity: 82.30%

NCBI accession number: KF530797

E value: 3.29E-07

This article is available from: 10.36648/1989-8436.11.4.117

Isolate sequences

GGCGGTATACTATGATGACATGGCTCGGTGCGGTTGGATCCCTCC
TAATATTTTGACCAGAGGCCAGTGGATGCCTATGACTCTACCAGGGC
CTGGGGGACGGCGTTTCCTAAGGGAGCTGATACCGCTTACGTCCTA
CGGCACAAAACAGGGGACCTTCACCTTTTGCCCTCTCGCATGAGCA
TGGGTCCATTTAGCTTTTTGTTGGGGTGGGGCTCACCATCGCCTCG
ATCACTATCTGTTCTTAGTCTATGATCATTCCCACTGACACTGAAACT
GGGACAAGACCCCTACTCCTGGCGGAGGTGGCGAATATTGATATTT
CGCCAAAAGCCTGATCCTGACGCGCCCCTGGCGTGAATGTGGTCAT
CGCCTTCAAATTCACTTTAACTTGCGGCGAAGAGCAGGGAGTTAAT
ACCTTGCTGTTTTGACTTTAACGACACTATAAGAACAAGCTAACTCT
GTCTCCGCGCCCGCGGCCATACTAATGGGGGCAAGTGTTAATCTTA
ATTACAGTTCCGTAAACGCTCCCCCCCCGTGCTTTTGTTTTTTTTAAA
TGTAGATACCCACGTGCCTCCGGGCTTGAACACGTGTTTCCGTATTT
TGTCACTGCTAGAGCTCCAGTACTCGGCAGAGGGGATTTTTTCCTTT
TCCCGCGGTATCACTGCAATATATATCACAGATGGAACCACCAATCCC
CCGAGGGCCACCCCCCTCCCCCCTGATACCATACACTGACGTCCCAG
ACGCCGGTGGGGGGCGCCACAAGATTTATTTAGCATTTCCCTGGCC
CTCCCTCCGCTCCCACGAGCGGATACCCCCTCTCTCCACACTTGTGG
AAATTCTCCTTTGCGACAATTTTTTGACTCGTGGCTTCACGAGAACG
C

Sample code: S5P

Identified organism: Pseudomonas monteilii strain DPA clone b

% Pairwise identity: 93.30%

NCBI accession number: KJ676707

E value: 0

Isolate sequences

GGTAGATATGTGGACATGGCTCAGGTGCGGTGGGACACCCCTTT
ATAGTTTGACGGCCTCAGGGGGGCGGGAAAACCCCTTAAAATATTG
GGCGACCCCCTTTCGAAAGGAACGCTAATACCTAATACGTCCTACG
GGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCT
AGGTCGGATTAACTAGTTGGTGGGGTAATGGCTCACCAAGGCGAC
GATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAG
ACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGA
CAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAG
GTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAA
GTTAATACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCT
AACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTA
ATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTT
GGATGTGAAAGCCCCCGGGCTCATCCTGCGAACTGCATCCAAAAAC
TGGCAAGCTAGCAGTACGGCTAGAGGGGTGGTGGAATTTCCCTGT
GTAGCGGTGAATGCGTAGATATAGGAAGGAACACCAGTGGCGAGG
CGACCACCCTGCACTGAATACTGACTACTGAGGTGGCGAAGCGTGG
GGGAGCAAACAAGCATGAGATACCCTTGGTAGTTCCCACCGCCCGT
CAGAACGGATGGACAACTTAGGCCGTAGGAAGTCCCTCGGAAGAT
GTTCAGTGGCAACGCTCAAGGTTGAAATCGCGCC

Results and Discussion

This study examined the antimicrobial activity based on the active components of fresh leaves of *Ocimum gratissimum* and *Azadirachta indica* [3-6] on Pseudomonas [7,8]. The zone of inhibition observed in this study is comparable to those obtained in other studies. This study showed that N hexane, ethanol and aqueous leaf extracts of *Ocimum gratissimum* and *Azadirachaindica* were resistant to the bacterial isolates.

In this study, the leaf extract did not show any inhibitory activity against any of the gram-negative bacteria. This could probably be due to the biochemical differences in the structure within the cell components of bacteria. The solvents used in this study was consistent with earlier studies [17-20]. The ethanol, aqueous, N-hexane extract of *Azadirachta indica* leaves were with different concentration (25, 50, 75, 100 g/ml) respectively against the bacteria of *E.coli* and *Salmonella spp* respectively in the present observation. This extract of *Azadirachta indica* showed no zone of inhibitory on extract but the control of each only inhibited, the statistical analysis of the extracts was not significant to inhibit the bacteria isolates. The ethanolic extract of the leaves of *Ocimium gratissimum,* used in traditional medicine for the treatment of several ailments such as urinary tract, wound, skin and gastrointestinal infections, was evaluated for its antibacterial properties against four clinical bacteria isolates namely: *Escherichia coli, Proteus mirabilis, Staphylococcus aureus and Pseudomonas aeruginosa* and the antifungal properties *[21]. A. indica* leaves have been reported to possessed good anti-bacterial activity and this lead [13] to conclude it confirmation as a great potential of bioactive compounds of primary health care [21]. Plant essential extracts have been used for many resins, thousands of years, in food preservation pharmaceuticals, alternative medicine and natural therapies. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. The studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness (control) varied [22].

Table 5: 16S rRNA sequence variability values based on partial sequence comparison of Pseudomonas aeruginosa.

Strains	% Similarity based on partial sequence of 16S rRNA	
Pseudomonas aeruginosa	KF530797.1	100
Bacterium Strain GXII	KF96438.1	94.43
Bacterium strain AS16	MN096674.1	94.28
P. mosselii strain BFPB7816S	EF600876.1	94.28
Bacterium strain F7-2	KT759002.1	94.27
Endophytic bacterium 126P-3	JF901369.1	94.08

The findings of this study donot agree with that of several researchers that demonstrated *Azadirachta indica* and *O. gratissimum* extracts have an antibacterial activity against several species of bacteria. Therefore, the efficacy of these extracts as reported the plant extract contradict the study of this [22-26]. May be due to the age of the plant, solvent extraction, extraction method and the period of harvest of plant materials. The traditional identification of bacteria based

on phenotypic characteristics is generally not as accurate as identification based on genotypic methods **(Tables 5 and 6)**.

Table 6: 16S rRNA sequence variability values based on partial sequence comparison of Pseudomonas monteilii.

Strains	% Similarity based on partial sequence of 16S rRNA	
Pseudomonas monteilii	KJ676707.1	100
P. putida	LR134299.1	99.93
P. spp ASDP1	KU375114.1	99.93
Bacillus spp SKM33	LM6553116.1	99.93
*P. monteilii*strain 170620603RE	CP043396.1	99.86
Bacterium B22-1	KC709506.1	99.79

Conclusion

The sensitivity of gram negative bacteria on the two organism used for this study are coded with N5P and S5P which is known as *Pseudomonas monteilii* and *Pseudomonas aeruginosa* after being taken for their sequencings which were showed that Gentamycin (GEN) and Ofloxacin (OFL) were susceptible to the bacteria. The observed sequence variability between the two species of Pseudomonas was still another testimonial of the fact that *Pseudomonas monteilii* and aeruginosa are still far apart comparing of 99.93% to 94.28%. The result confirmed the earlier studies.

Conflict of Interests

The authors declared that there is no conflict of interest whatsoever

References

1. Cowman MN (1999) Plant products as antimicrobial agents. Clinical Microbiology Review 12:564–582.
2. EdeogaHO, Okwu DE, MbaebieBO (2005) Phytochemical constituents of some Nigerian Medicinal Plants. Afr J Biotech 4:685-688.
3. AdebajoAO, Adewunmi CO, Essien EE (1983) Anti-infective agent of higher plants. International Symposium of MediumPlants. 5th edn. University of Ife, Nigeria: 152-158.
4. Abdullahi M, Muhammed G, Abdulkadir NU (2003) Medicinal and Economic plants of Nupe land, 1st edn, Jube Evans publisher, Bida, Niger state, Nigeria, 139.
5. Idris LO, Uzoamaka N (2006) Phytochemical screening and anti-inflammatory evaluation of methanolic and aqueous extracts of Euphorbia heterophylla. Afri J Biotech 5:529-531.
6. Adegboye MF, Akinpelu DA, Okoh AI (2008) The bioactive and phytochemical properties of Garcinia kola seed extract on some pathogens. African J Biotech 7:3934-3938.
7. Hilario E, Buckley TR, Young JM (2004) Improved resolution on the phylogenetic relationships among Pseudomonas by the combined analysis of atpD, carA, recA and 16S rDNA. Antonie van Leeuwenhoek 86:51-64.
8. Frimmersdorf E, Horatzek S, Pelnikevich A, Wiehlmann L, Schaumburg D (2010) How Pseudomonas aeruginosa adapts to various environments: a metabolomics approach. Env Microbiol 12:1734-1747.
9. Yamamoto S, Harayama S (1998) Phylogenetic relationships of Pseudomonas putida strains deduced from the nucleotide sequences of gyrB, rpoD and 16S rRNA genes. Int J SystemBacteriol 48:813-819.
10. Tayeb L, Ageron E, Grimont F, Grimont PAD (2005) Molecular phylogeny of the genus Pseudomonas based on rpoB sequences and application for the identification of isolates. ResMicrobiol156:763-773.
11. Tayeb LA, Lefevre M, Passet V, Diancourt L, Brisse S, et al. (2008) Comparative phylogenies of Burkholderia, Ralstonia, Comamonas, Brevundimonas and related organisms derived from rpoB, gyrB and rrs gene sequences. Res Microbiol 159:169-177.
12. Mulet M, Gomila M, Scotta C, Sánchez D, Lalucat J, et al. (2012a). Concordance between whole-cell matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry and multilocus sequence analysis approaches in species discrimination within the genus Pseudomonas. Systematic Applied Microbiol35:455-464.
13. Sánchez D, Mulet M, Rodríguez AC, David Z, Lalucat J, et al. (2014) Pseudomonas aestusnigri sp. nov., isolated from crude oil-contaminated intertidal sand samples after the Prestige oil spill. Systematic Applied Microbiol 37:89-94.
14. Mulet M, Gomila M, Lemaitre B, Lalucat J, García-Valdés E (2012b) Taxonomic characterization of Pseudomonas strain L48 and formal proposal of Pseudomonas entomophila sp. nov. Systematics and Applied Microbiology 35:145-149.
15. Yamamoto S, Kasai H, Arnold DL, Jackson RW, Vivian A, et al. (2000) Phylogeny of the genus Pseudomonas: intrageneric structure reconstructed from the nucleotide sequences of gyrB and rpoD genes. Microbiol 146:2385-2394.
16. Kovacs N(1956) Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature (Lond.)178:703
17. Juliana O, Paulo L, Giuliana LS (2000) Antibacterial activity of plant extracts and phytochemicals on Antibiotic Resistant Bacteria. Brazilian J Microbiol 31:247-256.
18. Price MN, Dehal PS, Arkin AP(2010) Fast Tree 2-approximately maximum-likelihood trees for large alignments. PLoS One 5:e9490
19. Udochukwu EK, Lola A, Adetuyi FO, Jude-Ojei B (2009) Assessment of the antibacterial activity of Vernonia amygdalina and Ocimum gratissimum leaves on selected food borne pathogens. International Journal of Third World Medicine 8:23-24.
20. Mehlika KB, Hadush G (2015) Antimicrobial activity of various extracts of Ocimum basilicum L. and observation International Journal of Basic and Clinical Pharmacology 4:875
21. Nweze EI (2017) Characteristics of chemotypes as found in the chemical com-position of Ocimum gratissimum. Pharmacology 81:61-80.
22. Sudevan S, Ekenza EC (2004) Medicinal evaluation of Xylopia aethiopica and Ocimum gratissimum. J Med Aromatic Scien 26:44.

This article is available from: 10.36648/1989-8436.11.4.117

23. Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG (2002) Pseudomonas aeruginosa displays multiple Phenotypes during development as a Biofilm. Journal of Bacteriology 1140-1154.

24. Ibrahim IAJ, Kareem TA, Azeez YM, Falhi HK (2019) Phylogenetic tree analysis based on the 16S sequence alignment for Klebsiella spp. Isolated from different sources. Iraqi J Scien 60:2618-2628.

25. Ramos JL (2004) Genomics, Life Style and Molecular Architecture. New York, NY, USA: Kluwer Academic/Plenum Publishers 369-571.

26. Klaif SF, Naser HH, Sadeq JN (2019) The genetic relationship for Klebsiella pneumoniae isolated from human urinary tract and beef. Iraqi J Vet Scien 33:75-80.