Antibacterial activity of five selected ethno-medicinal plants **against extreme multi-drug resistance strains of *pseudomonas aeruginosa* and *Staphylococcus aureus***

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Abstract

Emergence of multidrug resistance strains of different kinds of virulent pathogens to various antibiotics worldwide had become one of the most concerning public health issues in Nigeria. This research is aimed at evaluating the antibacterial activity of five selected ethno-medicinal plants against multi-drug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Ficus sycomorus*, *Vitex doniana*, *Jatropha curcas*, *Acacia nilotica* and *Prosopis africana* leafs were extracted using chloroform, to get the crude extracts of the various plants material. Exactly 150 clinical and environmental samples each, was collected for the isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* each. The antibiogram profile and resistance level of the isolated strains was ascertained using the standard disc diffusion bioassay. Multi-drug resistance genes; BLA-TEM, BLA-SHV and CTX-M presence were evaluated and also served as another form of selection protocol for extremely resistant strains of the selected pathogenic bacterial species. In-vitro and in-vivo bioassay was done to evaluate the antimicrobial potential of the plants extracts on the most resistant strains of the two pathogenic bacteria. Tannins, saponin, alkaloids, flavonoids, phenols, terpenoids,

quinones, phytosterols and phlobatanins were found present in these plants extracts. Eleven *Pseudomonas aeruginosa* and four *Staphylococcus aureus* extreme multi-drug resistance strains were successfully isolated and identified. The in-vitro bioassay reveals that *F. sycomorus* and *J. curcas* extracts has the highest antimicrobial activity against the various multi-drug resistant strains, while *A. nilotica* extract also maintains some level of activity against the resistant strains. The in-vivo bioassay reconfirmed the antibacterial effects of the three most active plants mentioned above, against all the resistant bacteria. Decoction of the three most active plants extracts also proves effective on the resistant strains of both bacteria, than the individual extracts. *F. sycomorus, J. curcas* and *A. nilotica* plant extracts could provide an alternative active pharmaceutical agents against multidrug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Keyword: Resistance, bioassay, antimicrobial, ethno-medicinal plant, phytometabolites, bacteria

Introduction

Plants are potential source of medical agents, as the ethno-medicinal plants are traditionally used to treatment and management many diseases and infections, such as diarrhea, fever and cold (Hassan *et al.,* 2023a). Nowadays, the herbal medicines are still widely used in conventional and alternative medical practices in developed and developing countries as a complementary medicine. Plants are been used as a source of inspiration in the development of novel drug or part of it (Abu *et al.,* 2020). Phytochemicals are metabolic chemical compounds that occur naturally in plants, which are responsible for a lot of plants peculiar features and capability, such as; color and organoleptic properties, like the deep purple of blueberries or the smell of garlic (Umaru *et al.,* 2022).

Phyto-metabolites are wide variety of chemical compounds, which can be sorted by their differential chemical class, biosynthetic origin and functional groups (Rivas *et al.,* 2021). Plant however also used in the treatment and management of zoonotic hazards such as bites from snakes, bees, scorpions and other zoonotic animals (Olanipekun *et al.,* 2021).

Pseudomonas aeruginosa and *Staphylococcus aureus* are virulent species of bacteria, which are two of the most causative agents of nosocomial infections (hospital acquired infections). They are aerobic, opportunistic

pathogens, whose infections are usually very difficult to treat, because of their elevated intrinsic resistance as well as their capacity to acquire resistance to different antibiotics (Breidenstein *et al.,* 2011). Bacterial multi-drug resistance genes like BLA-TEM, BLA-SHV and CTX-M genes usually encode production of Extended Spectrum Beta Lactamase, which confers virulent resistance to various forms of Beta-lactams antibiotics among others, this is one of the most important form of antibiotic drug resistance. The Bla-SHV gene together with other functional agents aids in maintaining bacterial genomic integrity, by inducing DNA damage repair mechanism and withstanding environmental stress by the host immune response systems (Ejaz *et al.,* 2021). The extended spectrum beta Lactamase enzymes of the TEM and SHV bacterial producing families are the mutant forms of beta-lactamases, while the CTX-M family initially originated from certain environmental bacteria (Ehlers *et al.,* 2009). Many researches had shown the economic and medical impacts of these resistance bacteria to the society (Hassan *et al.,* 2023a).

Ethno-medicinal plants have since been exploited as potential sources of pharmaceutical agents and traditionally used in the treatment and management different kinds of diseases and infections, such as diarrhea, fever and cold (Rivas *et al.,* 2021). These plants parts, decoction, phyto-metabolites (which are metabolic chemical compounds that occur naturally in plants, responsible for a lot of plants peculiar features and capability, such as; medical defense, color and organoleptic properties) and other matter are nowadays, widely used in conventional and alternative medical practices in developed and developing countries as a complementary medicine garlic (Umaru *et al.,* 2022). However emergence of new strains of pathogenic agents, couple with the detrimental effects of multi-drug resistance, especially among gram negative and positive bacteria, which now adays always increasing by the day, making even some of the most effective drugs and antibiotics available useless.

Medicinal plants are the biggest bio-resource of drugs of traditional systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates/semi-antibiotics and chemical entities for synthetic drugs (Shikov *et al.,* 2022). Medicinal plants like *Ficus sycomorus* is a medicinal plant which is a semi-deciduous spreading savannah tree, growing up to 21 m with a maximum height of 46 m (Hassan *et al.,* 2020). In Nigeria, the plant is

mostly found in the semi-arid regions. The plant has different local names such as; sycamore fig (English), Baure (Hausa), Tarmu (Kanuri), and Kamada (Babur) among others (Kassa *et al.,* 2015). *F. sycomorus* is used traditionally in treating different type of diseases, illness and infections including treatment of snake bites, jaundice, chest pains, dysentery, cool, coughs and throat infections (Sofowora, 1993). In northern Nigeria, the stem bark, leaf and root of *F. sycomorus* is used traditionally to treat fungal infections, jaundice, snake bite and dysentery (Wogenie, 2008). While *Vitex doniana* (Black Plum) is another medicinal plant, of the family verbanaceae (Sathiamoorthy *et al.,* 2007). In Nigeria, *Vitex doniana* plant is used in treating different diseases, such as diarrhea, diabetes and hypertension (Dharmasiri *et al.,* 2003). Other medicinal plants of great traditional importance includes; *Prosopis africana* (Orwa *et al.,* 2009, Weber *et al.,* 2008 and Bosha and Asuzu, 2015), *Jatropha curcas* (Pecina *et al.,* 2014 and Gamal *et al.,* 2016) and *Acacia nilotica* (Kalaivani, 2010, Jigam *et al.,* 2010 and Aliyu, 2006).

More often literatures and researches mostly dwell on percentage presence of these bacterial multidrug resistance strains, not the effects or relationships of ethno botanical plants extracts on these isolates however this study furthermore researched the relationship between those agent that are extremely multi-drug resistance, their nucleotide sequence and the specific kinds of resistance genes they contain and the synergistic effects of plants phyto-metabolites on the extremely multi drug resistance strains of the nosocomial agents. This research aimed at characterizing the antibiogram profile of *Pseudomonas aeruginosa* and *Staphylococcus aureus* clinical and environmental isolates, from different hospitals in Kaduna state metropolis, and to evaluate the antibacterial effects of five selected ethno-medicinal plants extracts on the extreme antibiotic resistance strains.

Materials and Method

Sample Collection and Processing

Plant Sampling and Authentication

Leafs of *Ficus sycomorus*, *Vitex doniana*, *Jatropha curcas*, *Acacia nilotica* and *Prosopis africana*, were collected from different parts of Katsina and Kaduna states. All selected plants were then identified and authenticated by a qualified botanist of the herbarium section of the Department of Plant Science, Bayero University Kano, Nigeria. Each plant sample was properly washed using tap water and rinsed with distilled water, before air drying under shade. Each dried plant sample material was then pulverized separately into

powder and stored in clean polythene bags at ambient temperature for further analysis (Hassan *et al.,* 2021).

Isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Pseudomonas aeruginosa and *Staphylococcus aureus* isolates were collected from different hospitals (urine, blood, wound swap, sputum) and their surroundings, refuse dumps, mechanic workshops and poultry houses in Kaduna metropolis. The samples were then further sub-cultured using cetrimide agar, blood agar and mannitol salt agar, where microbiological and biochemically reconfirmations analyses were carried out on the individual isolates, before storage on slants of nutrient agar and refrigerated at 4°c, until required for use (Breidenstein *et al.,* 2011).

Plant Material Extraction

A portion (500g) each of the respective processed stored powder of the plants materials, were separately percolated in a maceration bottle, using 300 ml of chloroform. The powdered plant sample was then allowed to macerate for a period of one week in the solvent inside the percolator. Each extract was filtered using a pressure suction pump and then evaporated to dryness at 40 ^{0}C using rotary evaporator. Individual residue produced were allowed to cool, weighed and stored in a refrigerator, until use (Hassan *et al.,* 2020).

Phytochemical Screening

Qualitative and quantitative phytochemical analysis were done, using standard protocols, as described by (Sofowora, 1993) and (Lakache *et al.,* 2016).

Microbiological Identification of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Test isolates was inoculated and sub-cultured on different kinds of culture mediums, which includes; nutrient agar, blood agar and the cetrimide agar, for Colony-morphology identification. In addition to macroscopic characteristics, gram-staining and microscopic examination was carried out with special regard towards cell shape and arrangement (Gellatly and Hancock 2013).

Biochemical Identification of the Bacterial Isolates

Biochemical analyses was carried out on each of the isolates, including the Nitrate Reduction, Catalase, Oxidase, Methyl-Red, Motility, Indole, Citrate, Voges Proskauer and Urease testes (Brown *et al.,* 2012).

Susceptibility Test

The antibiotic susceptibility was tested on Mueller–Hinton agar plates, using the standard disk diffusion technique (Kirby-Bauer test) (Sarwat *et al.,* 2015). Briefly, the confirmed isolates were grown on Mueller-Hilton agar and incubated at 37°C for 18–24h. After incubation, few colonies was reconstituted on sterile physiological saline to a diluent approximating to 0.5 McFarland standards. The bacterial suspension was spread onto a Mueller–Hinton agar plate surface to form a confluent lawn and incubated for approximately 15 min, afterwards, the agar plates was impregnated with standard antibiotic discs for both the bacteria. Plates were read after 24h of incubation and results was recorded (Sarwat *et al.,* 2015). Isolates that resisted 97 % of all antibiotics were considered extreme resistance strains.

Preparation of Fraction Stock Solution

Five gram (5 g) of each extract fraction was diluted with 10 mL of 10% dimethyl sulfoxide (DMSO), which serves as stock Solution (500mg/ml), and sterilized by filtration through a bacterial filter of pore size 0.45µm using positive pressure. The filtrate were then kept at 4°C in refrigerator until used (Kossah *et al.,* 2013).

Inoculum Standardization

A loopful of the confirmed test isolates was picked using a sterile wire loop and emulsified in 3-4mls of normal saline and properly shaken. The suspension turbidity was then matched with the 0.5 McFarland standard (Cheesbrough, 2000).

Identification of Resistance Genes

The evaluation for the presence of resistance genes in the identified isolates of *P. aeruginosa* and *S. aureus* was done using multiplex polymerase chain reaction technique as described by Shaebth, (2018), with specific synthesized primers for the following resistance genes:

i. BLA TEM F-ATGAGTATTCAACATTTCCG
   R-CTGACAGTTACCAATGCTTA
ii. BLA SHV F-CGCCTGTGTATTATCTCCCTGTTAGCC
   R-TTGCCAGTGCTCGATCAGCG
iii. CTX-M F-CGCTTTGCGATGTGCAG
   R-ACCGCGATATCGTTGGT

From Inqaba Biotec African Genomics Company. The final amplified products were electrophoresed through agarose gels (1%) containing 0.5% ethidium bromide and visualized under UV transilluminator Documentation unit (Shaebth, 2018).

Antimicrobial Assay

The antimicrobial activity of selected extracts was determined using the disc diffusion method according to Black and Black, (2018). Mueller Hinton agar was sterilized by autoclaving at 121°C for 15min, cooled, poured into Petri dishes and inoculated with the selected isolates by striking the swab over the surface of the medium in three directions to confirm a complete distribution. Sterile filter paper discs (Whatman No. 3, 6 mm diameter and three layers) was saturated by stock solutions of 100µL of each extract (10 mg/mL); the disks were allowed to dry for one hour, then placed on the surface of inoculated plates. The used organic solvents and distilled water disks served as negative controls. The plates were kept in a refrigerator for one hour to allow better diffusion of the extract prior to incubation at 37°C/24h. After incubation, the inhibition zones formed around disks were measured in millimeters (including the diameter of the disk (6 mm). Each experiment was run in triplicates and the means calculated.

***In-Vivo* Antimicrobial Assay**

All the applicable guidelines for the care and use of animals were followed in the study, meanwhile an ethical clearance letter was collected from the state animal health research control committee. Seven days prophylactic and 1h post infection effects of isolates and plants extracts was investigated using the method previously described by Oke, (2006).

Statistical Analysis

Results obtained were presented as mean ± standard deviation of three determinations. Means were also analyzed using one-way analysis of variance to determine significant differences in all the parameters using SPSS. Differences with values of $P<0.05$ will be considered statistically significant.

Results

Plant Extraction Result

Extraction of *Ficus sycomorus*, *Vitex doniana*, *Jatropha curcas*, *Acacia nilotica* and *Prosopis africana*, chloroform extract, shows that some of the leaf extracts has a dark brownish and/or greenish colour, with a gummy or crystalline texture and different percentage yields.

Table 1: Percentage yield and physical properties of *Ficus sycomorus*, *Vitex doniana*, *Jatropha curcas*, *Acacia nilotica* and *Prosopis africana* Leaf Extract

Plant Name	Initial Weight of Plant Material (g)	Total yield (g)	Yield (%)	Colour	Texture
Ficus sycomorus	500	26.73	5.35	Greenish	Crystalline
Vitex doniana	500	37.81	7.56	Dark Greenish	Gummy
Jatropha curcas	500	51.99	10.40	Dark Brownish	Crystalline
Acacia nilotica	500	67.03	13.41	Dark greenish	Gummy
Prosopis africana	500	38.52	7.70	Greenish	Crystalline

Result for Phytochemical Analysis

Table 2-6, shows the result for qualitative and quantitative phytochemical screening of *Ficus sycomorus, Vitex doniana*, *Jatropha curcas*, *Acacia nilotica* and *Prosopis africana* leaf extract respectively. Qualitative screening of the plants extracts reveals the presence of numerous phytometabolites, among which; tannins, saponin, alkaloids, flavonoids, phenols, terpenoids, quinones, phytosterols and phlobatanins, are all found to be present in all the extracts. However phytochemicals like resisns, chalcones, vitamin D, acidic compounds and anthoquinones are only found in some of the extracts. Quantitative phytochemical analyses of the plants extracts indicates that phenolics have a relatively high percentage concentration in all the extracts.

Table 2: Qualitative and Quantitative Phytochemical Content of Chloroform extract of *F. sycomorus* Plant Parts

S/N	Phytochemical	Leaf Extract (mg/g dry wt)	
		Qualitative	Quantitative
1	Flavonoid	+	6.02 ± 2.35
2	Alkaloid	+	5.75 ± 0.37
3	Saponins	+	2.49 ± 0.21
4	Phytosterols	+	1.83 ± 1.45
5	Phenols	+	14.47 ± 2.01
6	Terpenoids	+	0.64 ± 2.05
8	Triterpenoids	+	NT
9	Tannins	+	6.53 ± 1.47
10	Cardiac glycoside	+	2.50 ± 0.11
11	Anthraquinones	+	0.79 ± 1.48
12	Anthocyanins	_	NT
13	Phlobatannins	+	NT
14	Flavonols/flavones	+	NT
15	Coumarins	_	NT
16	Quinones	+	NT
17	Resins	_	NT
18	Amino acids	+	NT
19	Chalcones	+	NT
20	Vitamin A	_	NT
21	Vitamin D	+	NT
22	Acidic compound	_	NT

Key:

+ = Presence - = Absence NT: Not tested

Results are presented as mean ± standard deviation

Table 3: Qualitative and Quantitative Phytochemical Content of Chloroform extract of *Vitex doniana* Plant Parts

S/N	Phytochemical	Leaf Extract (mg/g dry wt)	
		Qualitative	Quantitative
1	Flavonoid	+	2.20 ± 1.13
2	Alkaloid	+	3.05 ± 1.23
3	Saponins	+	1.94 ± 1.22
4	Phytosterols	+	1.46 ± 2.05
5	Phenols	+	8.35 ± 2.17
6	Terpenoids	+	1.46 ± 0.49
8	Triterpenoids	+	NT
9	Tannins	+	5.50 ± 2.18
10	Cardiac glycoside	+	1.83 ± 0.55
11	Anthraquinones	+	2.05 ± 1.80
12	Anthocyanins	_	NT
13	Phlobatannins	+	NT
14	Flavonols/flavones	+	NT
15	Coumarins	+	NT
16	Quinones	+	NT
17	Resins	+	NT
18	Amino acids	_	NT
19	Chalcones	+	NT
20	Vitamin A	+	NT
21	Vitamin D	+	NT
22	Acidic compound	_	NT

Key:

+ = Presence - = Absence NT: Not tested

Results are presented as mean ± standard deviation

Table 4: Qualitative and Quantitative Phytochemical Content of Chloroform extract of *Jatropha curcas* Plant Parts

S/N	Phytochemical	Leaf Extract (mg/g dry wt)	
		Qualitative	Quantitative
1	Flavonoid	+	4.05 ± 1.28
2	Alkaloid	+	9.27 ± 0.70
3	Saponins	+	2.90 ± 2.92
4	Phytosterols	+	0.63 ± 0.73
5	Phenols	+	9.55 ± 2.35
6	Terpenoids	+	1.68 ± 0.94
8	Triterpenoids	+	NT
9	Tannins	+	2.53 ± 1.36
10	Cardiac glycoside	+	0.18 ± 0.18
11	Anthraquinones	+	0.59 ± 1.77
12	Anthocyanins	_	NT
13	Phlobatannins	+	NT
14	Flavonols/flavones	+	NT
15	Coumarins	_	NT
16	Quinones	+	NT
17	Resins	+	NT
18	Amino acids	_	NT
19	Chalcones	_	NT
20	Vitamin A	_	NT
21	Vitamin D	_	NT
22	Acidic compound	+	NT

Key:

+ = Presence - = Absence NT: Not tested

Results are presented as mean ± standard deviation

Table 5: Qualitative and Quantitative Phytochemical Content of Chloroform extract of *Acacia nilotica* Plant Parts

S/N	Phytochemical	Leaf Extract (mg/g dry wt)	
		Qualitative	Quantitative
1	Flavonoid	+	1.97 ± 1.64
2	Alkaloid	+	1.53 ± 2.85
3	Saponins	+	1.32 ± 2.75
4	Phytosterols	+	0.29 ± 1.64
5	Phenols	+	9.92 ± 1.15
6	Terpenoids	+	1.32 ± 1.74
8	Triterpenoids	_	NT
9	Tannins	+	1.36 ± 1.82
10	Cardiac glycoside	+	0.83 ± 1.28
11	Anthraquinones	+	0.06 ± 0.94
12	Anthocyanins	_	NT
13	Phlobatannins	+	NT
14	Flavonols/flavones	+	NT
15	Coumarins	_	NT
16	Quinones	+	NT
17	Resins	_	NT
18	Amino acids	_	NT
19	Chalcones	_	NT
20	Vitamin A	_	NT
21	Vitamin D	_	NT
22	Acidic compound	_	NT

Key:

+ = Presence - = Absence NT: Not tested

Results are presented as mean ± standard deviation

Table 6: Qualitative and Quantitative Phytochemical Content of Chloroform extract of *Prosopis africana* Plant Parts

S/N	Phytochemical	Leaf Extract (mg/g dry wt)	
		Qualitative	Quantitative
1	Flavonoid	+	1.99 ± 0.91
2	Alkaloid	+	5.32 ± 1.74
3	Saponins	+	2.97 ± 2.07
4	Phytosterols	+	0.06 ± 0.51
5	Phenols	+	7.31 ± 2.17
6	Terpenoids	+	0.28 ± 1.91
8	Triterpenoids	+	NT
9	Tannins	+	4.06 ± 1.05
10	Cardiac glycoside	+	0.08 ± 0.16
11	Anthraquinones	+	0.85 ± 1.09
12	Anthocyanins	_	NT
13	Phlobatannins	+	NT
14	Flavonols/flavones	+	NT
15	Coumarins	_	NT
16	Quinones	+	NT
17	Resins	_	NT
18	Amino acids	_	NT
19	Chalcones	+	NT
20	Vitamin A	+	NT
21	Vitamin D	_	NT
22	Acidic compound	+	NT

Key:

+ = Presence - = Absence NT: Not tested

Results are presented as mean ± standard deviation

Result for the Isolation of *Pseudomonas aeruginosa* clinical Isolates

A total of 108 clinical samples were collected and processed from different general hospitals in Kaduna state metropolis, of which 43 *Pseudomonas aeruginosa* strains were identified. There was a considerable variation in the infection of *Pseudomonas aeruginosa* as classified by the clinical sources. Wound samples however recorded the highest figures (18), while the least was found in stool and sputum (Table 7). Socio-demographic characteristics of all the participating patients, their gender, age and a statistics of the bacterial isolation from the different groups.

Table 7: *Pseudomonas aeruginosa* clinical isolates from different General Hospitals in Kaduna metropolis

Sources (s)	**No of Samples**	***P. aeruginosa* Isolates (%)**
Wound	38	18 (16.67)
Stool	14	3 (2.78)
Oral	7	5 (4.62)
Fluids	9	5 (4.62)
Ear Swap	11	6 (5.56)
Urine	21	6 (5.56)
Sputum	8	- (0)
Total	108	43 (39.81)

Result for the Isolation of *Pseudomonas aeruginosa* Environmental Isolates

A total of 42 environmental samples were collected and processed from different parts of Kaduna state metropolis, of which 22 *Pseudomonas aeruginosa* strains were identified. Refuse Dump samples recorded the highest figures of 7, while the least was found in Hospitals Surgical Equipment's (Table 8).

Table 8: *Pseudomonas aeruginosa* Environmental isolates from different parts of Kaduna metropolis

Sources (s)	**No of Samples**	***P. aeruginosa* Isolates (%)**
Refuse Dump	16	7 (16.67)
Hospital Refuse Dump	10	6 (14.28)
Poultry Houses	6	6 (14.28)
NNPC Waste Water Outlet	2	1 (2.38)
Mechanic Workshop	5	2 (4.76)
Hospitals Surgical Equipment's	3	0 (0)
Total	42	22 (50.00)

Result for the Isolation of *Staphylococcus aureus* Clinical Isolates

A total of 89 clinical samples were collected and processed from different general hospitals in Kaduna state metropolis, of which 43 (48.31%) were identified as *Staphylococcus aureus*. There was a considerable variation in the infection of *Staphylococcus aureus* as classified by the clinical sources. Wound samples however recorded the highest figures 13, while the least was found in sputum sample (Table 9). Socio-demographic characteristics of all the participating patients, their gender, age and a statistics of the S. aureus isolation from the different groups.

Table 9: *Staphylococcus aureus* clinical isolates from different General Hospitals in Kaduna metropolis

Sources (s)	**No of Samples**	***Staphylococcus aureus* (%)**
Wound	21	13 (14.61)
Stool	11	6 (6.74)

Oral	8	4 (4.49)
Fluids	9	6 (6.74)
Ear Swap	8	4 (4.49)
Urine	22	8 (8.99)
Sputum	10	2 (2.25)
Total	89	43 (48.31)

Result for the Isolation of *Staphylococcus aureus* Environmental Isolates

A total of 61 environmental samples were collected and processed from different parts of Kaduna state metropolis, of which 44 (72.13%) were identified as *Staphylococcus aureus*. Refuse dump samples recorded the highest figures of 24 (39.34%), while the least was found in hospitals surgical equipment's 2 {3.28%} (Table 10).

Table 10: *Staphylococcus aureus* Environmental isolates from different parts of Kaduna metropolis

Sources (s)	**No of Samples**	***Staphylococcus aureus* (%)**
Refuse Dump	28	24 (39.34)
Hospital Refuse Dump	14	4 (6.56)
Poultry Houses	7	7 (11.48)
NNPC Waste Water Outlet	4	4 (6.56)
Mechanic Workshop	3	3 (4.92)
Hospitals Surgical Equipment's	5	2 (3.28)
Total	61	44 (72.13)

Bioassay Result for the most Resistant Strains of *Pseudomonas aeruginosa*

Antimicrobial activity assay done on all the clinical and environmental isolates done on all the 150 collected samples, reveals that eleven of the isolates are extreme multi-drug resistant, based on the bioassay analyses carried out using standard antibiotics (Table 11).

Table 11: Bioassay for the most extremely resistant strains of *P aeruginosa*

Resistant Isolates	**Standard Antibiotics Zone of Inhibition in cm**												
	NF	**CXM**	**CRO**	**ACX**	**ZEM**	**LBC**	**AUG**	**CTX**	**IMP**	**OFX**	**GN**	**NA**	**VA**
P_3	R	R	R	R	R	S	R	R	R	R	R	I	R
P_{15}	R	R	R	R	R	R	I	R	R	I	R	R	R
P_{16}	R	R	R	R	R	R	R	R	I	R	R	R	R
P_{20}	R	R	I	R	R	R	R	R	R	R	R	R	R
P_{26}	R	R	R	R	R	I	R	R	R	S	R	R	R
P_{42}	R	R	R	R	R	R	R	R	I	R	S	R	R
P_{47}	R	I	R	R	R	R	I	R	R	R	R	R	R
P_{88}	R	R	R	R	R	I	R	R	R	R	I	R	R
P_{100}	R	R	R	R	R	R	R	R	R	I	I	R	R
P_{125}	R	R	R	R	R	R	R	R	R	R	R	R	I
P_{146}	R	R	R	R	R	S	R	R	R	R	I	R	R

Key: S- Sensitive ≥ 1.5, I- Intermediate 0.5 – 1.4, R- Resistance ≤ 0.4

Antibiotics: NF - Nitrofurantoin (300µg), CXM – Cefuroxime (30µg), CRO – Ceftriaxone Sulbactarm (45µg), ACX - Ampiclox (10µg), ZEM - Cefexime (5µg), LBC – Levofloxacin (5µg), AUG - Amoxicilin Clavulanate (30µg), CTX – Cefotaxime (25µg), IMP - Imipenem/Cilastatin (10/10µg), OFX - Ofloxacin (5µg), GN – Gentamycin (10µg), NA - Nalidixic Acid (30µg) and VA – Vancomycin (30µg)

Bioassay Result for the most Resistant Strains of *Staphylococcus aureus*

Antimicrobial activity assay done on all the clinical and environmental *S. aureus* isolates done on all the 150 collected samples, reveals that four of the isolates are multi-drug resistant, based on the bioassay analyses carried out using standard antibiotics (Table 12).

Table 12: Bioassay for the most resistant strains of *S. aureus*

Resistant Isolates	Standard Antibiotics Zone of Inhibition in cm										
	PEF	**CN**	**APX**	**Z**	**AM**	**R**	**CPX**	**S**	**SXT**	**E**	**M**
S_{37}	R	S	R	R	R	R	R	R	R	R	R
S_{64}	R	R	R	R	R	R	I	I	R	R	R
S_{72}	I	R	R	R	R	R	R	R	R	I	R
S_{105}	R	R	R	I	R	R	R	R	R	R	R

Key: S- Sensitive ≥ 1.5, I- Intermediate 0.5 – 1.4, R- Resistance ≤ 0.4

Antibiotics: PEF - Pefloxacin (10µg), CN - Gentamycin (10µg), APX - Ampiclox (30µg), Z - Zinnacef (20µg), AM - Amoxacillin (30µg), R - Rocephin (25µg), CPX - Ciprofloxacin (10µg), S - Streptomycin (30µg), SXT - Septrin (30µg), E – Erythromycin (10µg) and M - Methicillin (30µg)

Results for the Resistance Genes Identification

The agarose Gel Electrophoresis of the PCR amplified products of Extended-Spectrum Beta-lactamase Genes done, revealed the presence of the selected resistance genes in virtually all the selected extremely multi-drug resistant bacterial strains (Figure 5).

M P_3 P_{15} P_{16} P_{20} P_{26} P_{42} P_{47} P_{88} P_{100} P_{125} P_{146} S_{37} S_{64} S_{72} S_{105}

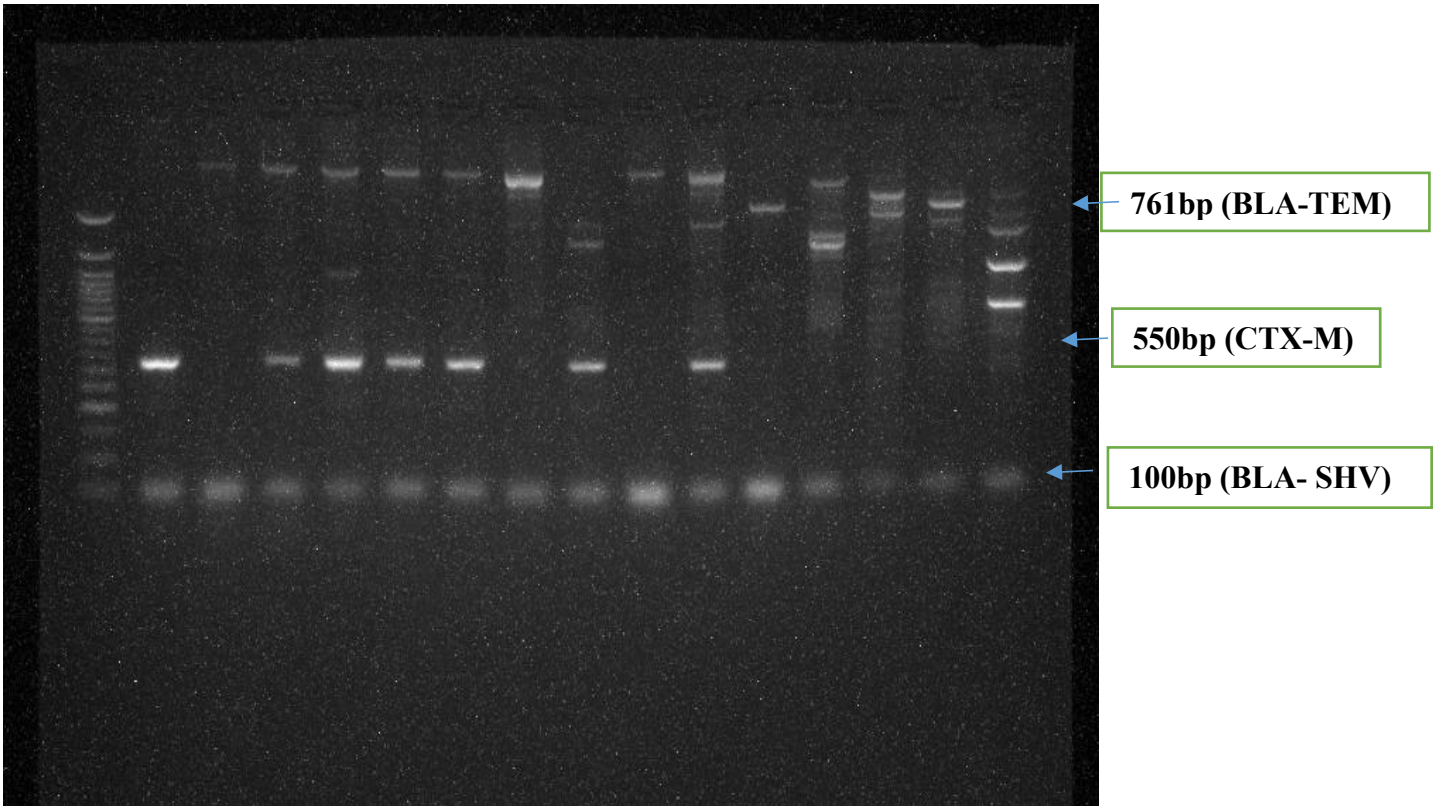

Plate 1: Agarose gel electrophoresis of CTX-M, *bla* SHV and *bla* TEM (multiplex PCR), M: BIONEER DNA Marker 100bp

In-vitro Bioassay of Plants Extracts against Multidrug Resistant Isolates of *Pseudomonas aeruginosa*

Mean Results for the In-vitro bioassay of *F. sycomorus, V. doniana, J. curcas, A. nilotica* and *P. africana* leaf extracts against multidrug resistant isolates of *Pseudomonas aeruginosa*, which reveals that *F. sycomorus* and *J. curcas* has the highest antimicrobial activity against the various multi-drug resistant strains (Table 13).

Table 13: Plants extracts bioassay against multidrug resistant isolates of *Pseudomonas aeruginosa*

S/N	Resistant Isolates	Isolate Source	Crude Extracts Zone of Inhibition (cm) (1000µg/ml)	Control (cm)

			Ficus sycomorus	***Vitex doniana***	***Jatropha curcas***	***Acacia nilotica***	***Prosopis africana***	**Gentamycin**
1	P_3	Clinical	2.0 ± 0.19	0.5 ± 2.11	1.3 ± 2.04	1.6 ± 1.05	-	-
2	P_{15}	Clinical	-	1.1 ± 0.51	1.2 ± 1.18	1.5 ± 1.72	-	-
3	P_{16}	Clinical	1.1 ± 0.51	-	1.5 ± 0.21	1.1 ± 1.49	-	-
4	P_{20}	Env.	0.7 ± 0.29	-	0.6 ± 1.05	1.0 ± 0.56	-	-
5	P_{26}	Clinical	1.8 ± 1.64	1.0 ± 0.32	2.0 ± 0.44	0.6 ± 1.43	0.7 ± 1.73	0.5
6	P_{42}	Clinical	2.5 ± 1.03	-	1.6 ± 1.10	1.7 ± 1.01	0.6 ± 0.25	1.4
7	P_{47}	Clinical	2.2 ± 1.17	0.7 ± 0.32	1.8 ± 0.04	2.7 ± 1.47	-	-
8	P_{88}	Clinical	2.5 ± 0.53	-	1.4 ± 1.62	1.9 ± 0.05	1.8 ± 0.62	0.8
9	P_{100}	Clinical	1.8 ± 0.25	2.0 ± 1.53	2.3 ± 1.03	1.7 ± 0.53	0.7 ± 1.83	1.1
10	P_{125}	Env.	1.3 ± 1.03	1.2 ± 1.22	1.4 ± 0.74	1.3 ± 1.46	1.0 ± 2.15	-
11	P_{146}	Clinical	1.2 ± 1.17	-	2.0 ± 1.02	0.9 ± 0.85	-	1.3

Key:

P: *Pseudomonas aeruginosa*

±: Standard Deviation

-: No zone of inhibition

Env.: Environmental

In-vitro Bioassay of Plants Extracts against Multidrug Resistant Isolates of *Staphylococcus aureus*

Mean Results for the in-vitro bioassay of *F. sycomorus, V. doniana, J. curcas, A. nilotica* and *P. africana* leaf extracts against multidrug resistant isolates of *Staphylococcus aureus* in cm. The analysis reveals that *J. curcas* has the highest antimicrobial activity against the different strains of multi-drug resistant *S. aureus* (Table 14).

Table 14: Plants extracts bioassay against multidrug resistant isolates of *S. aureus*

S/N	Resistant Isolates	Isolate Source	Crude Extracts Zone of Inhibition (cm) (1000µg/ml)	Control (cm)

			Ficus sycomorus	***Vitex doniana***	***Jatropha curcas***	***Acacia nilotica***	***Prosopis africana***	**Gentamycin**
1	S_{37}	Clinical	1.6 ± 1.13	0.6 ± 1.08	2.1 ± 1.11	1.9 ± 0.18	0.8 ± 1.64	1.5
2	S_{64}	Clinical	1.4 ± 1.98	0.6 ± 1.63	1.2 ± 1.80	0.7 ± 0.56	-	-
3	S_{72}	Env.	2.2 ± 0.97	-	1.9 ± 0.83	1.9 ± 1.03	1.3 ± 2.29	-
4	S_{105}	Clinical	1.9 ± 1.63	0.7 ± 0.05	2.1 ± 2.07	0.9 ± 0.55	-	-

Key:

S: *Staphylococcus aureus*

±: Standard Deviation

-: No zone of inhibition

Env.: Environmental

In-vitro Bioassay of Decocted Extract of *F. sycomorus, J. curcas* and *A. nilotica* against Extreme Multidrug Resistant Isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Mean Results for the in-vitro bioassay of a decocted extract of the most active plant extracts (*F. sycomorus, J. curcas* and *A. nilotica*), against the isolated multidrug resistant isolates of *Pseudomonas aeruginosa*, shows that combining the three most bio-active extracts proofs to be more efficient in inhibiting the growth of the extreme multi-drug resistant strains of both the virulent bacteria (Table 15).

Table 15: Extracts decoction of *F. sycomorus, J. curcas* and *A. nilotica* bioassay against extreme multidrug resistant isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

S/N	Resistant Isolates	Isolate Source	Decocted Extract Zone of Inhibition (cm) (1000µg/ml)	Control Gentamycin (cm)
1	P_3	Clinical	2.6 ± 1.53	-

2	P_{15}	Clinical	2.8 ± 0.64	-
3	P_{16}	Clinical	2.4 ± 1.32	-
4	P_{20}	Env.	1.3 ± 1.03	-
5	P_{26}	Clinical	1.4 ± 0.24	1.0
6	P_{42}	Clinical	2.5 ± 2.72	1.1
7	P_{47}	Clinical	1.1 ± 1.29	-
8	P_{88}	Clinical	2.0 ± 0.34	0.5
9	P_{100}	Clinical	2.6 ± 1.35	1.2
10	P_{125}	Env.	1.7 ± 2.31	-
11	P_{146}	Clinical	2.4 ± 1.07	1.0
12	S_{37}	Clinical	2.9 ± 2.05	1.2
13	S_{64}	Clinical	2.3 ± 1.01	-
14	S_{72}	Env.	2.8 ± 1.15	-
15	S_{105}	Clinical	3.1 ± 1.78	-

Key:

P: *Pseudomonas aeruginosa*

S: *Staphylococcus aureus*

±: Standard Deviation

-: No zone of inhibition

Env.: Environmental

Results for the In-vivo Bioassay of the Plants Extracts against Resistant Isolates of *Pseudomonas aeruginosa*

The in-vivo bioassay of *F. sycomorus, V. doniana, J. curcas, A. nilotica* and *P. africana* leaf extracts against multidrug resistant isolates of *Pseudomonas aeruginosa* in rats model (**3 rats per group**) revealed that *J. curcas* and *A. nilotica* extracts are the most active bio-metabolites against the activity of the resistant strains of *P. aeruginosa.* Two hundred and one albino rats were used in the analyses (198 rats were part of the infected groups), among which 92 rats survived, while 106 died. Each individual group is composed of 33 rats, with the exception of the

positive control group of 3 rats. *Acacia nilotica* and *Jatropha curcas* have the highest survival rate of 78.79 and 69.70 % respectively (Table 16).

Table 16: In-vivo bioassay of the plants extracts against *P. aeruginosa* in rats model

S/N	Resistant Isolates	Isolate Source	Infected Groups + Crude Extracts (500µg/ml)					Control	
			Ficus sycomorus	***Vitex doniana***	***Jatropha curcas***	***Acacia nilotica***	***Prosopis africana***	+	-
1	**P_3**	Clinical	2	0	3	2	1		0
2	**P_{15}**	Clinical	2	1	2	3	1		0
3	**P_{16}**	Clinical	2	3	1	3	0		0
4	**P_{20}**	Env.	1	0	1	2	1		0
5	**P_{26}**	Clinical	3	1	3	2	1		0
6	**P_{42}**	Clinical	3	0	3	2	1		1
7	**P_{47}**	Clinical	3	1	3	3	0	**3**	0
8	**P_{88}**	Clinical	1	2	2	3	1		0
9	**P_{100}**	Clinical	0	0	3	3	1		1
10	**P_{125}**	Env.	1	1	0	2	2		0
11	**P_{146}**	Clinical	2	2	2	1	1		0
Survival rate (%)			**60.60**	**33.33**	**69.70**	**78.79**	**30.30**	**100**	**6.06**

Key:

P: *Pseudomonas aeruginosa*

Env.: Environmental

+: Positive Control (Un-infected)

-: Negative Control (Infected)

Results for the In-vivo Bioassay of the Plants Extracts against Resistant Isolates of *S. aureus*

The in-vivo bioassay of plants leaf extracts against multidrug resistant isolates of *S. aureus* in rats model (3 rats per group). Seventy five (75) albino rats were used in the analyses (72 rats

were part of the infected groups), among which 41 rats survived, while 31 died. Each individual group is composed of 12 rats infected with different strains of *S. aureus*, with the exception of the positive control group of 3 rats. *Jatropha curcas* and *Ficus sycomorus* have the highest survival rate of 83.33% each (Table 17).

Table 17: In-vivo bioassay of the plants extracts against *S. aureus* in rats model

S/N	**Resistant Isolates**	**Isolate Source**	**Infected Groups + Crude Extracts (500µg/ml)**					**Control**	
			Ficus sycomorus	***Vitex doniana***	***Jatropha curcas***	***Acacia nilotica***	***Prosopis africana***	+	_
1	S_{37}	Clinical	2	2	3	2	1		0
2	S_{64}	Clinical	3	2	3	1	0		0
3	S_{72}	Env.	3	3	2	3	1	**3**	1
4	S_{105}	Clinical	2	1	2	3	1		0
Survival rate (%)			**83.33**	**66.67**	**83.33**	**75.00**	**25.00**	**100**	**8.33**

Key:

S: *Staphylococcus aureus*

Env.: Environmental

+: Positive Control (Un-infected)

-: Negative Control (Infected)

Discussion

Resistant bacterial strains like *P. aeruginosa* and *S. aureus* can use both intrinsic, acquired and adaptive microbial antibiotic resistance mechanisms to rapidly develop resistance to various kinds of bacterial antibiotics, such as the cephalosporin's, aminoglycosides, quinolones and β-lactams (Gong *et al.,* 2021). Plants phytometabolites can however be exploits, in the treatment and management of such ailments cause by multi-drugs resistant pathogens, which other available pharmaceutics have failed to manage (Michael *et al.,* 2023). In this research antibacterial activity of five selected ethno-

medicinal plants have been research, against some of the most multi-drug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, isolated from various general hospitals, clinical environment and different kinds of environmental focal points in Kaduna state metropolis. Plant extraction was done using chloroform as the extraction solvent, in which *Acacia nilotica* plant material extraction yielded the highest percentage yield of 13.41 %. Qualitative phytochemical screening of the plants extracts done, shows that all the five plants contain; tannins, saponin, alkaloids, flavonoids, phenols, terpenoids, quinones, phytosterols and phlobatanins in them. While quantitative phytochemical analyses reveals that all the extracts contains phenolic chemicals as the highest containing chemicals in them, however *F. sycomorus* and *J. curcas* extracts appears to be the two most chemically enriched extracts among the five selected plants. Some of these findings were similarly reported by researchers like; (Hassan *et al.,* 2020), (Braide *et al.,* 2018), (Hassan *et al.,* 2023b), (Bunu *et al.,* 2021), (Makholwa *et al.,* 2023), (Perumal *et al.,* 2023) and (Alagbe *et al.,* 2022). There are some difference between this study and some of these reported researches, this might be as a result of the geological differences or the plants health or available soil enrichment (Anil *et al.,* 2017).

One hundred and eight (108) clinical samples were collected from different general hospitals for the isolation of *P. aeruginosa*, out of which 39.81% were found to *P. aeruginosa* strains, of which most the strains were of Wound swap samples. This gives a similar outcome with the research done by Sarwat *et al.* (2015). While isolation of same strains from environmental origins was also done using forty two (42) collected samples of *P. aeruginosa* strains were successfully isolated from 50% of the sample. Refuse dump samples however gave the highest percentage isolates from the various samples collected. On the other hand Eighty nine (89) clinical samples were collected from different general hospitals also for the isolation of *S. aureus*, out of which 48.31% were found to be positive for *S. aureus* presence, of which most the strains are also of wound swap origin, just like that of *P. aeruginosa* isolation. While isolation of same strains from environmental origins was also done using 61 collected samples of *S. aureus* strains were successfully isolated from 72.13% of the samples. Refuse dump samples however also gave the highest percentage isolates from the various environmental samples collected. Standard antimicrobial bioassay done on all the clinical

and environmental *P. aeruginosa* isolates, reveals that eleven of the isolates are multi-drug resistant, based on the bioassay analyses carried out using gram negative standard antibiotic assay disc. Eleven clinical and environmental *P. aeruginosa* strains were found to be extremely multi-drug resistant to more than ten (10) standard antibiotics, including drugs of choice for the bacteria (Imipenem/Cilastatin). While bioassay sensitivity analyses done on *S. aureus* isolates reveals that four (4) of the 150 isolated strains are also extremely multi-drug resistant to numerous antibiotics, including methicillin. of which clinical samples; S_{37} and S_{105} are the most resistant isolates, which are only susceptible to gentamycin and Zinnacef respectively. These results reveals that most of the isolated multidrug resistance isolates were gotten from the hospital community. This corresponds with the research done by Sarwat *et al.* (2015), were a high percentage of multidrug resistance bacteria was isolated from the hospital community.

Resistance genes identification analyses done reveals that most of the isolated resistance strains have either two or three of the BLA-TEM, BLA-SHV and CTX-M resistance genes (Plate 1). These genes have been reported to be responsible for multiple antibiotic drug resistance mechanisms including protein activators for multiple efflux pump systems and quorum sensing activation and enhancement (Nilsson, 2007). Similar outcome was reported by Ehlers *et al.* (2009), which support the pre-existence of resistance genes in pathogenic nosocomial bacteria like; *P. aeruginosa* and *S. aureus.*

In-vitro analyses done on the multi-drug resistant isolates against the five selected ethno-medicinal plants leaf extract shows that three (*F. sycomorus, J. curcas* and *A. nilotica*) of the five plants extracts have a relatively high antimicrobial effects against some of the resistant strains, however *F. sycomorus* and *J. curcas* extracts has the highest antimicrobial activity against the various multi-drug resistant strains. The antibiotic gentamycin was use as a reference control drug, as it was initially used on both the gram positive and negative isolates during the anti-biogram analyses. *Prosopis africana* extract have the least antimicrobial effect against all the selected resistance strains, while *J. curcas* has the highest antimicrobial effect on all the extracts. This findings were similar with the research done by Henik *et al.* (2023), on biodiesel industrial waste based on *Jatropha curcas* as a fungicide to control *Fusarium oxysporum* and *Alternaria solani*. The analyses also shows that *P. aeruginosa* strain 'P_{20}', which is an environmental isolate from

a mechanic workshop, is the most dominantly multi resistant strain of bacteria among all the selected resistant isolates. The resistant strains in this research are quite aggressively resistant to almost all kinds of antibiotics including the drug of choice for the multi-drug resistant isolates. For example in contrast to a research done by Rubina *et al.,* (2014), which reported that most extremely resistant strains of *P. aeruginosa* and *S. aureus* were susceptible to Vancomycin and Imipenem, which is not in line with these isolated strains of multi-drug resistant bacteria.

This aggressive resistibility might be as a result of an acquired mutation from multiple contacts with some of the various chemical compounds from the mechanic workshop, couple with the *P. aeruginosa* natural multi-drug resistance genes and intrinsic factors. This can be compared with some of the research outcome by Gamal *et al.* (2016), where biofuel produced using *J. curcas* aid in mutating some bacterial pathogens to develop multidrug resistance. Decoction of the three most active plants extracts (*F. sycomorus, J. curcas* and *A. nilotica*), proof effective, revealing a relatively high synergistic effect of the extract to each other. These shows that these bioactive plants extracts has a very good synergistic effect to each other, than when used individually. The statistical inhibitory difference is significant enough, to conclude that, the plants extracts will have a better antibacterial activity against the two bacterial multidrug resistance pathogens, the individual extracts.

In-vivo antimicrobial bioassay done, using albino rats shows the pathogenic infectivity of all the resistance strains, as the isolates were able to kill all the infected groups after one days post infection study. *Acacia nilotica* extracts however recorded the highest in-vivo anti-bacterial effects on the various resistance strains, followed by *F. sycomorus* and *J. curcas* extracts, which activity has already been established from the in-vitro bioassay done. One important observed phenomenon in the in-vivo analyses is that *Prosopis africana* extract seems to be a little bit toxic to the positive administered groups. This might be as a results of a number of factors, such as maceration solvent, processing protocols and toxic by-products of processing. This research is also compare with the research done by Braide *et al.* (2018), on the phytochemical properties, toxicological screening and antibacterial qualities of various parts extracts of *Ficus sycomorus* and the research done by Hassan *et al.* (2021), on the antimicrobial activities of *Cymbopogon citratus* and *Ximenia americana* leaf extracts against some selected bacterial and yeast clinical isolates.

Conclusion

F. sycomorus, J. curcas and *A. nilotica* plant extracts could provide an alternative natural pharmaceutical agent or semi decoction part of a drug formulation for the management and treatment of various kinds of ailments cause by multi-drug resistance *P. aeruginosa* and *S. aureus* bacterial pathogens.

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Competing interests

Authors have declared that no competing interests exist.

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