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ORIGINAL RESEARCH

In Vitro Inhibitory Potential of *Lawsonia inermis* Extracts against Multidrug Resistant Clinically-Relevant Bacteria: a Phytochemical, Quantitative Antimicrobial and Toxicological Assessment

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Abstract
Objective: Majority of the current antibiotics have become less effective due to widespread of multidrug-resistant microorganisms. Medicinal plants are promising candidates that could be used to manage this menace. Therefore, phytochemical, toxicological and antimicrobial potentiality of *Lawsonia inermis* extracts against MDR clinical bacteria were carried out.
Material-Method: Henna leaf and seed were extracted by cold maceration technique using methanol and water and screened phytochemically. Eight MDR isolates, four of which are ESβL-producers were used for this study. *In vitro* antimicrobial efficacy and quantitative antimicrobial potency of extracts were estimated. MIC and MBC were determined using broth macrodilution technique. Cytotoxicity test was conducted using brine shrimp lethality assay and LC_{50} was determined.
Results: The findings of this study revealed that aqueous leaf extract possesses maximum percentage yield of 25.58%. Tannins and phenolic compounds were detected in all extracts, while steroid was absent. Methanol seed extract showed the highest antimicrobial efficacy against all bacteria with 100 percent activity. The highest and lowest zones of inhibition were recorded at 30.0±0.00 and 10.0±0.00 mm, respectively. The zones of inhibition of extracts differed significantly. All extracts displayed highest activity index against the ESβL-producing *Enterobacter aerogenes* 196 that was isolated from wound with highest value at 4.28. *Pseudomonas aeruginosa* U_{109} showed maximum susceptibility index (93.75%); majority of MIC values recorded were within the range of 1.95-62.5 mg/mL. Cytotoxicity test of methanol and aqueous extracts displayed $1000<LC_{50}>1000$, respectively.
Conclusion: Findings from this study elucidate the efficacy of *Lawsonia inermis* as a potential remedy to manage MDR-related infectious bacteria.
Keywords: Antimicrobial Resistance, Multidrug Resistant Microorganisms, ESBL, Antimicrobial Activity, Indigenous Plant, Phytotherapy.

INTRODUCTION

Antibiotics, the 20th-century wonder drugs, have played a major role in the treatment of infectious diseases the world over[1]. However, in part, as a result of irregular, irrational, inappropriate and extensive use of these drugs, antimicrobial resistance (AMR) has surfaced and has led to the development and widespread of multidrug-resistant (MDR) pathogenic bacteria[2]. Antimicrobial resistance is now a leading cause of death globally. Typically of COVID-19 that has swept across the globe; another pandemic (AMR) is spreading silently and rapidly with no regard for border, race or colour[3]. In the Review on Antimicrobial Resistance, 700,000 deaths a year are attributed to AMR and regrettably estimated more 10 million lives each year to be lost in 2050[4]. Not far-fetch, report from first comprehensive global burden associated 4.95 million lives with and attributed 1.27 million deaths to bacterial antibiotic-resistant infections, in 2019 alone[5], hence corroborating earlier report. Yet, the invention of new drugs has reduced drastically in the last three decades[6], and resistance to microorganisms continues at a faster pace. The development of new therapy threatens global efforts to contain drug-resistant infections, where previously treatable illnesses are (becoming) hard-to-treat and even kill[7].
Medicinal plant, however, shows promising effect

in mitigating, if not eradicating the problems of antibiotic resistance. Unlike conventional medicines that microbes find easy to develop resistance to due to a single active ingredient for the same therapeutic target, herbal medicine uses a combination of efficacious natural active ingredients to breakdown the cell wall and cell membrane of microorganisms, which can lead to the release of cellular contents, protein binding domain disruption, enzyme inactivation, and ultimately leading to cell death[8,9]. More specifically, a 1000-year-old antimicrobial remedy was formulated from Bald Leechbook using the ancient's technique and found to be more effective than conventional drug, vancomycin, against methicillin-resistant *S. aureus*[10]. Moreover, medicinal plants continue to enjoy human patronage because they are cheap, readily available and free from side effects often associated with conventional antibiotics[11]. Antimicrobial activities of useful plants vary: the majority act in synergy, reducing the side effect of synthetic drugs while others act as quorum quenchers[9,12].

Lawsonia inermis L. (synonym *Lawsonia alba*), is the sole species of the genus *Lawsonia* belonging to the family *Lythraceae*[13]. It is popularly called 'Mehndi' or 'Henna'; 'Laali' among the Yoruba-speaking people, 'Lalle' among the Hausa-speaking people. Henna is famed for its cosmetic uses worldwide and continuous use for celebrations of women's fertility and marriage in the eastern Mediterranean since the Bronze Age[14,15]. Its seeds have been reported to possess deodorant action and are used in most cases of gynecological disorders[16]. The potency of the plant has been evaluated against an extremely large variety of human pathogenic bacteria. In most cases, aqueous extracts, employing hot or cold water, methanol, ethanol and even acetone, of the leaves or whole plant, and in a few cases, the stems and bark, have been found to have the highest efficacies[14]. This corresponds to the traditional intake of decoctions prepared from the leaves of henna for variety of ailments associated with bacterial infections[17,18]. Studies on *L. inermis* leaf extracts demonstrated antibacterial activity against Gram-positive and Gram-negative bacteria[19,20]. Its extract act against pathogenic organisms from Urinary Tract Infection and found methanol leaf extracts had various degrees of antibacterial activities[21]. Similarly, clinical isolates from wound infection were treated with extracts of the leaves using agar well diffusion methods[22]. Results showed that the henna leaves extracts were able to inhibit the growth pattern of *A. niger* and *F. oxysporum, Streptococcus* sp. and *S. aureus*. Decoction of its leaf is used for aseptic cleaning of wounds and healing[23], and this suggests the wound healing management and potential of the plant.

With the current scientific and ethnomedicinal report of *L. inermis*, we therefore proceeded to investigate the phytochemical, antimicrobial and toxicological properties of aqueous and methanol extracts of this plant (leaf and seed), against multidrug resistant and ESβL-producing clinical bacteria that were isolated from urine, wound, sputum and amniotic fluid samples.

MATERIAL AND METHODS

Collection and processing of plant materials

L. inermis plant was collected from a nearby plantation in Akinyele Local Government, Ibadan with the assistance of the Chief Technologist, Herbarium, Department of Botany, UI. The leaves and seeds of the plant were immediately plucked from the stem, washed thoroughly with clean water and then air-dried away from the sun- and fluorescence light. The plant materials were milled twice into a very fine powder using an electrical blender (Model: BL1085BA-CB) disinfected with 70% ethanol. It was then stored in a sealed air-tight container under dark conditions at room temperature for further use.

Extraction of plant materials

The plant materials were weighed using an electric weighing balance (Model: YP-B100002) into sterile bottles for the cold maceration technique, as described by Gull *et al.*[20] and Habbal *et al*[24] with slight modifications. A ratio of 1:6 plant materials to solvents was employed; this is presented in Table 1. Methanol was used as an organic solvent while sterile distilled water served as an aqueous extractant. The extracts were chosen based on the ethnomedicinal preference as reported by Idowu[25]. All bottles were properly covered and left for three days with frequent agitation. After 72 hours of cold maceration with frequent agitation, muslin cloth was first employed to remove particles and ease the passage through filter paper. Accordingly, the contents were filtered through a four-layered muslin cloth, then through a Whatman filter paper No.1 and, where necessary, cotton pluck was employed. All filtrates of the same extract were pooled together and concentrated. While all organic extracts were concentrated *via* a vacuum rotary

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evaporator; the aqueous extracts were freeze-dried to avoid the denaturation of the active constituents. These were stored at 20ºC for further use.

The percentage yields of crude extracts were calculated as follows:

$$\textbf{Extraction yield}\ (\%) = \frac{\text{Weight of the concentrated extract (g)}}{\text{Weight of the milled dried plant sample used for the extraction (g)}} \times 100$$

Table 1. Plant to solvent ratio (1:6) of *L. inermis* extraction

Plant Part	Plant material (g)	Methanol (mL)	Aqueous (mL)
Leaf	321	1926	1926
Seed	450	2700	2700
Overall (solvent)	NA	4626	4626

NA: Not applicable

Phytochemical screening of *Lawsonia inermis* (leaf and seed) extracts

The following phytochemical screening of aqueous and methanol extracts of *L. inermis* was performed to determine the phytoconstituents present in the plant materials, using standard methods: terpenoids, tannins[26]; phenolic compounds[27]; quinones, cardioglycosides[20]; saponins, alkaloids[28]; steroids[29]; flavonoids[30]; glycosides[31]; proteins and amino acids[32].

Source and maintenance of isolates

All the isolates used in the present investigation were eight (8) MDR bacterial strains from clinical sources; four (4) of which are ESβL-producers. They were obtained from the Microbiology Department Culture Collection, UI and have been characterized up to molecular level. Table 2 shows their names, assigned codes, resistance phenotype as well as the specific niche they were isolated from. Upon plating, they were preserved on nutrient agar slant. Pure cultures obtained by subculturing on the same medium were used for further studies.

Table 2. Source of isolates used in this study

	Assigned code	Name of Isolate	Source	Resistance Phenotype
	U_9	*Acinetobacter baumannii*	Urine	AMC, CTX, CPD, CIP, GEN, TET
	U_{30}	*Klebsiella oxytoca*	Urine	CTX, CPD, CIP, GEN, TET
	U_{87}	*Salmonella enterica*	Urine	CTX, CPD, CIP, GEN, TET
	U_{109}	*Pseudomonas aeruginosa*	Urine	AMC, CTX, CPD, CIP, GEN, TET
ESβL-PRODUCERS	129	*Klebsiella pneumoniae*	Urine	CRO, CIP, CN, FEP, SAM, AMC, ATM
	190	*Escherichia coli*	Amniotic fluid	AZM, CAZ, CPD, CTX
	195	*Enterobacter cloacae*	Sputum	FOX, CN, FEP, SAM, AMC
	196	*Enterobacter aerogenes*	Wound	CRO, CIP, CAZ, FOX, CN, FEP, SAM, AMC, ATM

AMC: Amoxicillin/Clavulanate
CTX: Cefotaxime
CPD: Cefpodoxime
CIP: Ciprofloxacin
GEN: Gentamicin
TET: Tetracycline
CRO: Ceftriaxone
CN: Cefalexin
FEP: Cefepime
SAM: Ampicillin + Sulbactam
ATM: Aztreonam
AZM: Azithromycin
CAZ: Ceftazidime
FOX: Cefoxitin

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Determination of antimicrobial efficacy

In vitro antimicrobial efficacy of extracts was evaluated by agar well diffusion method, as described by Ali *et al*.[33] and Rajput and Kumar[34] with minor modifications. Ciprofloxacin disc (Oxoid) was used as positive control while 40% (v/v) methanol served as the organic diluent and negative control[35] as it was expected to be inactive against the isolates.

Standardization of inoculum

McFarland standard was used as a reference to adjust the turbidity of microbial suspension so that the number of microorganisms will be within a given range (1.5×10^8 CFU/mL). The standard was prepared as previously described by Andrews[36] and Cheesbrough[37]. The test isolates were resuscitated from agar slant using a sterile wire loop to touch the surface and streaked on a nutrient agar plate. Upon 18-24 hours of incubation period, an inoculum suspension equivalent to 0.5 McFarland standards was prepared.

Preparations of test samples

The test extracts were prepared by dissolving the crude extracts in sterile distilled water (aqueous extract) or 40% methanol (v/v) (organic extract). Forty percent (40% v/v) methanol was prepared by measuring 40 mL of methanol and dispensed into 60mL of sterile distilled water. Four different concentrations were prepared which include 1000 mg/mL, 500 mg/mL, 250 mg/mL and 125 mg/mL for each extract.

Performance of test assay

After preparation and standardization of inoculum and test extracts, the bioassay was conducted to determine the antimicrobial activity of the extracts. Accordingly, a sterile swab stick was dipped into the prepared inoculum suspension to take up the cells. The swab stick was used to spread (lawn) the cell suspension evenly on the entire surface of the Mueller Hinton agar (Oxoid) plate from edge to edge, turning the plate at every 60° between streaking while turning the swab stick, too. Then, using a sterile cork borer, a well of 8mm diameter was aseptically bore on the Mueller Hinton agar plate and labeled appropriately. Thereafter, an aliquot of 100µL of each test sample of varying concentrations was carefully pipetted into each well. Plates were left to diffuse at room temperature for 1-2 hours, and they were subsequently incubated at 37°C for 24 hours. Ciprofloxacin disc served as the positive control while 40% methanol as the negative control. Results of zone of inhibition were read and reported in millimeters. The mean and coefficient of variation of replicate values were recorded.

Statistical analysis

The raw data of replicate values of the zones of inhibition were computed using Microsoft Excel (2010). Data were then exported to IBM SPSS Statistical Package (25.0 version) for statistical analysis. The effects of all the four extracts on the isolates, Multiple Comparison Tests within each test sample and concentration against each isolate were analyzed using two-way analysis of variance (ANOVA) adopting Univariate General Linear Model at significant value $P < 5\%$.

Quantitative estimation of antimicrobial potency

The following quantitative estimate was performed on the antimicrobial susceptibility testing to determine the percentage and activity index of the extracts, and bacterial susceptibility index of the isolates, as adopted by Rajput and Kumar[34].

Percentage activity

Percentage activity (PA) shows the total antimicrobial potential of a particular extract on test microbial strains or, it demonstrates the number of test isolates susceptible to all concentrations of a particular extract. This ranges from 100 (where all the concentrations of extract tested were effective against all test isolates) to 0 (where all tested concentrations of a particular extract did not exhibit any inhibitory activity against all test isolates).

Mathematically expressed as:

$$PA= \frac{\text{No of isolates susceptible to all tested concentration of a specific extract}}{\text{Total number of concentrations of specific extract tested against each isolate}} \times 100$$

Activity index

The activity index (AI) was calculated to express the relationship between the zones of inhibition of the extract to that of reference antibiotics. When AI is greater than 1, the test extract is better in activity than the reference antibiotic; however, where the result is less than 1, it reveals that the reference antibiotic is better. This is to determine how efficacious the test extracts are in inhibiting test bacteria based on zones ratio.

$$AI = \frac{\text{Mean of diameter of the zone of inhibition with regards to each concentration of extract}}{\text{Diameter of the zone of inhibition of the reference antibiotic}}$$

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Bacterial susceptibility index
Bacterial susceptibility index (BSI) is used to compare the relative susceptibility pattern between all isolates tested against each extract. The value of BSI may range from 0 (resistance to all extracts) to 100 (susceptible to all the tested concentrations of extracts). It estimates how susceptible the test bacteria are to the test extracts.

$$\text{BSI \%} = \frac{\text{Number of concentration of extracts effective against each isolate}}{\text{Number of concentration of specific extract tested}} \times 100$$

Determination of minimum inhibitory concentration and minimum bactericidal concentration
The minimum inhibitory concentration (MIC) was employed to determine the lowest concentration of an extract that will inhibit the visible growth of an organism after incubation. The MIC was determined using the broth macrodilution method as described by Andrews[36] with slight modifications.
Inoculum suspension and standardization for MIC
Isolates were resuscitated from agar slant as described above. After 18-20 hours of incubation, a loopful of inoculum was transferred into Mueller Hinton broth (Oxoid) and incubated to match 0.5 McFarland standards.
Preparation of test extract for MIC
The test extract was prepared by a double (two-fold) serial dilution in Mueller Hinton broth for a range of 10 concentration gradients (1000 to 1.95 mg/mL). A concentration of 20% (v/v) of methanol was used to prepare the stock solution of organic diluent (methanol extract) which also serve as negative control while sterile distilled water was used as aqueous stock solution diluent. The 20% methanol was used here because of its inability to interfere with the result in MIC, unlike the 40% in agar well diffusion.
Determination of MIC
One milliliter of each test extract was pipetted into sterile test tubes followed by the addition of an equal volume of test isolates. The content of the tubes was thoroughly mixed to achieve an even distribution. For each evaluation, methanol (20% v/v) plus each test isolate (MI) was used as a negative control to ascertain the influence of methanol on the assay; ciprofloxacin plus each test isolate (CI) serve as the positive control. Additionally, another set of control assays were used which include: test isolates plus broth (BI) in one part (to observe the adequacy of the broth in supporting the growth of the test isolates); and extract alone, sterility test (the lowest, 10th concentration gradient, i. e. 1.95 mg/mL) in another part (to check for the sterility of test extract). All tubes including inoculated and uninoculated extract-free tubes were incubated at 37ºC for 24 hours. The MIC endpoint was read as the lowest concentration of test extract at which there was no visible growth.
Determination of MBC
The minimum bactericidal concentration (MBC) is the least concentration at which there was no obvious growth on the agar plate from MIC tube suspension. This was determined by considering the least inhibitory tube (MIC) and other tubes with a higher concentration gradient (that is, the tubes with no visible growth/turbidity). Specifically, a sterilized wire loop was dipped into each corresponding test tube that shows no turbidity, it was then streaked on nutrient agar plates and they were incubated at 37 ºC for 24 hours. At the end of the incubation period, the plates were examined for the presence or absence of growth. MBC was recorded as the least concentration at which no bacterial growth was observed on the plate.
Brine shrimp lethality assay (Cytotoxicity Test)
The cytotoxicity assay was performed to determine the toxicity profile of the test extracts as previously described by Ojewunmi *et al.*[26] with minor modifications. Seawater was obtained from Lagos Bar Beach and cleared off of any obvious impurities by filtering dirties and sand particles.
Hatching of shrimp eggs
A rectangular container with an unequal-internal-demarcation was procured and perforated for the hatching process. Accordingly, the container was half-filled with seawater, and Brine Shrimp Eggs (*Artemia salina* Leach) was gently sprinkled into the smaller compartment of the container. Then, using a blank sheet, the sprinkled side was covered leaving the other side opened. It was expected that the nauplii, upon hatching, would swim to the other light portion as a result of their phototropicity. This procedure was conducted in an undisturbed, well-ventilated and constantly illuminated environment.

After 48 hours of incubation, there were enough freshly hatched nauplii, and these were used for the bioassay.

Performance of brine shrimp lethality assay

In a sterile bottle, 0.05g of each test extract was weighed and 10 mL of seawater was added to make a stock solution of 5000 µg/mL. Potassium dichromate solution was used as a positive control, while seawater served as a negative control. Various concentrations: 1000, 100 and 10 µg/mL of the test extract was prepared from the stock solution. Each test tube contained a final volume of 5 mL each plus 10 nauplii with the aid of a Pasteur dropper, and was carried out in triplicate. The setup was allowed to stand in a ventilated, undisturbed space for 24 hours under constant illumination. After 24 hours of incubation at room temperature, the survived nauplii in each tube assay was counted with a source of light and the average of each of the three (3) tubes was determined. The percentage (%) mortality of the Brine Shrimp nauplii was calculated for each concentration accordingly using the following formula:

$$\% \text{ Mortality} = N_1/N_0 \times 100$$

Where,
N_1 = Total number of killed nauplii after 24 hours of incubation at room temperature
N_0 = Total number of nauplii transferred.

Probit was calculated using the standard probit table. Median Lethal Concentration (LC_{50}) was computed using probit analysis by plotting the mortality rate against dose.

RESULTS

Plant extraction

The total weight of the plant material used, yields, extraction yields and the morphological characteristics of all the four extracts are summarized in Table 3.

Table 3. Percentage yields and morphological observation of *L. inermis* extracts

Parameters	SA	LA	SM	LM
Weight (g [W_1])	450	321	450	321
Yields (g [W_2])	36.8	82.1	83.5	54.8
% yields	8.18	25.58	18.56	17.07
Consistency	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Texture	Gummy/jelly	Gummy/jelly	Neither gummy/nor jelly	Gummy/jelly
Appearance	Light brown	Yellowish-brown	Light brown	Dark brown

SA: Aqueous extract of *L. inermis* seed SM: Methanol extract of *L. inermis* seed
LA: Aqueous extract of *L. inermis* leaf LM: Methanol extract of *L. inermis* leaf

It was observed that, despite the least dry weight of plant material used in leaf extraction (321g) compared to seed (450g), aqueous leaf extract (LA) had highest percentage yield (25.58%), and it was observed to be yellowish-brown, while aqueous seed extract (SA), had the least (8.18%). Methanol leaf and seed extract (LM and SM) had similar yield (with 1.5% differences). The consistency of all the extract remained in semi-solid form till the end of the study.

Phytochemical Screening of *L. inermis* extracts

Of all the eleven phytochemical compounds screened, tannins and phenolic compounds were present while steroids were absent in all (Table 4). All extracts had at least five of the screened phytoconstituents. Terpenoids were only detected in both LM and SM. There was an absence of quinones and alkaloids in all extracts excluding SA which also had the highest screened phytoconstituents (9).

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Table 4. Phytoconstituents present in the aqueous and methanol extracts of *L. inermis* (leaf and seed)

S/N	Screening	Reactions	SM	LM	SA	LA
1	Terpenoids (Salkowski's test)	5mL extract + 2mL Chloroform + 3mL conc. H_2SO_4	+	+	–	–
2	Tannins	Extract + few drops 0.1% $FeCl_3$	+	+	+	+
3	Phenolic compounds	1mL extract + 3 drops 5% $FeCl_3$	+	+	+	+
4	Quinones	1mL extract + 1mL NaOH	–	–	+	–
5	Steroids (Salkowski's test)	1mL extract + 1mL H_2SO_4	–	–	–	–
6	Saponins	Extract + H_2O (Shake vigorously)	+	–	+	+
7	Alkaloids	Extract + Chloroform + HCl + allow to stand + Chloroform layer + Dragendoff reagent	–	–	+	–
8	Flavonoids	1mL extract + 3 drops NH_3^+ + 0.5mL conc. HCl	+	–	+	+
9	Cardioglycosides	5mL extract + (2mL glacial acetic acid + a drop $FeCl_3$) + 1mL conc. H_2SO_4	+	+	+	–
10	Proteins	Extract + few drops conc. HNO_3	–	+	+	+
11	Glycosides	Extract + $FeCl_3$ + boiled$_{5mins}$ + cooled + equal volume of benzene + benzene layer separated + NH_3^+	+	+	+	–

SA: Aqueous extract of *L. inermis* seed
LA: Aqueous extract of *L. inermis* leaf
SM: Methanol extract of *L. inermis* seed
LM: Methanol extract of *L. inermis* leaf
+: present
–: absent

Source and percentage occurrence of the multi-drug resistant bacterial strains

A total number of eight (8) MDR and ESβL-producing clinical bacterial strains were obtained for the present study. The percentage occurrences are as follows: urine with 62.5% and others (amniotic fluid, sputum and wound) only had 12.5% each.

Antimicrobial efficacy of *Lawsonia inermis* extracts

The *in vitro* antibacterial activity of *L. inermis* extracts (leaf and seed) and the reference antibiotics against MDR isolates were evaluated based on the presence or absence of a clear zone of inhibition. This is summarized in Table 5. SM had the highest zone of activity against all tested MDR bacteria. It can be noticed from the results that ESβL-producing *Enterobacter aerogenes* 196 was the most sensitive strain with 30.0±0.00 mm zone of inhibition while *Klebsiella oxytoca* U_{30} was the least sensitive with 11.5±0.06 mm. The SA had the highest zone of inhibition (26.5±0.02 mm) against *Pseudomonas aeruginosa* U_{109}, and the least (10.0±0.00 mm) against each of ESβL-producing *E. aerogenes* 196 and *E. cloacae* 195.

The resistance pattern of the clinical isolates was so pronounced as depicted by the results of reference antibiotics (where 62.5% of the isolates were found to be resistant). The leaf extracts (LA and LM) revealed huge variations in their potency. *Pseudomonas aeruginosa* U_{109} was the only susceptible isolate to all tested concentrations of LM; ESβL-producing *Escherichia coli* 190 on the other hand, was only susceptible to LA at 1000 mg/mL (15.5±0.04 mm). As fathomed from the study, the majority of the clinical isolates showed no activity to LA at 250 mg/mL and below, in fact, none was susceptible at 125 mg/mL. As expected, the 40% (v/v) methanol was not active against all isolates.

All the four extracts at different concentrations tested against all isolates differed significantly at $p < 5\%$. In addition, when multiple comparisons of the activity of the extracts and concentrations against each isolate were analyzed, all were found to be statistically significant at $p < 5\%$.

Quantitative estimation of antimicrobial efficacy

As observed from Table 5 below, the percentage activity reveals the totality or effectiveness of an extract to all tested MDR microbial strains. SM showed the most efficacious antibacterial activity against all the multiple drug-resistant and ESβL-producing isolates. That is, the isolates were 100% sensitive to all the tested concentrations of the crude extract. SA gave the second maximum activity (93.75%) against all strains followed by LM (71.88%) while LA was found to possess the least (56.25%) but still better than the reference antibiotic (37.5%).

Table 5. Antimicrobial Efficacy of aqueous and methanol extracts of *L. inermis* against MDR clinical isolates

Extract	Conc. tested (mg/mL)	Diameter of zones of inhibition (in mm)* on test isolates								Percent Activity (%)
		ESβL-Producing isolates								
		196	**129**	**190**	**195**	**U_{109}**	**U_{9}**	**U_{30}**	**U_{87}**	
SA	1000	13.5±0.15	15.0±0.09	21.0±0.06	20.0±0.07	26.5±0.02	22.5±0.03	18.0±0.00	20.0±0.20	93.75
	500	15.0±0.18	14.0±0.10	17.5±0.04	16.5±0.12	21.0±0.06	19.5±0.03	13.5±0.05	11.0±0.12	
	250	11.0±0.00	10.5±0.06	13.5±0.05	10.0±0.00	16.0±0.00	17.0±0.00	13.0±0.00	12.0±0.23	
	125	10.0±0.00	—	13.0±0.00	—	14.5±0.04	13.0±0.10	12.0±0.00	11.0±0.00	
LA	1000	11.5±0.06	14.0±0.00	15.5±0.04	13.0±0.00	13.5±0.05	12.0±0.11	13.0±0.10	11.0±0.00	56.25
	500	11.0±0.00	12.5±0.16	—	10.5±0.06	14.0±0.10	12.0±0.11	11.5±0.06	11.0±0.00	
	250	—	—	—	—	11.0±0.00	11.0±0.12	11.0±0.00	—	
	125	—	—	—	—	—	—	—	—	
SM	1000	30.0±0.00	19.5±0.03	20.0±0.00	22.5±0.03	22.0±0.06	21.0±0.00	18.5±0.03	15.5±0.04	100
	500	28.0±0.00	19.0±0.00	15.0±0.00	22.5±0.03	23.0±0.06	17.5±0.04	18.5±0.03	14.0±0.00	
	250	22.5±0.03	19.0±0.00	19.0±0.00	24.5±0.02	20.0±0.00	16.0±0.00	14.5±0.04	15.0±0.00	
	125	20.5±0.03	12.0±0.11	12.0±0.00	20.0±0.00	16.0±0.00	15.0±0.00	11.5±0.06	13.0±0.00	
LM	1000	14.5±0.04	15.0±0.00	14.5±0.04	16.5±0.04	13.0±0.00	14.0±0.00	16.5±0.12	14.0±0.00	71.88
	500	11.0±0.00	12.0±0.11	15.0±0.00	15.5±0.04	13.0±0.00	15.0±0.18	16.5±0.12	11.5±0.06	
	250	11.0±0.00	10.0±0.00	11.0±0.00	—	14.0±0.00	12.5±0.28	15.0±0.00	—	
	125	—	—	—	—	14.5±0.04	—	—	—	
Control	Cipro (5µg)	7	12	—	18	—	—	—	—	37.5
	Methanol (40% v/v)	massive growth	massive growth	massive growth	massive growth	massive growth	massive growth	massive growth	massive growth	0.0

SA: Aqueous extract of *L. inermis* seed
LA: Aqueous extract of *L.* inermis
SM: Methanol extract of *L. inermis* seed
LM: Methanol extract of *L. inermis* leaf
—: no inhibitory activity

196: *E. aerogenes*
129: *K. pneumonia*
190: *E. coli*
195: *E. cloacae*
(in mm)*: Mean of replicate value ± coefficient of variation

U_{109}: *P. aeruginosa*
U_{9}: *A. baumannii*
U_{30}: *K. oxytoca*
U_{87}: *S. enterica*

The antimicrobial susceptibility patterns of the isolates are presented in Table 6 and 7. Activity index reveals the efficacy of plant extract against MDR bacterial strains in comparison to the reference antibiotic. From Table 6, SM showed a very robust activity index of 4.28 against the ESβL-producing *E. aerogenes* 196 that was isolated from wound, which means that the extract is above four times more effective than the reference antibiotic (1.0). In addition, it was astonishing to find out that all the other extracts (SA, LM and LA) had the highest activity index against the same ESβL-producing *E. aerogenes* 196 that was isolated from wound (2.14, 2.07 and 1.64, respectively). More than 60% of the clinical isolates were resistant to the reference antibiotic, hence difficult to estimate their AI as the ratio of extract to zero (0) will result in a mathematical error, not determinable, thus denoting resistant with 'R'.

The result of the bacterial susceptibility index (BSI) is shown in Table 7. BSI indicates how susceptible an isolate is to all tested treatments. None was 100% sensitive; *P. aeruginosa* U_{109} however, was the most susceptible (93.75%) of all the isolates, followed by *K. oxytoca* U_{30} and *Acinetobacter baumannii* U_9 (87.50%) and ESβL-producing *E. aerogenes* 196 and *E. cloacae* 195 exhibited the least (68.75%).

Minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous and methanol extracts are presented in Table 8. The least MIC value was found with LM within the range of 1.95-31.25 mg/mL. While it has the least value against *K. oxytoca* U_{30} and the ESβL-producing *E. aerogenes* 196, the highest are against all the other ESβL-producers; and its MBC range from 125-500 mg/mL. SM had MIC value within the range of 1.95-62.5 mg/mL, with the least against *K. oxytoca* U_{30} (1.95 mg/mL) and highest against *P. aeruginosa* U_{109} (62.5 mg/mL) and MBC range of 125-1000 mg/mL.

Both aqueous extracts (SA and LA) had similar MIC and MBC range (7.81-250 mg/mL; 125-1000 and 125- >1000 mg/mL, respectively): SA had the least MIC against the ESβL-producing *E. aerogenes* 196 (7.81 mg/mL) and highest against the ESβL-producing *K. pneumoniae* 129 (250 mg/mL), while LA had the least against the ESβL-producing *K. pneumoniae* 129 and *K. oxytoca* U_{30} (7.81 mg/mL) and the highest against the ESβL-producing *E. aerogenes* 196 and *P. aeruginosa* U30 (250 mg/mL).

Table 6. Activity index (AI) of aqueous and methanol extracts of *L. inermis* against MDR clinical isolate

Extract	Conc. tested (mg/mL)	ESβL-Producing organisms							
		196	129	190	195	U_{109}	U_9	U_{30}	U_{87}
SA	1000	1.92	1.25	R	1.11	R	R	R	R
	500	2.14	1.16	R	0.91	R	R	R	R
	250	1.57	0.87	R	0.55	R	R	R	R
	125	1.42	—	R	—	R	R	R	R
LA	1000	1.64	1.16	R	0.72	R	R	R	R
	500	1.57	1.04	—	0.58	R	R	R	R
	250	—	—	—	—	R	R	R	—
	125	—	—	—	—	—	—	—	—
SM	1000	4.28	1.62	R	1.25	R	R	R	R
	500	4	1.58	R	1.25	R	R	R	R
	250	3.21	1.58	R	1.36	R	R	R	R
	125	2.92	1.00	R	1.11	R	R	R	R
LM	1000	2.07	1.25	R	0.91	R	R	R	R
	500	1.57	1.00	R	0.86	R	R	R	R
	250	1.57	0.83	R	—	R	R	R	—
	125	—	—	—	—	R	—	—	—

SA: Aqueous extract of *L. inermis* seed
LA: Aqueous extract of *L. inermis* leaf
SM: Methanol extract of *L. inermis* seed
LM: Methanol extract of *L. inermis* leaf
—: no inhibitory activity
196: *E. aerogenes*
129: *K. pneumonia*
190: *E. coli*
195: *E. cloacae*
R: Isolate resistant to reference antibiotic
U_{109}: *P. aeruginosa*
U_9: *A. baumannii*
U_{30}: *K. oxytoca*
U_{87}: *S. enterica*

Table 7. Bacterial susceptibility index (BSI %) of aqueous and methanol extracts of *L. inermis* against MDR clinical isolates

Extract	ESβL-Producing organisms							
	196	129	190	195	U_{109}	U_9	U_{30}	U_{87}
SA	100	75	100	75	100	100	100	100
LA	50	50	25	50	75	75	75	50
SM	100	100	100	100	100	100	100	100
LM	75	75	75	50	100	75	75	50
Total BSI	81.25	75	75	68.75	93.75	87.50	87.50	75

SA: Aqueous extract of *L. inermis* seed
LA: Aqueous extract of *L. inermis* leaf
SM: Methanol extract of *L. inermis* seed
LM: Methanol extract of *L. inermis* leaf
196: *E. aerogenes*
129: *K. pneumonia*
190: *E. coli*
195: *E. cloacae*
U_{109}: *P. aeruginosa*
U_9: *A. baumannii*
U_{30}: *K. oxytoca*
U_{87}: *S. enterica*

For control, as expected, broth plus inoculum (BI); and 20% (v/v) methanol plus inoculum (MI) showed growth, which, respectively, indicated that the broth supported the growth of the bacteria and the organic diluent (20% methanol) is not the acting principle that's inhibiting the organism. Ciprofloxacin plus inoculum (CI) showed variation with susceptibility and resistant pattern. The sterility test (extracts only), despite being the least concentration (the 10th gradient i.e. 1.95 mg/mL) revealed that the test extracts are free from bacterial colonization, hence sterile.

Brine shrimp lethality assay (cytotoxicity test)
Cytotoxicity assay reveal the toxicity profile of the extract (Table 9). It displays the percentage mortality of the shrimp, probit and LC_{50}. The LC_{50} is the least concentration at which 50% of the test organisms die. The LC_{50} of both aqueous extracts (LA and SA) is above one thousand ($LC_{50} > 1000$), which means that the extracts are safe, while the methanol extracts (LM and SM) is less than one thousand ($LC_{50} < 1000$). The positive control ($K_2Cr_2O_7$) had LC_{50} of 10, while the negative control (seawater) did not affect *Artemia salina.*

Table 8. MIC and MBC of aqueous and methanol extracts of *L. inermis* against MDR clinical isolates

Extract	Conc (mg/mL)	ESβL-Producing organisms								
		196	129	190	195	U_{109}	U_9	U_{30}	U_{87}	Sterility
SA	MIC	7.81	250	15.63	15.63	125	62.5	15.63	62.5	—
	MBC	500	500	1000	1000	500	125	125	500	NG
LA	MIC	250	7.81	15.63	15.63	250	62.5	7.81	31.25	—
	MBC	1000	1000	1000	>1000	1000	500	125	500	NG
SM	MIC	31.25	15.63	31.25	31.25	62.5	7.81	1.95	15.63	—
	MBC	500	1000	500	500	250	125	125	500	NG
LM	MIC	1.95	31.25	31.25	31.25	15.63	15.63	1.95	15.63	—
	MBC	250	250	250	250	250	500	125	500	NG
Control	CI	G	G	NG	G	G	G	G	G	NA
	MI	G	G	G	G	G	G	G	G	NA
	BI	MG	MG	MG	MG	MG	MG	MG	MG	NA

SA: Aqueous extract of *L. inermis* seed
LA: Aqueous extract of *L. inermis* leaf
SM: Methanol extract of *L. inermis* seed
LM: Methanol extract of *L. inermis* leaf
—: no inhibitory activity
NG: No growth
MI: Methanol + test isolate
196: *E. aerogenes*
129: *K.* pneumonia
190: *E. coli*
195:*E. cloacae*
U_{87}: *S. enterica*
G: Growth
BI: Broth + test isolate
U_{109}: *P. aeruginosa*
U_9: *A. baumannii*
U_{30}: *K. oxytoca*
NA: Not applicable
MG: Massive growth
CI:Cipro + test isolate

Table 9. Brine shrimp lethality assay of aqueous and methanol extracts of *Lawsonia inermis*

Extract	Concentration	Numbers of survived nauplii			No. of Dead nauplii	% Mortality	Probit	LC_{50}
		1st test tube	2nd test tube	3rd test tube				
SM	1000	0	0	0	30	100	8.09	27.799
	100	7	5	6	12	40	4.75	
	10	5	7	3	15	50	5.00	
LM	1000	6	6	2	16	53.33	5.08	942.640
	100	7	5	10	8	26	4.36	
	10	9	8	7	6	20	4.16	
LA	1000	8	7	7	8	26.67	4.36	37735179.299
	100	7	5	6	12	40	4.75	
	10	9	8	7	6	20	4.16	
SA	1000	7	6	3	14	46.67	4.90	8317.419
	100	8	8	7	7	23.33	4.26	
	10	9	6	6	9	30	4.48	
$K_2Cr_2O_7$	1000	0			10	100	8.09	10
	100	0			10	100	8.09	
	10	5			5	50	5.00	

SA: Aqueous extract of *L. inermis* seed
SM: Methanol extract of *L. inermis* seed
$K_2Cr_2O_7$: Potassium dichromate
LA: Aqueous extract of *L. inermis* leaf
LM: Methanol extract of *L. inermis* leaf

DISCUSSION

The emergence of antibiotic resistance has necessitated the continuous search for new and effective antibiotic alternatives to battle the menace of antimicrobial resistance, worldwide. This can be observed in the urge for continuous investigation of traditional medicines to exploit for safe and effective remedies of microbial and non-microbial ailments[9,38]. This study, therefore, elucidated the therapeutic potential of an indigenous plant, *Lawsonia inermis* extracts to combat multidrug-resistant and ESβL-producing clinical bacteria. To benefit from the usage of long-lasting medicinal plants in the treatment of infectious diseases, as experienced in folkloric medicines, it is essential to mimic, to the maximum possible extent, the traditional method employed[8,10]. It is for this reason, therefore, that the present study followed the ethnobotanical survey as documented by Idowu[25] that substantiated that many individuals use water and alcohol with this plant, but majority prefers water. This was supported in the work of many other researchers[21,39-42] that utilised methanol and aqueous extraction; hence, the choice of solvents employed in our study.

Furthermore, documented by Heinrich *et al.*[8], the success rate of extraction depends on the initial preparation process – the size of the biomass particles. With this in mind, the plant materials were milled twice, first by coarse mill and then a fine mill to generate a fine powder as large particles usually result in poor extraction, whereas small particles do have higher surface area and will therefore be extracted more efficiently. Thus, the application of the cold maceration technique in our investigation corroborated the traditional mimicry, as cold maceration, which allows for soft extraction, has been found to retain most, if not all of the phytoconstituents present in plant materials[8,10]. The variation in percentage yields as depicted in Table 3 could be attributed to different plant parts and solvents used[8]. The leaf aqueous extract showed the highest extraction yields which demonstrated that its constituents are relatively polar, and buttress the artistic preparation process of the plant, thereby supporting the preference of water as solvent of choice in traditional practice.

Phytochemical screening of *L. inermis* extracts revealed the presence of tannins and phenolic compounds in all test extracts, and this is in harmony with the work of Gull *et al.*[20] who also detected these compounds in their study. However, in contrast to their report and that of Usman and Rabiu[43] who reported non-detection of protein as well as alkaloids

in all of their crude extracts, because proteins and alkaloids were detected in our study. The latter detected steroids in their study and this disagrees with our study as there was absence of steroids in all the extracts. In addition, the report from Ali et al.[33] corroborates our study with the presence of glycosides in three of our extracts. All variations, as observed, are tenable as the extraction of phytochemicals has been reported to be affected by pre-extraction factors: plant part used, its location and particle size, method of drying, diurnal and seasonal variation, degree of processing, among others; and extraction-related factors – extraction method adopted, solvent chosen, solvent-to-sample ratio, pH and temperatures of solvent, and length of extraction[11,44].

The phenolic compounds observed in this study may be responsible for the antimicrobial properties exhibited by *L. inermis* extracts as these compounds have been reported to enhance antimicrobial activity against resistant pathogens through mechanisms of action that are not limited to inhibiting and reducing the activity of the efflux pump and interacting with some crucial enzymes that are precursors of the bacterial cell membrane[45]. Tannins, detected in all the screened extracts, have been documented to bind microbial proteins thereby inhibiting protein synthesis[46]. In addition, tannins are astringent and are used for treating intestinal disorders such as diarrhoea and dysentery thus exhibiting antibacterial activity[47]. Tannins are also widely used in traditional medicine in treating wounds and arresting bleeding[48]. The presence of glycosides moieties like saponins, anthraquinones, cardiac glycosides, and flavonoids are known to inhibit tumor growth and serve also to protect against gastrointestinal infections[47], this supports the ethnobotanical use of *L. inermis* to treat different gastrointestinal diseases. Cardioglycosides are active principle that functions in blocking the channels regulating the electrochemical state of heart muscle cells. One of the effects of this activity is the generation of increased pressure in the heart's pumping ability. Plants that possess these phytoconstituents have been used in the treatment of dropsy, a condition also called oedema[49]. The presence of these secondary metabolites is of pharmacognostic importance and this gives credence to the use of Henna in ethnomedicine.

Antimicrobial efficacy of different *L. inermis* extracts against eight (8) multidrug-resistant and ESβL-producing clinical isolates depict different bioactive compounds, and on that basis, variation in their antimicrobial potency. This variation has been documented by other researchers[20,21,24,50]. The variation in the activity of crude extracts is probably due to the different solvents used as well as plant parts that yield varieties of bioactive compounds. Many previous studies indicated that medicinal plant extract contains several phytochemicals that synergistically show remarkable antimicrobial properties against MDR organisms[9,10,21,34,51]. This might be because of the holistic formation of these complex bioactive compounds that synergistically modulate multiple targets to produce overall inhibitory actions[52]. Oftentimes, the bioassay-led method of investigation narrowing activity to a single compound fails because, often times, activities are lost during fractionation[8,9,10,38]. Therefore, the synergistic combination of different phytocompounds as observed in our investigated plant extracts might be responsible for the antibacterial activity.

Only a study[24] has been documented on antimicrobial activity of *L. inermis* seed, and found minimal activity compared to its leaf. However, we reported the best antimicrobial activity of seed methanol extracts against all the MDR bacteria for the first time. SA, as well as LM showed high antimicrobial potential against the multiple drug-resistant bacterial strains. Although previous studies have documented antimicrobial potential of leaf extract of *L. inermis*; methanol extract showed broad-spectrum antibacterial activity against *P. aeruginosa, E. coli,* MRSA, and MDR *E. coli* [21]. Gull *et al*.[20] employed the cold maceration technique, as used in our study, recorded good antimicrobial activity of all four (4) tested extracts (methanol, chloroform, aqueous, and acetone) against all bacteria strains used in their study. However, Elgailany and Elnin[21] reported inactivity of leaf aqueous extract at all tested concentrations (50, 25, 12.5, and 6.25%) against *E. coli* and MDR *E. coli* strains. In a like manner, Habbal *et al*.[24] documented better activity or higher antimicrobial activity of dry and fresh leaves of *L. inermis* than its seeds. This is not inconsonant with our findings, we revealed that seed extracts, which had the highest numbers of phytochemicals, exhibited the most profound and remarkable antibacterial activity against all tested strains.

The variation reported in the two studies might be as a result of the Soxhlet apparatus and water bath respectively used in extraction procedure that is likely to have denatured the heat-labile active principle that is expected to be present in order to

have antimicrobial effect. Rani and Khullar[39] and Sharmeen *et al*.[53] also reported the ineffectiveness of aqueous extract against all tested strains in their study, but the method of extraction was not disclosed in the latter's report. A similar hot method of extraction, Soxhlet, was employed by Kannahi and Vinotha[41] and Rotary evaporator by Al-Rubiay *et al*.[40] and these researchers also reported inactivity of their aqueous extract against all tested isolates. By cold-macerating and freeze-drying our aqueous extracts, we might have preserved most, if not all of the bioactive compounds, hence evident of robust activity recorded in our study. This therefore suggests that, as much as possible, a mild extraction method should be employed, most importantly, if the crux of the study is to derive and buttress the ethnomedicinal benefit, as demonstrated in an 'AncientBiotic' research[10].

Shahabinejad and Kariminik[42], who employed the cold maceration technique, as done in our investigation, reported good antibacterial activity of *L. inermis* extracts against all fifty (50) uropathogenic bacterial strains. Worthy of note from their study, *Acinetobacter, E. aerogenes,* MDR *E. coli,* MDR *P. aeruginosa,* and MDR *K. pneumoniae* showed varying zones of inhibition which ranged from 10-30 mm. This corroborates our findings that contribute to the robust antibacterial activity of the extracts against MDR pathogens isolated from urine with zone of inhibition ranging from 11±0.00-26.5±0.02 mm. Additionally, Aqil and Ahmad[54] evaluated the antibacterial potency of this plant extract against some standard and MDR bacteria and observed *L. inermis* to possess impressive activity against all the eight tested isolates ranging from less than 10 mm to above 40 mm.

In addition to extraction technique, solvent and part of the plant used outlined above, other factors that might have cumulated to the discrepancy in result include, but not limited to, *in vitro* antimicrobial method employed; variation in phytochemicals of the extract; density and size of the inoculum; concentration of test extract; volume of test extract pipetted in agar well or disk; temperature and diffusion period before incubation, composition of medium and incubation temperature[21,55]. Furthermore, a recent study shows that extracts of *L. inermis* demonstrated interesting antimicrobial activities at increasing concentrations[50] as noted in our investigation. We felt that the multiple drug-resistance properties of the test organisms might have contributed to activity at increase dose because from our preliminary lab demo, we observed that if a plant would not be active, even at a similar increase dose, it would still be ineffective.

The sensitivity of the MDR bacteria to the test extracts differed significantly at P <0.05, which indicates the likely different mode of action in respect to the extract and individual bacterium. Also, the phytocompounds might have acted differently based on the multiple drug-resistant strains as opined by Aqil and Ahmad[54].

It was indeed, in reality, astonishing to figure out that all the four extracts had the highest activity index against the ESβL-producing *E. aerogenes* 196 that was isolated from wound. The AI is a qualitative index to evaluate the efficacy of the test extract to the reference antibiotic. This is to say, when the AI is less than 1, it shows that the reference antibiotic possesses good activity than the test extract. However, when AI is greater than 1, it shows that the extract has better activity against the isolates than the reference antibiotic. All extracts indicated tremendous activity index, in particular, SM was above 4 – which means the extract is more than four times better than the reference antibiotic. It, in addition, suggests that the seed methanol (SM) could be better employed in the treatment of wound and skin-related diseases. Furthermore, our findings elucidate one of the most widely recognized ethnobotanical usages of the Henna plant in the management of wounds and other skin-related diseases. Our report attests to the remarkable wound healing potential of *L. inermis* which has also been mentioned by several other researchers[22,33,40,41,43,50]. Recently, Daemi *et al*.[56] elucidated the wound healing mechanism of *L. inermis*, and observed that their extract healed better than their control group.

The present study recorded some varying MIC and MBC values against the MDR isolates. MIC is generally defined as the lowest concentration of extract that inhibits the growth of the test organism[57]. Majority of the MIC of the four extracts recorded in the present study is within the range of 7.81-62.5 mg/mL for both aqueous extracts (SA and LA) and 1.95-31.25 mg/mL for methanol extracts (SM and LM). We established that the methanol extracts (LM and SM) had a better inhibitory potency on the isolates by exhibiting the least MIC values (1.95 mg/mL). This is in contrast to Al-kurashy *et al*.[58] whose MIC values for aqueous and alcoholic extract are within the range of 8-64 mg/mL and 32-64 mg/mL, respectively, for the following non-resistant organisms – *E. coli, S. aureus, P. aeruginosa* and *E.*

faecalis. The wide variation in the MIC and MBC values as observed in this study might be as a result of the invincible acquired multiple drug-resistance capability of the test isolates. Moreover, the presence of various phytoconstituents and their combined activity as well as the intrinsic tolerance of the individual MDR test bacterium as earlier stated by Aqil and Ahmad[54] might have also played a major part, as different bacterium acts differently to test samples. Thormar[57] postulated that the MIC values of antimicrobial agents attracting the most attention are the ones that inhibit or kill bacteria *in vitro* at concentrations below 1% vol/vol (10000 ppm). However, it has also been put forward that the *in vitro* result does not usually correspond to the *in vivo* situation, in which antibacterial and bacterial concentrations in different body fluids and tissues may fluctuate widely[11], hence not making those values the absolute constant. Besides, being an MDR isolates, higher concentrations of MIC or MBC might not be far-fetched.

Artemia salina has been well established first by Michael *et al*.[59] and Meyer *et al*.[60] and several others later[26,61,62] as a general biological assay, convenient for active plant constituents. Specifically, it was proposed as a simple bioassay for natural product research. In our findings, the Brine Shrimp Lethality assay showed that the aqueous extracts of *L. inermis* (SA and LA) which are greatly above 1000 (LC_{50} > 1000) infer that they are very much safe[60,63]. This report is in unison with Ojewunmi *et al*.[26], in their ethanol extract, who documented that the plant is safe and non-toxic. LM was virtually non-toxic on the shrimp with over 940 as LC_{50}[63]. While the SM was, however, found moderately toxic to the nauplii (LC_{50} = 27.799)[64], it was less toxic compared to the control (LC_{50} = 10). Extracts of alcohol or organic solvent are often seen moving more toward toxicity compare to aqueous that is recounted to be less toxic[38,65], this is demonstrated in our study.

CONCLUSION

With the current increase in hard-to-treat infections due to the global mess of antibiotic resistance, the present study investigates the antimicrobial potential of an indigenous plant, *Lawsonia inermis* (Henna), against multidrug-resistant bacteria including ESβL-producers. Our investigation reveals that Henna seed extracts (SM and SA) exhibited the highest antibacterial activity against all tested MDR bacteria than the leaf extracts, which also had the highest number of screened phytochemicals. In addition, *L. inermis* showed good activity not only against the wound- and UTI-causing bacteria; but also against sputum- and amniotic fluid-implicated organisms. The extracts, aqueous in particular, are non-toxic and very safe. Our findings further demonstrate the potential of *L. inermis* in the treatment of MDR-related infectious diseases and provide scientific rationale for medicinal use of this plant. This therefore suggests that "Laali", as it is commonly called in Yoruba, could be used as a cheap and potential strategy to manage infections, when compared to ineffective conventional antibiotics.

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