Antibacterial activity of *Lawsonia inermis* leaf extracts against Multidrug-resistant *Pseudomonas aeruginosa* from infected wounds

ABSTRACT

Background: Wound infection comprises numerous different organisms that have the ability to surface colonization of wounds. Multidrug-resistant *Pseudomonas aeruginosa* is one of the pathogenic bacteria associated with wound infections

Aim: This study isolated and identified multidrug-resistant *Pseudomonas aeruginosa* from infected wounds and determine the antibacterial activity of *Lawsonia inermis* leaf extracts against it.

Design: This is a Clinical and laboratory-based study involving patients with defined cases of wound infections.

Place and Duration of study: This study was conducted in the Microbiology (Bacteriology) laboratory of Specialist Hospital, Bauchi, Nigeria, from February to November 2021.

Methods: Twenty-eight (28) *Pseudomonas aeruginosa* isolates were recovered from 179 wound swabs using standard laboratory procedures and were screened for multidrug-resistant patterns according to the Kirby-Bauer disc diffusion method. Antibacterial efficacy of the aqueous, ethanolic, and methanolic leaf extracts of *Lawsonia inermis* was tested against the multidrug-resistant isolates using agar well diffusion techniques. The zone of inhibition was measured and the differences between means were statistically analyzed ($p<0.05$).

Results: A total of twenty-eight (28) multidrug-resistant *Pseudomonas aeruginosa* were confirmed, showing resistance to Amoxicillin (64.3%), Ceftazidime (85.71%), and Cefotaxime (78.57%) but sensitivity to Imipenem (95.5%). The phytochemical screening revealed the presence of flavonoids, glycosides, saponins, steroids, and tannins among others. MDR *P. aeruginosa* was inhibited at varied concentrations of the extracts with the diameter mean zone of inhibition increasing as the concentration increased. The Methanol extracts showed the highest antibacterial activity against MDR *P. aeruginosa* with a mean zone of inhibition of 9.500±0.288mm at 400mg/ml.

Conclusion: These results indicated that *Lawsonia inermis* leaf extracts possess antibacterial activities on Multidrug-resistant *Pseudomonas aeruginosa* which could be a good source for the production of plant-based antibacterial drugs., although somewhat less than the synthetic standard drugs (Imipenem) having a mean of 13.83±0.288mm.

Keywords: Wound infection, multidrug-resistant, Kirby-Bauer disc diffusion method, Pseudomonas aeruginosa

1.0 INTRODUCTION

The largest organ in the body and the body's first line of defense is the skin. The skin's surface is populated by a variety of bacteria known as normal flora, even when it is clean. The majority of the time, these microorganisms do not infect people or boost immunity, but a bre ak in the skin's surface (whether caused by trauma, an accident, surgery, or a burn) opens the doo r for microbial diseases [1]. According to Matsuura and Barg [2], Numerous different organisms have the ability to surface colonization of chronic wounds. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Stre*

ptococcus pyogenes, Proteus species, Streptococcus species, and Enterococcus species are frequ ent bacterial pathogens linked to wound infection [3].

Pseudomonas aeruginosa is a Gram-negative, aerobic bacillus that belongs to the *Pseudomonadaceae* family. It is a common pathogen that may be isolated from soil, water, and the gastrointestinal system. It is also frequently discovered in wet locations, such as hospitals, sinks, cleaning buckets, drains, and other damp areas. *P. aeruginosa* has been identified as the third most common cause of hospital-acquired urinary tract infections and is the second most frequent cause of hospital-acquired pneumonia [4]. The virulence factors of this organism have a variety of impacts; for example, pyocyanin suppresses cellular respiration, elastase damages blood vessels and breaks down the extracellular matrix of epithelial cells, and rhamnolipids disrupt cells and aid *P. aeruginosa* in invasion [5]. Multidrug-resistant (MDR*) P. aeruginosa* is closely linked to nosocomial infections, which are a global health problem due to the emergence of MDR strains (i.e., resistance to at least three antibiotics). Due to the lack of sufficient therapy options, MDR *P. aeruginosa* presents a number of therapeutic problems. Due to a poor permeability in the outer membrane, which functions as a selective barrier, P. aeruginosa is innately resistant to certain antibiotics [6].

Medicinal herbs are essential for meeting the requirements of people worldwide, particularly in developing nations [7]. In most poor nations, almost 80% of the populace still relies on using traditional medication made from plants. The utilization of plants for therapeutic purposes has a long history on the African continent. In the past, traditional medicine was the only available form of healthcare in Nigeria. Plants have long been utilized by traditional healers to prevent or treat infectious diseases. Nearly half of today's medications come from the plant kingdom. According to Gonzalez-Lamothe *et al.* [8], plants are abundant in a variety of secondary metabolites called polyphenols, including tannins, terpenoids, alkaloids, and flavonoids, all of which have been shown to have in-vitro antibacterial activities.

Lawsonia inermis, often known as *L. alba* or henna, is a blooming plant that grows 2–6 meters tall. It is the only species in the *Lawsonia* genus of the *Lythraceae* family. *Lawsonia inermis*, creates the maroon dye molecule lawsone [9]. Terpenoids, glycosides, quinones, tannin, saponins, flavonoids, phytosterols, alkaloids, sterols, fatty acids, and amino acids are only a few of the many phytochemicals present in the plant [9]. The plant was found to have antifungal, antibacterial, antiparasitic, antiviral, anticancer, antidiabetic, and anti-inflammatory activities according to numerous *in-vitro* and in *vivo* research Rao *et al.* [10]. *Lawsonia inermis* is commonly cultivated in numerous tropical locations of Asia, America, and Africa. In this area, apart from decoration, henna is used in the treatment of various skin diseases and wound infections, but information on the potency and contribution of each bioactive components remain scanty. Therefore, the present study aimed to determine the antimicrobial efficacy of phytochemical components of henna on MDR *Pseudomonas aeruginosa* isolates from cases of wound infections.

2.0 MATERIALS AND METHODS

2.1 Bacterial Isolates

A total of 28 MDR *Pseudomonas aeruginosa* isolates were recovered from hundred and seventy-nine (179) aseptically collected wound swabs and processed using standard microbiological techniques, as described by Cheesbrough [11]. The specimens were collected from all groups of in and out-patients attending the Specialist Hospital, Bauchi. Ethical clearance, verbal and written consent of the patients was sought before the collection of specimens and data. Patients with a history of antibiotic usage within the last 48 hours were excluded from the study.

2.1 Preparation of Bacterial Inoculum

Standard preparation of inoculum was prepared according to CLSI [13]. A loopful of each isolated *P. aeruginosa* colonies was inoculated into 4 ml peptone water and incubated at 37^{o}C for 4 hours. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland, which was prepared by mixing 0.5 ml of 1.75% (w/v) Barium chloride dehydrates with 99.5 ml 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately 1×10^{8} colony-forming units per milliliter (cfu/ml) [11].

2.2 Antimicrobial Susceptibility Testing

The *P. aeruginosa* isolates were tested for MDR pattern on Muller-Hinton agar media (Oxoid, UK) plates using the MacFarland standard inoculum. The following antibiotic discs (maxi disc) were used: Ceftazidime (30µg), Cefuroxime (30µg), Cefotaxime (30µg), Gentamycin (10µg), Ciprofloxacin (5µg), Ampicillin (10µg), Erythromycin (30 µg), Amoxicillin (30µg), Imipenem (10µg), Cefepime (30µg), Amikacin (30 µg), Chloramphenicol (30µg), Levofloxacin (5µg) according to Kirby-Bauer disc diffusion methods as described by González-Lamothe *et al.* [12]. *Pseudomonas aeruginosa* isolates that were resistant to 2 or more antibiotics were selected as multidrug-resistant (MDR) and preserved for further use.

2.3 Collection and Processing of Plant Leaves

The fresh leaves of the *Lawsonia inermis* were collected from Bayara within the Bauchi metropolis in polythene bags and transported to Abubakar Tafawa Balewa University Herbarium for identification using relevant keys after then they were taken to Microbiology Laboratory for further processing as described by Jeyaseelan *et al.* [14]. The leaves were cleaned and rinsed thoroughly with tap water and air dried under shade for 15 days. The dried plant material was ground into a fine powder using mortar and pestle in the laboratory and stored in air-tight polythene bags until use.

2.4 Extraction of Plant Leaves

Extraction of plant leaves was carried out using the maceration technique as described by Selvamohan *et al.* [15] with little modification. A 50 grams powder of the dried leaves was weighed using a digital scale and placed in a bottle containing 300ml each with separate solvents (absolute methanol, absolute ethanol, and water). The bottles were capped with aluminum foil and kept at ambient temperature with constant agitation for 72 hours. The macerates were filtered using No. 1 Whatman filter paper and funnel (Pyrex) and kept in a water bath at 45^{0}C for 3 days to evaporate till a semi-solid extract was obtained. The semi-solid extract was weighed, labeled, and stored for further use.

2.5 Phytochemical Screening of the leaf Extracts

The extracts were subjected to various preliminary phytochemical tests to determine the active constituents present in the different extracts, following standard procedures as described by Sofowora [16].

2.6 Preparation of Stock Solution of Extracts

Stock solutions were prepared from the plant leaf extracts by weighing 0.4g of the solid leaf extract and dissolved in 1ml of Dimethyl sulfoxide (DMSO), making a solution of 400mg/ml from where two-fold serial dilution of different concentrations was made (200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml). This was done for each of the leaf extracts (water, methanol, and ethanol) Sofowora, [16].

2.7 Anti-bacterial Screening of the Extracts

The *in-vitro* antibacterial activity of the crude leaf extract of *Lawsonia inermis* was determined using the agar-well diffusion method as described by Akpotu *et al.* [17] with little modification. Mueller-Hinton agar was prepared according to the manufacturer's instructions. The medium was allowed to solidify and the plates were placed in a drier to remove excess moisture. Exactly 0.1ml of the test organism was taken from the standard inoculums of the isolate and streaked over the entire surface of the dried Mueller-Hinton agar. Four holes of 6mm in diameter were made on each plate containing the organism using a cork borer and the plates were marked to indicate the position of the four holes with different concentrations (400, 200, 100, 50, and 25) mg/ml. Exactly 0.2mls of the different concentrations of the leaf extracts were introduced into the holes in the medium. The medium was allowed to stand for 1hr for complete diffusion and was incubated at 37^0C for 24hrs. Imipenem was used as the positive control while dimethyl sulphoxide as the negative control. The zone of inhibition around each well was measured in mm and the experiment was performed in triplicates.

2.8 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the broth dilution method [18,31]. A quantity of 2ml of the varied (concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml) was introduced into 2mls of Mueller- Hinton broth separately in a test tube given ratio 1:1. After which, 0.1ml of the standard suspension of the test organism was added to the test tubes except for the negative control tube. The tubes were then incubated aerobically at 37^0C for 24hrs. The presence of growth or absence of growth at the end of the incubation period was recorded. The least concentration of the extract showing no detectible growth was regarded as the minimal inhibitory concentration.

2.9 Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration of MBC was determined by subculturing 0.1ml from the MIC tubes. The dilution showed no detectable growth on a fresh extract-free solid medium (Mueller- Hinton Agar). The plates were incubated at 37^0C for 24hrs. The lowest dilution that shows no single growth was considered the minimum bactericidal concentration [18, 31, 32].

3.0 RESULTS AND DISCUSSION

3.1 Multidrug Resistant (MDR) characteristics of *P. aeruginosa* isolates

The result of the resistance profile of MDR *P. aeruginosa* is shown in table 1 indicating that *P. aeruginosa* was totally resistant to all the amino-penicillin (Ampicillin and Amoxicillin).

Table 1: Antimicrobial Susceptibility Pattern of *P. aeruginosa* isolates in this study

	No. (%) of *P. aeruginosa* isolates (n=28) and susceptibility pattern	
Antibiotics (µg)	**Sensitive**	**Resistant**
Ampicillin (10)	10(35.7)	18(64.3)
Amoxycillin (30)	10(35.7)	18(64.3)
Cefuroxime (30)	10(35.7)	18(64.3)
Ceftazidime (30)	4(14.29)	24(85.71)
Cefotaxime (30)	6(21.43)	22(78.57)
Cefepime (30)	11(39.2)	17(60.7)
Chloramphenicol (30)	18(64.3)	10(35.7)
Erythromycin (30)	10(35.7)	18(64.3)

Gentamycin (10)	11(39.3)	17(60.7)
Amikacin (30)	12(42.9)	16(57.1)
Imipenem (10)	28(95.5)	0 (0.0)
Ciprofloxacin (5)	16(57.1)	12(42.9)
Levofloxacin (5)	20(71.4)	8(28.6)

(CLSI, 2019)

The isolates also showed high resistance to third-generation cephalosporins, ceftazidime (85.71%), and cefotaxime (78.57%). while the isolates showed moderate activity to the fourth-generation cephalosporins (cefepime), aminoglycosides (gentamycin and amikacin), and fluoroquinolones ranging from 64.3% to 35.7%. *P. aeruginosa* showed high activity with imipenem (95.5%) among all the antibiotics and is generally regarded as the preferred agent for the treatment of infection due to MDR *P. aeruginosa*. The high resistance of our isolates to common drugs used in this area can be attributed to indiscriminate usage like underdosing/incomplete treatment, self-medication, and use of sub-standard antibiotics.

The present study investigated the susceptibility of 28 *P. aeruginosa* to 13 antimicrobial agents. *P. aeruginosa* was observed to be resistant to classes of antibiotics that have been shown by many studies to be active. The result of the resistance profile shows that the MDR *P. aeruginosa* is totally resistant to Ampicillin, Amoxicillin, Erythromycin, and Cefuroxime. High resistance to 85.71% and 78.57% was exhibited by ceftazidime and cefotaxime respectively, while cefepime gentamicin and amikacin show moderate resistance ranging from 64.3% to 35.7%. Although previous reports in Nigeria by Brown *et al.* [19] reported low susceptibilities in their studies with ceftriaxone and cefotaxime. Aibinu *et al.* (2007) [20] also reported close results of these drugs against *P. aeruginosa* in their study. A contrary report or rather decrease in activity was observed for ceftazidime as compared to a previous report (Nwankwo *et al.* [21].

The highest number of susceptibilities of *P. aeruginosa* isolates was recorded for imipenem (95.5%). These results agreed with the findings of Aibinu *et al.* [21] and Iliyasu *et al.* [22] who reported 95.6% and 94.6% susceptibility to imipenem respectively. This implies that imipenem remains a potent anti-pseudomonal antimicrobial agent in Nigeria.

3.2 Phytochemical Screening of the Plant Leaf Extracts

The results of this research revealed the presence of phytochemicals that are present in the plants leaf extracts of *Lawsonia inermis*. The leaf extracts were found to contain saponins, tannins, glycoside, steroids, quinones, flavonoids, terpenoids, and alkaloids (Table 2).

Table 2: Phytochemical Constituents of *Lawsonia inermis* Leaf Extracts

Phytochemicals	***Lawsonia inermis* Leaves extracts and tests inference**		
	Methanol	**Ethanol**	**Aqueous**
Alkaloids	+	_	_
Flavonoids	+	_	+
Tannins	+	+	+
Saponins	+	-	+
Glycosides	+	+	+

Steroids	-	+	-
Quinones	+	-	+
Terpenoids	+	-	-

Key: (+) present, (-) absent

Except for methanolic extract, which possesses alkaloids, flavonoids, glycosides, quinones, saponins, tannins, and terpenoids, alkaloids, and terpenoids are absent in ethanol and aqueous solvent extracts. Flavonoids, glycosides, saponins, tannins, and quinines were present in the aqueous extract while the ethanol extract showed the presence of flavonoids, saponins, tannins, glycosides, and steroids. Similarly, Khaled *et al*. [23] reported the phytochemical analysis of *Lawsonia inermis* which showed the presence of quinones, saponins, tannins, flavonoids, terpenoids, glycosides and, alkaloids which all have good sources of antimicrobial and antioxidant activity [24, 30, 31]. It was observed that all the extracts showed prominent antibacterial activity. Therefore, the chemical constituents present in *Lawsonia inermis* leaf can be used as potential antibacterial compounds for the treatment of various diseases, like wound infections.

3.3 Antibacterial efficacy of *L. inermis* leaf extracts

The antibacterial susceptibility pattern of the methanolic, ethanolic, and aqueous leaf extracts of *L. inermis* confirmed the antibacterial activity against MDR *Pseudomonas aeruginosa* and, the higher the concentration of the extract, the higher the zone of inhibition. The zones of inhibition obtained on the tested isolates were expressed as mean ± SEM (Table 3). The results showed that alcoholic extracts of *L. inermis* had more antibacterial activity than the aqueous extracts, which is similar to that previously reported by Al-Rubiay *et al.* [25]; Jothipraksam *et al.* [26]. On the other hand, Merdaw [27] reported that the water extract of henna was clearly superior. These compounds are most often obtained by ethanol or methanol extraction, so it is not surprising that the result of this study and some other studies show more antibacterial activity than the aqueous. This could be due to the better solubility of the active components in organic solvent De Boer *et al.* [28].

Table 3: Mean zone of inhibition of *L. inermis* on MDR *Pseudomonas aeruginosa*

Concentrations (mg/ml)	S.E±Mean Effects		
	Methanol	**Ethanol**	**Aqueous**
400	9.500 ± 0.167^{b}	7.667 ± 0.167^{b}	7.333 ± 0.208^{b}
200	7.667 ± 0.167^{c}	6.167 ± 0.167^{c}	3.833 ± 0.208^{c}
100	5.500 ± 0.167^{d}	4.333 ± 0.167^{d}	2.500 ± 2.500^{d}
50	3.333 ± 0.167^{e}	2.667 ± 0.167^{e}	1.667 ± 0.208^{e}
25	2.333 ± 0.167^{f}	1.167 ± 0.167^{f}	0.833 ± 0.208^{e}
Positive control	13.83 ± 0.167^{a}	13.83 ± 0.167^{a}	13.83 ± 0.208^{ab}

Negative control	0.000 ± 0.167^{g}	0.000 ± 0.167^{g}	0.000 ± 0.208^{f}

Each value is a mean of ± standard error of three replicates. Means followed by same superscripts in the column are not significantly different from each other.

Table 4: Minimum Inhibitory and Minimum Bactericidal Concentrations of *Lawsonia inermis* on MDR *P. aeruginosa* isolates

Extracts	Test and Concentration (mg/ml)	
	MIC	MBC
Methanol	50	100
Ethanol	100	200
Aqueous	200	400
+ve control	25	50
- ve control	0.00	0.00

3.6 Minimum Inhibitory and Bactericidal Concentration of *L. inermis* leaf extracts against MDR *Pseudomonas aeruginosa*

The minimum inhibitory and minimum bactericidal concentration of *Lawsonia inermis* leaf extracts against MDR *Pseudomonas aeruginosa* are summarized in table 4. The methanolic extract was more active with lower MIC and MBC when compared to the other extracts. However, the antibiotic used as a control (Imipenem) was more active against MDR *Pseudomonas aeruginosa* when compared with all the extracts tested.

Conclusion

According to the findings of this study, it was concluded that alcoholic leaf extracts of *L. inermis* possess high antibacterial activities on MDR *P. aeruginosa* than the aqueous which may be due to the active contents present the leaf extracts, although somewhat less than the synthetic standard drugs (Imipenem). Hence, if further purified, it would be a good source for the formulation of the drug for the treatment and management of wound infections. Further search for novel natural drugs as an alternative therapeutic option in the management of *P. aeruginosa* infections is highly advocated.

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Competing Interests:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Omole A, Stephen E. Antibiogram profile of bacteria isolated from wound infection of patients in three hospitals in Anyigba, Kogi Sate, Nigeria. FUTA J Res. Sci. 2014; (2), 258–266.
2. Matsuura GT, Barg N. Antimicrobial management of foot infections in patients with diabetes. J. Clin. Diabetes. 2013; 31, 59-65.
3. Sani RA, Garba SA, Oyewole OA. Antibiotic resistance profile of gram-negative bacteria isolated from surgical wounds in Minna, Bida, Kontagora, and Suleja Areas of Niger State. American J. Med. Sci. 2012; 2(1), 20–24.
4. Behnke M, Aghdassi SJ, Hansen S, Diaz L, Gastmeier P, Piening B. The prevalence of nosocomial infection and antibiotic use in German hospitals. Deusches Arzteblatt Intl.., 2017; 114(50):851-7.
5. Zulianello L, Canard C, Köhler T, Caille D, Lacroix JS, Meda P. Rhamnolipids are virulence factors that promote early infiltration of primary human airway epithelia by *P. aeruginosa*. J. Infect. Immunol. 2006; 74. 3134-3147.
6. Okeke I, Lamikanra A, Adelman R. Socioeconomic and behavioural Factors Leading to acquired bacterial resistance to antibiotics in developing Countries. Emerg. Infect. Dis.1999; 5(1): 18-27
7. Bekalo TH, Woodmatas DS, Woldemariam ZA. An ethnobotanical study of medicinal plants used by local people in the lowlands of Konta Special Woreda, Southern Nations, nationalities and peoples regional state, Ethiop. J. Ethnobiol. Ethnomed. 2009; 5(10):4269-26.
8. González-Lamothe R, Mitchell G, Gattuso M, Moussa S, Malouin DF, Bouarab K. (2009). Plant antimicrobial agents and their effects on plant and human pathogens. Intl. J. Molec. Sci. 2009;10(8):3400–3419.
9. Abdulmoneim M, Saadabi MA. (2007). Evaluation of *Lawsonia inermis Linn*. (Sudanese Henna) leaf extracts as an antimicrobial agent. Res. J. Bio. Sci. 2(4), 419–423.
10. Rao SS, Regak Pl, Signh YV. Angotecnques foe henna (*Lawsonia inermis*) cultivation improvement and trade. Central Arid Zone Research institute, Palinmarwar, 2005; 25-27.
11. Cheesbrough M. District laboratory practice in tropical countries Part 2. 2016; Cambridge University Press
12. González-Lamothe R, Mitchell G, Gattuso M, Moussa S, Malouin DF, Bouarab K. (2009). Plant antimicrobial agents and their effects on plant and human, J. Molec. Sci. 2009;10(8):3400–3419.
13. Clinical and Laboratory Standards institute. (2019). Performance standards for antimicrobial susceptibility testing. Twentieth informational supplement ed. CLSI document M100-S20. Wayne, PA: CLSI.93, 456-789.
14. Jeyaseelan EC, Pathmanatan MK, Jeyadevan JP. inhibitory effect of different solvent extracts of negundo L. and Allium Sativum L. against phytogenic bacteria. Arch. Applied Sci. Res. 2010; 2(6): 325-331.
15. Selvamohan T, Ramadas V, Kishore SS. Antimicrobial activity of Selected Medicinal Plants against some Selected human Pathogenic bacteria. Adv. Appl. Sci. Res. 2012; 3(5): 3374-3381

16. Sofowora A. Medicinal Plants and Traditional Medicine Africa. Spectrum books Ltd. Ibadan, Nigeria. 1984; Pp 135-221
17. Akpotu, M.O, Eze P.M, Abba C.C, Nwachukwu C.U, Okoye F.B. C. & Esimone, C.O Metabolites of endophytic fungi isolated from *Euphorbia hirta* growing in Southern Nigeria. Chem. Sci. Rev. Lett. 2017; 21:12-19.
18. El-Mahmood AM, Ameh JM. *In-vitro* antibacterial activity of *Parkia biglobosa* (Jacq) root, bark extract against some microorganisms associated with Urinary tract infections. Afr. J. Biotechnol. 2007; 6(11):195-200.
19. Brown BJ, Asinobi AO, Fatunde OJ, Osinusi K, Fashina NA. Antimicrobial sensitivity pattern of organisms causing urinary tract infection in children with sickle cell anaemia in Ibadan, Nigeria. WAJM. 2003; 22, 110-113.
20. Aibinu I, NwannekaT, Odugbemi T. Occurrence of ESBL and MBL in Clinical Isolates of *Pseudomonas aeruginosa* from Lagos. Nig. J. American Sci. 2007; 3, 81-85.
21. Nwankwo EOK, Shuaibu SA. Antibiotic susceptibility pattern of clinical isolates of *Pseudomonas aeruginosa* in a tertiary health institution in Kano. Nigeria. J. Med. and Biomed. Sci. 2010; 37-40.
22. Iliyasu MY., Mustapha I., Yakubu H., Shuaibu, H. M., Umar, A. F., Agbo , E. B., Uba A., & Deeni, Y. Y.. Genotypic Detection and Characterization of Adhesins in Clinical Escherichia coli Isolates. 30(8): 84-93, 2020; MRJI.59461 ISSN: 2456-7043.
23. Lambert PA. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa. J.* Royal Socie. Med. 2002; 95 (suppl. 41), 2-6.
24. Parivuguna V. Antimicrobial properties and Phytochemical constituents of Rheo discolor Hance. Ethnobot. Leaflets. 2008; (1), 114.
25. Al-Rubiay KK, Jaber NN. Al-Mhaawe BH `Alrubaiy, L. K. "Antimicrobial efficacy of Henna extracts". Oman Med. J. 2008; 23(4), 1-4.
26. Jothipraksam V, Ramesh S, Kumarn RS. Preliminary phytochemical screening and antibacterial activity of *Lawsonia inermis* (henna) leaf extracts against reference bacterial strains and clinically important AMPC beta-lactamases producing Proteus mirabilis. Intl. J. Pharm. Pharm. Sci. 2013; 5(1):219–222.
27. Merdaw MA. Inhibition of bacterial growth by *Lawsonia inermis* (henna) leaf extracts in vitro. Ibn Al-Haitham J. Pure and Appl. Sci. 2009; 22. (4), 32
28. De-boer JH, Kool A, Broberg A. William RM. Anti-fungal and antibacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol. 2005; 96 (3), 461-469.
29. Garba L, Lawan HS, Yusuf I, Abdullahi MM, Mukhtar MD, Puma HU. Phytochemical screening and *in vitro* Bacteriostatic effects of *Syzygium aromaticium* on clinical bacterial isolates. J. Biochem. Microbiol & Biotechnol. 2019; 17(1):5-9.
30. Kabeer ZM, Iliyasu MY Abdulrazak MH, Umar AF Antibacterial Activity of the Composite Mixture of *Senna siamea* Leaves and Tamarind Extracts on Multidrug Resistant *Salmonella typhi*. Eur. J. Med. Plants 2022, 33(3), 1-7.
31. Salisu A, Iliyasu MY, Sahal MR, Titus I, Ismai'l S, Umar RD, Tahir H, Kabeer ZM, Idris AH, Musa HS, Agbo EB Antimicrobial Potential of *Syzygium aromaticium* (Clove) Extracts on Multidrug-Resistant (MDR) Uropathogenic Bacteria Isolated

from Clinical Specimens in Bauchi, Nigeria. *Journal of Advances in Medical and Pharmaceutical Sciences*, 24(11), 2023, 45-57.
32. Ishaque M, Bibi Y, Moshood S, Nisa S, Qayyum, A. (Iriflophenone-3-C-β-d Glucopyranoside from *Dryopteris ramosa* (Hope) C. Chr. with Promising Future as Natural Antibiotic for Gastrointestinal Tract Infections. Antibiotics, 2022, 11(3), 301-308.