Antibacterial Effects of Lalley Leaves (*Lawsomiainermis*) against Isolates from Vaginal Infections

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ABSTRACT

This investigation looked at the effects of L. inermis extracts on vaginal isolates in terms of their antibacterial activity. Cold maceration was used to extract L. inermis leaf powder, and the resultant ethanol, aqueous, and chloroform fractions were separated. To produce residues that could be kept at 4°C, the resulting extracts were dried in an oven and then concentrated to a dry state. Saponin, tannin, alkaloids, terpenoids, and steroids were found in the residues after a phytochemical screening. Following accepted microbiological procedures, filthy HVS samples were randomly obtained from students, staff members, and inhabitants of hostels. They were then cultured. The samples showed the presence of Proteus spp., E. coli, Pseudomonas spp., Klebsiella spp., and Staph spp. The antibacterial activity that was concentration-dependent and varied with the organisms found was assessed using the agar diffusion technique on a broth culture, and it produced a result of 9.46%. For Proteus species and Staphylococcus species, the MIC of the crude extract and aqueous extract was 50 mg/ml, whereas it was 25 mg/ml for Klebsiella and Pseudomonas. For all of the isolated species, the aqueous extract showed a high MIC of 100 mg/ml. The chloroform extract (12.5 mg/ml) was shown to have the optimum minimal inhibitory concentration profile for Proteus species (8.0 + 1 mm), Staphylococcus species (12.0 + 0 mm), Klebsiella species (8.5 + 1.5 mm), and E. coli species (7.5 + 1.5 mm), as well as 6.25 mg/ml for Pseudomonas species (6.0 + 0). This study demonstrated that the leaves of Lawsomiainermis contain phytoconstituents that might cause an antibacterial response.

Key Words: *Lawsomiainermis, Antimicrobial, Inhibition zone diameter, Phytoconstituents, MIC*

eIJPPR 2023; 13(4):40-47

HOW TO CITE THIS ARTICLE: Oghenemaro EF, Oyemaechi OI, Erhomosele LE, Oghenejobo M. Antibacterial Effects of Lalley Leaves (*Lawsomiainermis*) against Isolates from Vaginal Infections. Int J Pharm Phytopharmacol Res. 2023;13(4):40-7. https://doi.org/10.51847/bvx5enhTZy

INTRODUCTION

For ages, man has relied on nature to provide for his fundamental necessities, including food, clothes, shelter, and medicine [1, 2]. Many people consider plants to be a natural remedy for treating and preventing a variety of ailments [3, 4]. Plants have been employed in traditional herbal medicine for a long time [5, 6] because they are now recognized as natural sources of powerful and novel antibacterial agents [7, 8]. Because of the wide variety of medicines that are easily available, these plants are sources of natural compounds that are utilized as ethnomedicine in several nations across the world [9] and provide limitless prospects for developing novel pharmaceuticals [10, 11]. Traditional medicine is thought to be used by 80% of people in underdeveloped nations for their primary healthcare, and it is becoming more and more popular as a result of the high toxicity and negative side effects of orthodox medication [10, 12]. Herbal medicine has long been characterized as a course of care that entails a coordinated pharmacological intervention of several distinct substances interacting with numerous targets, as opposed to a single medicament engaging with a single target [13, 14]. Natural active compounds found in plants

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Received: 23 May 2023; **Revised:** 01 August 2023; **Accepted:** 08 August 2023

may be utilized to cure ailments and improve health, which is the basis for herbal treatments. Utilizing plants and plant extracts to treat a variety of illnesses [15].

Henna, mehndi, and mignonette trees are all common names for the tall shrub plant Lawsoniainermis, which is a member of the Kingdom. Plantae, Division: Angiospermae, Class: Dicotyledoneae, Order: Myrtales, Family: Lythraceae, Genus: Lawsonia, and Species: L. inermis L. A blooming plant with a height of 2–7 meters, henna is found in subtropical and tropical climates all throughout the world, including South Asia, Africa, Oasis in the Sahara Desert, and even northern Australia. The elliptic, obovate to oblanceolate, acute at the apex, sometimes mucronulate, constricted into a short petiole, pinnately nerved, and submembranous leaves of henna plants [16] have these characteristics. Lawsone (2-hydroxynaphthoquinone), mucilage, mannitol, gallic acid, and tannic acid make up the majority of henna's chemical components [17]. According to reports, henna has antibacterial [18], immunostimulant [17], and hypoglycemic effects. The goal of the current study is to ascertain if Lawsoniainermis leaves have any antibacterial properties against isolates of vaginal infections.

MATERIALS AND METHODS

Reagents and equipment

Ethanol (99.9%) (Loba Chemie PVT Ltd, Mumbai, India), Lawsomia inermis leaves, Petri dishes, test tubes, swap stick, cork-borer, spatula, precision balance, agar medium (Loba Chemie PVT Ltd, Mumbai, India), autoclave, flame, incubator, rotary evaporator, wireloop, meter rule, and agar broth. The different chemicals and tools needed for this project were acquired from the laboratories of the Pharmaceutical Microbiology Department of the Faculty of Pharmacy at Delta State University, Abraka (DELSU).

Extraction of lawsomia inermis leaves

The pulverized leaves of L. inermis were collected from a local outlet in Kaduna, Nigeria. The leaves were then divided into ethanol, aqueous, and chloroform fractions after being extracted using cold maceration. The fractions obtained were filtered and concentrated in an oven to 101.2g dryness, and the residues recovered were refrigerated at 4°C [19].

Phytochemical screening

L. inermis leaf extract was prepared for phytochemical analysis using the techniques described by Enwa *et al*. (2016) and Okafor *et al*. (2021), with a little modification.

Fractionation of lawsomia inermis extract

About 45.04g of the extract was weighed and transferred into a separating funnel. The aqueous phase was prepared with 100ml methanol and 75ml of distilled water. About 200ml of chloroform was measured and transferred into the separating funnel which was shaken vigorously for 5 minutes. The upper layer was decanted anda bottom layer containing the chloroform fraction was carefully transferred into a beaker. This process was repeated four times and the fractions were allowed to concentrate at room temperature for 72 hrs. Furthermore, the fractions were packaged and stored in separate beakers for further microbiological studies.

Identification of bacteria ısolate

Biochemical tests such as indole, catalase test, citrate utilization test, H2S test, urease, oxidase, MOT, coagulase, and gram staining were carried out. Glucose, lactose, and sucrose tests were carried out to determine the presence of these sugars [20].

Preparation of broth culture

15g of the nutritional broth powder were dissolved in 1 L of distilled water to create the nutritional broth. After thoroughly blending the solution for 5 minutes to achieve total dissolution, the mixture was placed into test tubes. The solution was then autoclaved for 15 minutes at 121°C. A sterile wire loop was used to inoculate the preparation with the microorganisms, and it was then left for 24 hours.

Evaluation of antimicrobial activity

Molten Mueller Hilton Agar (MHA) was allowed to solidify. Fifteen (15) test tubes were sterilized and labeled crude (CR 100 - 6.25 mg/ml), Aqueous (Aq 100 - 6.25 mg/ml), and Chloroform (Chl 100 - 6.25 mg/ml). Thereafter 0.4g of the extracts weredissolved in non-concentrated ethanol. Two-fold serial dilution was carried out for the crude, aqueous, and chloroform extracts inside the test tubes from a range of 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml. The broth was streaked on the plates and a cork borer 6 mm diameter was used to punch the sides of the plate which was labeled according to the various concentrations used. The holes were sealed with molten agar to close up cracks. The various concentrations of the extract and the control (ciprofloxacin) were introduced into the holes and the plates were incubated for 24 hours. The zone of inhibition was read in duplicate and the average was taken.

Evaluation of the minimum ınhibitory concentration (MIC)

Determination of minimum inhibitory concentration (MIC) was done using the method described by Enwa *et al*. 2023 with minimal adjustment.

Data analysis

In order to describe the data, graphs, frequency tables, means, and standard deviations were used. The data were

examined using SPSS 22, the Statistical Package for Social Sciences. With a p-value of 0.05, an independent t-test was used for inferential statistics.

RESULTS AND DISCUSSION

Yield

The initial weight of the powdered leaves collected was 1100g. The weight obtained after concentrating was 104.04g. The yield was calculated using the formula below:

Yield = Weight of concentrated extract/Initial weight collected × 100%

$$\text{Yield} = 104.04\text{ g} / 1100\text{ g} = 0.09458 \times 100\ \% \quad (1)$$

Yield = 9.46 %

Phytochemical screening

The results of the phytochemical screening revealed that saponins, tannins, alkaloids, terpenoids, and steroids were present while flavonoids and reducing sugars were absent **(Table 1)**.

Table 1. Phytochemical Screening

Phytochemicals	Inference
Saponin	+
Tannin	+
Alkaloid	+
Terpenoids	+
Steroids	+
Flavonoids	-
Reducing sugars	-

+ means Present
- means Absent

Identification of bacteria isolate

The bacteria isolate was identified using various biochemical tests. The result is presented in **Table 2**. Klebsiellaspp, Proteus spp, Staphylococcus spp, and E. coli were mostly encountered.

Table 2. Bacteria isolate identified

S/N	CODE	Catalase	Indole	H_2S	Citrate	Urease	Oxidase	M.R	MOT	Coagulase	Gram Stain	Glucose	Lactose	Sucrose	Inference
	FSC	+	-	-	+	+	-	-	-	-	-Rods	A	AG	AG	*Klebspp*
	FC6	+	-	-	+	+	-	-	-	+	+cocci	A	A	AG	*Staph spp*
	F7C	+	+	+	+	+	-	+	+	-	-Rods	A	A	A	*Proteus spp*
	RS	+	+	+	+	+	-	+	+	-	-Rods	AG	A	AG	*Proteus spp*
	FW_1	+	+	-	+	+	-	-	-	-	-Rods	AG	AG	AG	*Klebspp*
	FW_2	+	+	+	+	+	-	+	+	-	-Rods	A	AG	A	*Proteus spp*
	FW_3	+	-	+	+	+	-	+	+	-	-Rods	AG	AG	A	*Proteus spp*
	FW_4	+	+	-	+	+	-	+	-	-	-Rods	AG	AG	AG	*Klebspp*
	FW_5	+	+	-	-	-	-	+	+	-	-Rods	AG	A	A	*E.coli*
	FW_6	+	-	-	+	+	-	-	-	-	-Rods	A	AG	A	*Klebspp*
	FW_7	+	+	-	+	+	-	-	-	-	-Rods	AG	AG	A	*Klebspp*
	FW_8	+	+	-	+	+	-	-	-	-	-Long rods	AG	A	AG	*Klebspp*
	FH_2	+	-	-	+	+	+	+	+	-	-Rods	AG	-	-	*Pseudomonas spp*
	FH_{20}	+	+	-	+	+	-	-	-	-	-Rods	A	AG	A	*Klebspp*
	FH_3	+	+	+	+	+	-	+	+	-	-Rods	AG	AG	AG	*Proteus spp*
	FH_5	+	+	-	+	+	-	-	-	-	-Rods	A	AG	A	*Klebspp*
	FH_7	+	+	-	-	-	-	+	+	-	-Rods	AG	A	A	*E.coli*
	FH_9	+	+	-	+	+	-	-	-	-	-Rods	AG	A	AG	*Klebspp*
	FH_{10}	+	+	-	+	+	-	-	-	-	-Rods	AG	AG	AG	*Klebspp*
	FH_{11}	+	+	-	+	+	-	-	-	-	-Rods	AG	AG	AG	*Klebspp*

FH_{12}	+	+	-	+	+	-	-	-	-	-Rods				*Klebspp*
FH_{13}	+	+	-	+	+	-	-	-	-	-Rods	A	AG	AG	*Klebspp*
FH_{14}	+	+	-	+	+	-	-	-	-	-Rods	AG	A	AG	*Klebspp*
FH_{15}	+	+	+	+	+	-	+	+	-	-Rods	AG	AG	AG	*Proteus spp*
FH_{17}	+	+	+	+	+	-	+	+	-	-Rods	AG	A	AG	*Proteus spp*
FH_{18}	+	+	+	+	+	-	+	+	-	-Rods	AG	A	AG	*Proteus spp*
FH_{21}	+	-	-	+	+	+	+	+	-	-Rods	AG			*Pseudomonas spp*
FH_{22}	+	+	-	-	-	-	+	+	-	-Rods	AG	A	A	*E.coli*

KEY:C: Coursemates; H: Hosteller; W: University workers

Inhibition zone diameter of the extracts

Table 3 displays the diameter of the inhibition zone for each extract. The width of the inhibitory zone varies depending on the microorganism detected **(Table 3)**.

Table 3. Inhibition Zone Diameter of the Extracts

S/N	CODE	Concentration (mg/ml) / Inhibition Zone Diameter (IZD in mm)																	
		100			50			25			12.5			6.25			Control		
		1	2	AV	1	2	AV	1	2	AV	1	2	AV	1	2	AV	1	2	AV
	F7C-Crude (mm)	16	16	16	14	13	13.5	12	12	12	10	12	11	5	6	5.5	26	25	25.5
	F7C-Chloroform (mm)	16	18	17	13	12	12.5	11	9	10	7	9	8	6	7	6.5	29	27	28
	F7C-Aqueous (mm)	16	14	15	10	9	9.5	-	4	2	-	4	2	-	2	1	26	27	26.5
	FC6-Crude (mm)	19	18	18.5	15	15	15	11	10	10.5	9	8	8.5	6	6	6	15	15	15
	FC6-Cloroform (mm)	15	14	14.5	12	12	12	12	10	11	11	10	10.5	7	6	6.5	15	18	16.5
	FC6-Aqueous (mm)	12	14	13	9	9	9	6	7	6.5	-	5	2.5	-	-	-	21	24	22.5
	FSC-Crude (mm)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29	27	28
	FSC-Chloroform (mm)	7	10	8.5	6	8	7	4	6	5		4	.5	-	-	-	30	28	29
	FSC-Aqueous (mm)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	31	31	31
	FW_1-Crude (mm)	14	15	14.5	12	12	12	10	11	10.5	7	8	7.5	4	6	5	26	27	26.5
	FW1-Chloroform (mm)	11	14	12.5	10	10	10	11	10	10.5	9	12	10.5	7	7	7	29	29	29
	FW1-Aqueous (mm)	11	11	11	8	8	8	5	4	4.5	-	4	2	-	-;8	-	24	26	25
	FH_2-Crude (mm)	14	14.5	14.3	15	14	14.5	10	9	9.5	7	7	7	4	4	4	28	29	28.5
	FH_2-Chloroform (mm)	13	12	12.5	11	11	11	9	10	9.5	9	9	9	6	6	6	27	26	26.5
	FH_2-Aqueous (mm)	11	12	11.5	6	6	6	6	5	5.5	-	4	2	-	3	1.5	26	24	25
	FW_5-Crude (mm)	15	16	15.5	12	12	12	10	11	10.5	9	6	7.5	6	6	6	27	27	27
	FW_5-Chloroform (mm)	15	13	14	11	12	11.5	11.5	11	11.	8	12	10	6	6	6	25	27	26
	FW_5-Aqueous (mm)	11	12	11.5	7	9	8	5	5	5	3	3	3	2	-	1	24	26	25

Control: Ciprofloxacin 2 mg/ml

F7C: Proteus spp; FC6: Staphylococcus spp; FSC: Klebsiella spp, FW_1: Klebsiella spp, FH_2: Pseudomonas spp, FW_5: Escherichia coli; AV: Average

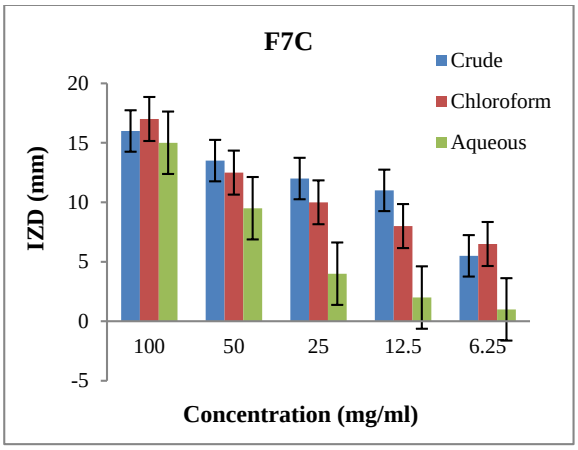

Figure 1. Graphical representation of the zone of inhibition of all extrat on isolate F7C

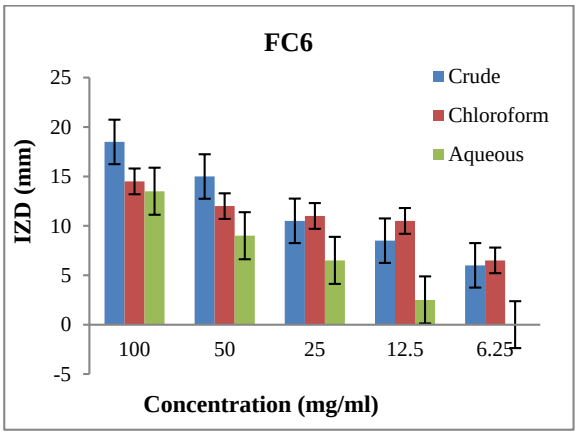

Figure 2. Graphical representation of the zone of inhibition of all extrat on isolate FC6

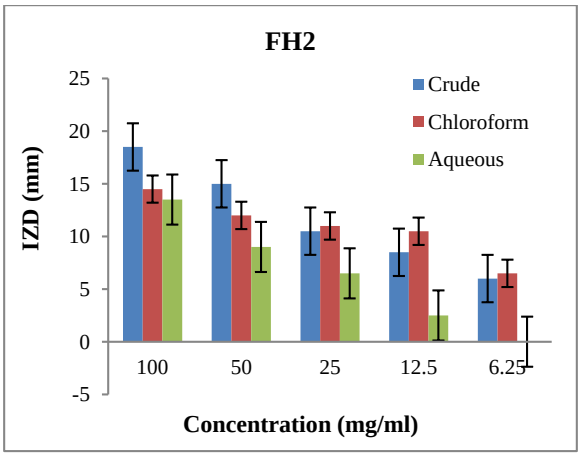

Figure 3. Graphical representation of the zone of inhibition of all extrat on isolate FH2

The figures above show the inhibition zone diameter of the extracts.

Minimum inhibitory concentration of lawsomiainermis extract

The minimum inhibitory concentration of the extracts is shown in **Table 4**. The chloroform extract produced the best minimal inhibitory concentration profile (12.5 mg/ml for Proteus species, Staphylococcus species, Klebsiella species, and E. coli, and 6.25 mg/ml for Pseudomonas species) **(Table 4)**.

Table 4. Minimum Inhibitory Concentration of the Extracts

Code	Sample	Organism	MIC (mg/ml)
F7C	Crude	*Proteus*	50
	Chloroform		12.5
	Aqueous		100
FC6	Crude	*Staphylococcus*	50
	Chloroform		12.5
	Aqueous		100
FSC	Crude	*Klebsiellaspp*	-
	Chloroform		12.5
	Aqueous		-
FW_1	Crude	*Klebsiellaspp*	25
	Chloroform		12.5
	Aqueous		100
FH_2	Crude	*Pseudomonas spp*	25
	Chloroform		6.25
	Aqueous		100
FW_5	Crude	*E.coli*	25
	Chloroform		12.5
	Aqueous		100

The secondary metabolites are mostly blamed for plants' antibacterial activities [21]. Steroids, tannins, and phenolic chemicals may be the active components of these secondary metabolites [22]. It was discovered that the sample contains saponins, tannins, terpenoids, flavonoids, alkaloids, steroids, and flavonoids, but reducing sugars were not present after a phytochemical examination was conducted to detect these metabolites present in various plant samples. This is in tandem with the previous work of Khawia and Wahiba [23] who identified terpenoids, phenols, and tannins in their samples. However, it was shown that the presence of these metabolites strongly depends on the extraction of the solvent. Cardio glycosides, terpenoids, polysaccharides, phenols, quinones, and tannins were present in the Lawsomiainermis methanol, acetone, and aqueous extracts but not in the chloroform extract [23]. This is due to the fact that metabolites are soluble in solvents based on their polarity. Starch, tannins, saponins, polypeptides, terpenoids, lectins, and other ions make up the majority of plant materials' water-soluble components, whereas flavonoids, alkaloids, tannins, sterols, polyphenols, and other substances are present in alcoholic extracts [24, 25]. Lawsone (2-hydroxynaphthoquinone), mucilage, mannitol, gallic acid, and tannic acid are the principal chemical components of henna [26]. The findings showed

that every extract has antibacterial activity against each and every bacterial strain found in the current investigation. Only one gram-positive bacterium strain (Staphylococcus spp.) was identified from the three populations of coursemates, hostel guests, and university employees. This supports previous findings that identified Staphylococcus species from clinical isolates [27], but most of the organisms in their study were Gram-negative bacteria. The reason for this trend has been attributed to the protective mechanisms and prevalence of gram-negative organismsinfectionacross populations, especially inextreme conditions and tropical regions [28]. Similarly, a study conducted on the antimicrobial evaluation of propolis ointment posited that the activity of a plant depends on the constituents present, the time of collection, the polarity of the extraction solvent as well as period of exposure to such solvent [29]. Furthermore, the phytochemical components of Lawsoniainermis are exclusively efficient against gram-positive bacteria and inactive against gram-negative bacteria [30]. In each instance, the chloroform extract outperformed the aqueous extract in terms of antibacterial inhibition. Additionally, it is important to highlight that Lawsoniainermis demonstrated antibiotic action against gram-positive (S. aureus) and gram-negative (E. coli, Proteus spp., Klebsiella spp., and Pseudomonas spp.) bacteria, which is consistent with Habbal's findings [31].

The inhibition zone diameter was concentration-dependent, hence higher concentrations of the crude, aqueous and chloroform extract had higher inhibition zone diameter. Several studies had established that the inhibition zone diameter ofplant extracts wasconcentration dependent. **Figure 1** displays the MIC values for each bacterial strain that was put to the test. **Figure 1**'s findings demonstrated that every tested bacterial isolate displayed the smallest MIC for the crude, chloroform, and aqueous extracts. The chloroform extract had the lowest MIC (6.25 mg/ml) for all organisms isolated. This is consistent with prior research by Iram [27], who found that L. inermis chloroform extract was a more effective antibacterial agent against Shigella spp., Klebsiella spp., S. aureus, and S. epidermidis. This explains why the aqueous extract was the least effective and the chloroform extract was more powerful. The polarity of the extraction solvent was the cause of the extract's variable antibacterial activity. **Figures 2 and 3** also indicated the efficacy of the extract on both isolates FC6 and FH2 with the crude extract been very active as compared to the chloroform and aqueous.

CONCLUSION

There is no question about L. inermis's antibacterial abilities, but a toxicological evaluation is also essential. Public health issues caused by multi-resistance bacteria always prompt researchers to turn to the natural resources of the plant kingdom, a crucial source for the creation of novel therapeutic treatments. The herb L. inermis, known for its numerous medicinal benefits, was the subject of the current study. Its potential for application as an antibiotic is shown by the phytochemical analysis and the evaluation of the antibacterial activity of its crude, aqueous, and chloroform extracts. The utilization of L. inermis extracts as a therapeutic alternative to antibacterial agents is extremely important as researched by this study in addressing issues related to virginal infections. Furthermore, efforts should be made towards isolating, characterizing, and identifying new active compounds from *Lawsonia inermis L*., that could lead to the new discovery of more efficacious compounds with therapeutic action. It is also pertinent to consider the toxicity profiles and potential side effects of *Lawsonia inermis L*., extracts to also determine it safe use. Finally, this research study showed that *Lawsomia inermis Leaves* has phytochemical constituent that can elicit antimicrobial activity against isolates from vaginal infections.

Acknowledgments: Faculty of Pharmacy, Delta State University.

Conflict of interest: None

Financial support: None

Ethics statement: Ethical approver was obtained from the ethical committee of the Faculty of Pharmacy, Delsu with Ref No. PHC/05/2002/2

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