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Pharmacognostic and Antimicrobial Activity of leaves *Lawsonia inermis* (Lythraceae)

[1*]Namadina, M. M., Haruna, H[1]., [2]Abdulrazak, M. H., Ibrahim, S[1]., Sunusi, U[3]., Kamal, R.M[4]., Nuhu, Y[1]., Balarabe, S[5]., Sani, S.B[10] ., Muttaka, A[13]., Sadau, S.B[8]., Fasogbon, A. O[7] ., Hamisu, H[9]., Tasiu, S[9]., Yahuza, S[6]., Nuhu, A.A[12]., Aminu, I.M[12]., Aminu, A[11]. and Yakubu, N[1].

[1]Department of Plant Biology, Bayero University, Kano
[2]Department of Biological Sciences, Sule Lamido University, Kafin Hausa
[3]Department of Biochemistry, Faculty of Basic Medical Science, Bayero University, Kano
[4]Department of Pharmacology, Federal University, Dutse, Jigawa State, Nigeria
[5]Department of Integrated Science, Federal College of Education, Kano
[6]Department of Microbiology, Federal University, Dutse
[7]Department of Microbiology, Bayero University, Kano
[8] Department of Science Technology Education, Bayero University, Kano
[9]Department of Microbiology, Federal University, Dutsinma, Katsina State
[10]Department of Biology, Kano University of Science and Technology, Wudil
[11]Department of Trypanosomiasis Research, Kano State Liaison Office Infectious Diseases Hospital
[12]Department of Biological Sciences, Yusuf Maitama Sule University, Kano State
[13]Department of Biochemistry, Federal University Gusau
Corresponding author: hajiyaiyalle@gmail.com, +2347035939668

Abstract

The lack of scientific standardization for the use of some herbal preparations, as well as possible therapeutic alternatives against antibiotic resistant microbial infections indicates a strong need for continuous effort to validate the use of plant material as alternative therapy regimens with similar or higher antibiotic beneficial properties. The objectives of this studies was to establish some important pharmacognostic profile and antimicrobial activities of *Lawsonia inermis* leaves. The phytochemical analysis was carried out using standard methods. The antibacterial potency was determined by the agar well diffusion method followed by quantitative evaluation of antibacterial and antifungal activities by Minimum Inhibitory Concentration and Minimum Bactericidal/Fungicidal Concentration. Chemomicroscopical evaluation revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain and mucilage with the exception of calcium carbonate. The average moisture contents, total ash, acid insoluble, water soluble ash, alcohol extractive value and water extractive values in the powdered plant material were 8.40%, 5.80%, 3.20%, 5.22%, 21.40% and 18.30% respectively. In addition, Fe, Mn, Ni, Pb, Zn, Cd and Cu were found to be within the safety limit. Carbohydrates, saponins, flavonoids and tannins, cardiac glycosides, triterpenes and alkaloids were observed to be present. Methanolic leaf extract of *Lawsonia inermis* was observed to be more effective than the aqueous extract due to its ability to inhibit all the test organisms. This study has proven the effectiveness and efficacy of *L. inermis* in inhibiting the growth of *S. aureus, E. coli, P. aeruginosa* and *Klebsiella* and *Candida albicans* pathogenic microorganisms of clinical origin.

Keywords: Phytochemical analysis, Chemomicroscopical, Minimum Inhibitory, *Lawsonia inermis*, Bactericidal, Fungicidal Concentration.
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Introduction

In the past decade, interest on the topic of antimicrobial plant extract has been growing, and the use of herbal medicines in world represents a long history of human interaction with the environment. The plant used for traditional medicine contains a wide range of substance that can be used to treat chronic as well as infectious disease (Al-Daamy *et al.,* 2016). Some bacteria and fungi are extremely pathogenic causing serious human infections. The discovery of antibiotics to combat these pathogens marked a resolution in the 20th century (Raja *et al.,* 2013). Unfortunately, because of the inappropriate use of antibiotics in human and veterinary medicine, certain strains of bacteria and fungi developed the ability to produced substances which block the action of antibiotics or change their target or ability to penetrate cells (Raja *et al.,* 2013). Therefore, disease causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. Gonorrhea, candidiasis and other sexually transmitted infections, malaria and childhood era infections are just a few of the diseases that have become hard to treat with antibiotics (Raja *et al.*, 2013). However, a large part of the problem is due to our increasing use and misuse of existing antibiotics in human and veterinary medicine. To substitute synthetic antibiotics, many of today modern and effective drugs have their origin in traditional folk medicine (Raja *et al.*, 2013). Therapeutic efficacy of many indigenous plants for many disorders has been described by traditional medicine practitioners (Raja *et al.*, 2013).

Lawsonia inermis Linn (Henna), the common names in different languages are: Henna (English), lalle (Hausa), lali (Yoruba), mehndi/heena (Urdu), mehndi (Hindi). It is a tropical and subtropical shrub, growing in North Africa. Henna leaves are very popular natural dye to color hand, finger, nails and hairs. The dye molecule, Lawson is the chief constituents of the plant; its highest concentration is detected in the petioles (Kannahi and Vinotha, 2013). In folk medicines, henna has been used as astringent, antihemorrhagic, intestinal antineoplastic, cardio-inhibitory, hypotensive, sedative and also as therapeutic against amoebiasis, headache, jaundice and leprosy (Kannahi and Vinotha, 2013). Modern pharmacological research on henna and its constituents has confirmed its anti-inflammatory, antipyretic and analgesic effects (Kannahi and Vinotha, 2013). It can also be used to treat pediculosis (Kannahi and Vinotha, 2013).

Widespread use of drugs is leading to the development of resistance against them in the pathogen and also the side effects associated with them is urging people not to use them. Therefore there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious disease from medicinal plant (Kannahi and Vinotha, 2013). The aim of this study is to determine the pharmacognostic profile and antimicrobial activities of *Lawsonia inermis* against some clinical bacterial and fungal isolates.

Materials and Methods

Collection and Identification of Plant Materials

The leaves of *Lawsonia inermis* were collected from local farm in May, 2018 at Babura Local Government Area, Jigawa state. The plant was identified and authenticated in the Herbarium of the Plant Biology Department of Bayero University, Kano and was compared with a voucher specimen number BUKHAN379.

Preparation of Plant extracts

Leaves powder (50 g) of *Lawsonia inermis* was extracted successively in 500 ml of methanol for 72hrs using cold maceration and the concentrate was evaporated to dryness on a water bath and stored in desiccator for further use.

Chemo-microscopic Studies of the Powdered Leaves of *Lawsonia inermis*

Powdered sample (5g) of plant species was used for this study to detect the presence of cell wall materials and cell inclusions. Finely ground sample of plant was cleared in a test tube containing 70% chloral hydrate solution. It was then be boiled on a water bath for about thirty minutes to remove obscuring materials. The cleared sample was mounted with dilute glycerol onto a microscope slide. Using various detecting reagents the presence of cell wall materials and cell inclusions was detected in accordance with WHO (2011) guidelines.

Cell wall Materials

Test for Cellulose

A drop or two of iodinated zinc chloride was added to the powdered sample and allowed to stand for a few minutes and observed under a microscope. It stained cellulose cell wall blue to blue- violet.

Test for Lignin

The powdered plant material was moistened on a slide with a small volume of phloroglucinol and allowed to stand for about two minutes or until almost dry. A drop of hydrochloric acid was added and viewed under a microscope. Pink stained or cherry red was observed for the presence of lignin.

Test for Suberized or Cuticular cell walls

A drop or two of Sudan red was added to the cleared powdered sample and allowed to stand for few minutes and observed under a microscope. Orange red or red colour was observed presence of suberin or cutin on the cell.

Test for Gum and Mucilage

To a small portion of the cleared powdered sample of the plant, a drop of ruthenium red was added. Appearance of pink coloration was considered positive for gums and mucilage.

Cell Inclusions/ Cell Contents

Test for Starch grains

To a small portion of the cleared powder sample of the plant, N/50 iodine was added. Appearance of blue-black or reddish-blue coloration on some grains would be considered positive for starch.

Test for Calcium oxalates and Calcium Carbonates

To a small portion of the cleared powdered sample of the plant, HCl was added, dissolution of crystals in the powdered drug without effervescence was considered positive for calcium oxalate while slow dissolution with effervescence was considered positive for calcium carbonate.

Inulin

A drop of 1-naphthol and that of sulphuric acid was added to the powdered sample and viewed under the microscope. Spherical aggregations of crystals of Inulin turned brownish red and dissolve.

Test for Tannins

To a small portion of the cleared powdered sample of the plant, 5% ferric chloride solution was added. Appearance of greenish black colour was considered as positive for tannins.

Determination of Physicochemical Constants of the Powdered Leaves of *Lawsonia inermis*

Moisture Content

This is the quantity of moisture present in a plant material. Moisture content of the powdered sample will be determined by loss on drying method.

3.0g each of the powdered sample was accurately weighed and placed in some clean, dried evaporating dishes of known weights. They were placed in an oven and heated at a temperature of 105ºC for 1 hour, then cooled in a dessicator and re-weighed. Heating and weighing were repeated until a constant weight was obtained. The weight loss on drying was computed following the formula below:

$$\% \text{ Moisture content} = \frac{\text{Initial Weight of Powder} - \text{Final Weight of Powder}}{\text{Initial Weight of Powder}} \text{ X } 100$$

Total Ash Value

2g of powdered plant materials was accurately weighed and placed separately in a crucible of known weight. It was heated gently and the heat gradually increased until it is white indicating the absence of carbon. It was allowed to cool in a desiccator and weighed; this was repeated until a constant

weight was obtained. The total ash value was determined as a percentage with the formula below

$$\% \text{ Ash Value} = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \text{ X } 100$$

Acid-insoluble ash

This is the residue that remains after boiling the total ash with dilute hydrochloric acid.

This was determined for the powdered plant material. 25ml of dilute hydrochloric acid was added to the crucible containing ash. It was covered with a watch glass and gently boiled for 5mins. The watch glass was rinsed with 5ml of hot water and the liquid added to the crucible. The insoluble matter was collected on an ash less filter-paper and washed with hot water until the filtrate is neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, dried in an oven and ignited to a constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes and then weighed without delay (Evans, 2002).

The acid-insoluble ash will then be calculated as a percentage for each of the two plants with the formula

$$\% \text{ Acid insoluble Ash} = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \text{ X } 100$$

Water soluble ash

To the crucible containing the total ash, 25ml of water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered glass crucible. It was then be washed with hot water and ignited in a crucible for 15 minutes at 105oC. The weight of the residue was subtracted from the weight of the total ash. The content of water soluble ash per air dried powdered sample was calculated and recorded (WHO, 2011).

$$\% \text{ Water Soluble Ash} = \frac{\text{Weight of Total Ash} - \text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \text{ X } 100$$

Alcohol-Soluble Extractive Value

This is the amount of extraction in percentage of a plant sample with alcohol.

4g of each of the plant material was separately weighed in a conical flask. 100ml of ethanol was added and macerated for 24 hours, during which the mixture was frequently shaken within the first 6hours using a mechanical shaker. It was filtered and 25ml of the filtrate transferred into an evaporating dish of known weight and evaporated to dryness on a water bath. It was dried to a constant weight, the percentage of alcohol-soluble extractive value was then determined for the plant as

$$\text{Alcohol-Soluble Extractive Value (\%)} = \frac{\text{Weight of Extract in 25ml X 4}}{\text{Original Weight of Powder}} \text{ X } 100$$

Water-Soluble Extractive Value

This is the amount of extraction in percentage of a plant sample with water. Same procedure as in alcohol-soluble extractive value was repeated here for the two plants, but solvent for extraction here was water.

$$\% \text{ Water Extractive Value} = \frac{\text{Weight of Extract in 25ml X 4}}{\text{Original Weight of Powder}} \text{ X } 100$$

Elemental analysis of the Powdered Leaves

The elemental analyses of the plant materials were carried out in Ahmadu Bello University Zaria, Multi-user Research Laboratory. Powdered plant material was digested using 2.5ml of hydrochloric acid (HCl) and 7.5ml Nitric Acid (HNO3). The concentration of Fe, Mg, Zn, Cu was read using the flame atomic absorption spectrophotometer (FAAS), AA 500 model, Atomic Emission Spectrophotometer. Atomic Absorption Spectrophotometer were used for other elements. Before determining the concentration of any element in the sample, calibration curve of the element in the sample was prepared using prepared standard stock solutions for the elements as reported by AOAC, 2000; 2005; Akpabio and Ikpe (2013).

Qualitative Phytochemical screening of the Aqueous and Methanolic extract of *Lawsonia inermis* Leaves

The plant extracts (aqueous and methanol) were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the standard method of (Evans, 1996 and Evans, 2009).

Antibacterial Susceptibility Test

Mueller Hinton Agar and Sabouraud Dextrose Agar were used for the antibacterial and antifungal susceptibility testing respectively. It was prepared according to manufacturer's instructions by suspending 38g of medium in 1000ml distilled water, sterilized at 121ºC, and cooled to room temperature prior to dispensing in Petri dishes.

Preparation of Extract Concentration

This was carried out according to the method described by Srinivasan *et al.*, (2009). Stock solution of the plant extracts were prepared by adding 0.5g of each crude plant extract in 1ml dimethyl sulphuroxide (DMSO). From each of the stock solutions, 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml concentrations were prepared using Two-fold serial dilution method. These concentrations were labelled and kept in bijou bottles for subsequent use.

Preparation of Turbidity Standard

McFarland standard are used as a reference to adjust the turbidity of microbial suspension so that the number of bacteria will be within a given range. Firstly, $BaCl_2$(1%w/v) and H_2S0_4(1% v/v)were prepared by dissolving 1g of $BaCl_2$ in 100ml of sterile distilled water and 1ml of concentrated H_2S0_4 in 99ml of sterile distilled water respectively to serve as stock solutions for the preparation of the McFarland standard. From the stock solutions, 0.5McFarland scale was prepared by adding 9.95ml of (1%v/v) H_2S0_4 to 0.05ml of (1% w/v) $BaSO_4$ whose density is equivalent to 1.5×108 CFU/ml approximate cell density of bacteria. The barium sulphate suspension in 6ml aliquots were transferred in to screw-cap tubes, tightly sealed, and stored at room temperature in order to prevent loss by evaporation. This was subsequently used for comparison with the turbidity of the bacterial and fungal inoculum (Cheesbrough, 2010).

Standardization of the Inoculum.

Using inoculum loop, enough material from an over-night culture of the test organism was transferred in to a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National committee for clinical laboratory standard (NCCLS, 2000).

Susceptibility Test of Clinical isolates to Plant extracts.

The antimicrobial activity of *Lawsonia inermis* crude extracts (Aqueous and Methanolic) against the test organisms was evaluated using agar well diffusion method of susceptibility test (Srinivasan *et. al.*, 2009*)*. Mueller-Hinton agar and Sabouraud Dextrose agar plates were inoculated with 0.1ml of standardized inoculum of each bacterium and fungus respectively (in triplicates) using 0.1ml pipette and spread uniformly with sterile swab sticks. Wells of 6mm size were made with sterile cork borer into the inoculated agar plates. Using micropipette, 0.1ml volume of the various concentrations; 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml each of the crude extracts were dispensed into wells of inoculated plates. The prepared plates were then left at room temperature for10 minutes, allowing the diffusion of the extracts into the incubation at 37 ºC for 24 hrs. The diameter of inhibition zones (DIZ) were measured and expressed in millimetres after incubation. The mean values of the diameter of inhibition zones were calculated to the nearest whole number. DMSO was used as negative control. Commercially available standard antibiotic, Ciprofloxacin (10mg) and Ketoconazole were used as positive control parallel with the extracts.

Determination of Minimum Inhibitory Concentration (MIC)

Extracts that exhibited activity against the test organisms were further assayed for their minimum inhibitory concentrations (MIC).The minimum inhibitory concentration (MIC) of the test organisms was determined using the test tube dilution technique. Nine milliliter (9ml) of the nutrient broth was pippeted in to various test tubes containing concentrations of 2000mg/ml, 1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml and 7.8125mg/ml of the extract, the remaining two as negative (containing only nutrient broth and positive

control (nutrient broth and organism) respectively. The overnight culture of the test organisms (0.1ml) aseptically obtained was added to the test tubes with exception of negative control and incubated at 37°C for 24hrs. The least concentration of the extract that did not indicated any visible growth of the incubated organisms in broth culture was taken as the minimum inhibitory concentration (MIC) (Abalaka *et al.,* 2012).

Determination of Minimum Bactericidal/Fungicidal Concentration

The bactericidal and bacteriostatic effect of the extract was determine by sub-culturing the well that showed no growth on the fresh medium and incubated it for 24hrs. If the growth is observed, the extract is considered to have only bacteriostatic effect while on the other hand, if the growth is observed, the extract is said to have bactericidal effect (Abalaka *et al.,* 2012).

Results

Chemo-microscopical studies on leaves of *Lawsonia inermis* were found to have cellulose cell wall, lignin, calcium oxalate crystals, tannins, starch and mucilage but calcium carbonate was absent and is presented in Table 1. The result of average moisture contents using loss on drying method was calculated to be 8.40% and the percentage yield of total ash, acid insoluble and water soluble matter were recorded in percentage values as 5.80%, 3.20% and 5.22% respectively. The extractives obtained were 21.40% and 18.30% for alcohol and water solvents respectively (Table 2). Elemental analysis of *L. inermis* powdered leaves is presented in Table 3. Trace metals which include Fe, Mn and Ni detected in *L. inermis* powdered leaves were below the FAO/WHO (1984) permissible limit for edible plants. Phytochemicals which include flavonoids, alkaloid, saponins, tannins, glycosides, triterpenes and steroid were detected in both aqueous and methanolic extracts. Anthraquinones was absent in both aqueous and methanolic extract of *L. inermis* as presented in Table 4. The result for activity of the leaf extracts on clinical isolates of *E. coli, P. aeruginosa, K. pneumonia, S. aureus* and *C. albicans* with methanol extract having the highest activity with mean inhibition zone range of 21, 25, 30, 29 and 28mm at 250mg/ml respectively which below the activity of the standard drugs.

Table 1. Chemo-microscopic Studies of Powdered Leaves of *L. inermis*

Constituents	Inference
Starch	+
Gum and Mucilage	+
Cellulose cell walls	+
Lignin	+
Aleurone grain	+
Calcium oxalate crystals	+
Calcium carbonate	-
Suberized/Cuticular cell wall	+
Inulin	+

Key: + Present, - Absent

Table 2. Physicochemical Constituents of Powdered Leaves of *L. inermis*

Parameters	Values (%w/w) ± SEM*	B.H.P Standard
Moisture content	8.40	10-12%
Ash content	5.80	6-19%
Acid insoluble ash	3.20	>1
Water soluble ash	5.22	-
Water extractive value	18.30	-
Ethanol extractive value	21.40	-

*****Average values of three determinations; BHP- British Herbal Pharmacopeia

Table 3. Elemental analysis of *L. inermis* powdered Leaves

Elements	Concentration (ppm)	FAO/WHO (1984) limit* (ppm)
Iron(Fe)	1.916	20.00
Copper (Cu)	0.041	3.00
Lead (Pb)	0.087	0.43
Zinc (Zn)	0.337	27.40
Nickel (Ni)	0.330	1.63
Manganese (Mn)	0.201	2.00
Aluminum (Al)	0.924	-
Cadmium (Cd)	0.001	0.21
Selenium (Se)	0.888	-
Chromium (Cr)	0.006	-
Arsenic (As)	-0.521	-

Table 4. Qualitative Phytochemical screening of Aqueous and Methanolic leaf extracts of *L. inermis*

Metabolite	Inference	
	Aqueous	Methanolic extract
Alkaloid	+	+
Flavonoid	+	+
Saponins	+	+
Cardiac glycoside	+	+
Tannins	+	+
Steroid	+	+
Triterpenes	+	+
Phenol	+	+
Anthraquinones	-	-
Carbohydrate	+	+

Key; + Present, - Absent

Table 5. Sensitivity/Inhibitory Results of Aqueous and Methanolic extracts of *Lawsonia inermis* Leaves

Extract	Diameter zone of inhibition/ Concentration (mg/ml)						
	Test organism	250	125	62.5	31.25	Amp/Ket	DMSO
Aqueous	*E. coli*	19	16	12	08	34/-	0
	P. aeruginosa	20	15	12	10	32/-	0
	K. pneumonia	23	20	17	12	34/-	0
	S. aureus	18	15	11	09	30/-	0
	C. albicans	24	20	16	13	-/34	0
Methanol	*E. coli*	21	18	15	11	34/-	0
	P. aeruginosa	25	22	16	12	32/-	0
	K. pneumonia	30	26	23	18	34/-	0
	S. aureus	29	24	18	15	30/-	0
	C. albicans	28	22	17	13	-/34	0

Key: Amp (Ampicillin); Ket (Ketoconazole)

Table 6: Minimum Inhibitory (MIC) and Bactericidal/Fungicidal Concentrations (MBC/MFC) of Crude Aqueous and Methanolic Extract of *Lawsonia inermis* on Clinical Isolates.

Clinical isolates	MIC		MBC/MFC	
	Aqueous	Methanolic	Aqueous	Methanolic
E. coli	31.25	31.25	62.5	62.5
P. aeruginosa	31.25	31.25	62.5	62.5
K. pneumonia	31.25	31.25	62.5	62.5
S. aureus	31.25	31.25	62.5	62.5
C. albicans	31.25	31.25	62.5	62.5

Anatomical and transverse section of *L. inermis* leaves revealed features such as stomata, epidermis, calcium oxalate crystals (as seen in Figure 1).

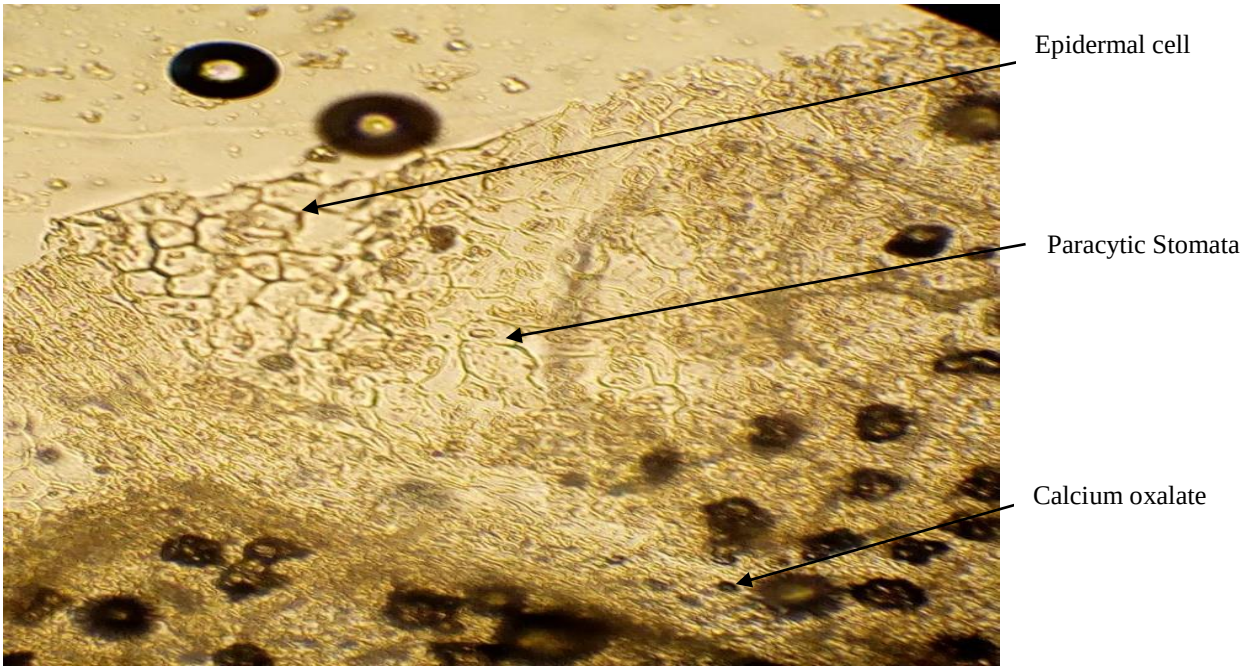

Figure 1: Photomicrograph of Transverse section of *Lawsonia inermis* leaves Magnification ×100

Discussion

The transverse section of *Lawsonia inermis* leaf revealed some prominent features like the stomata and the lower and upper epidermal layers. Anatomical features of the internal structures of plant drugs provides an important diagnostic features for the identification of both entire and powdered crude drugs and detection of adulterants in plant materials (Jeremiah *et al.,* 2019). Chemo-microscopical examination of the powdered leaves of *L. inermis* revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain and mucilage but calcium carbonate was absent. The chemo-microscopic features are most valuable in the identification of powdered drug as their identification is largely based on the form, the presence or absence of certain cell types and cell inclusions. These are very important diagnostic pharmacognostic parameters for the identification and authentication of crude drugs especially in powdered plants (Chanda, 2011).

The physicochemical constants of *Lawsonia inermis* leaves determined include the moisture content, total ash value, acid

insoluble ash, water soluble ash, alcohol (ethanol) extractives value and water extractives value. These values are useful as criteria to evaluate the identity and purity of crude drugs (Evans, 2009; WHO, 1996). It also indicates the presence of various inorganic materials like carbonate, oxalate and silicate in plant materials.

The average moisture content of the powdered plant material using loss on drying method was found to be 8.40%, and this value is within the permissible limits because B. H. P, (1990) and WHO, (2011) recommend the percentage of moisture content in any crude drug to be within 12-14 %. Low or permissible moisture in crude drugs may discourage the growth of bacteria, yeast, mould and fungi and will stand for long period of time during storage without spoilage or suggesting better stability against degradation of product (WHO, 1996). Ash values obtained include total ash as 5.80%, acid insoluble ash 3.20% and water soluble 5.22%. These Ash values indicate the presence of various impurities such as carbonate, oxalate, sand and silicate in plant materials (Kaneria and Chanda, 2011). The non-physiological ash is the inorganic residues in water soluble ash after the plant drug is burnt while the acid insoluble ash indicated that the plant was in good physiological condition and it contained little extraneous matters compared to the total ash content. The total ash value is used as a standard to assess the identity and purity of crude drugs (WHO, 1996, WHO, 2011).

The alcohol and water extractive values were 18.30% and 21.40% respectively. It was observed that alcohol had a higher extractive value (21.40%). Despites alcohol's low extraction capacity, it is sometimes more preferred than water especially in researches that deals with natural products because it serves as preservative against microbial growth and easy to evaporate and handle while water is a universal solvent that has high polarity and is able to extract more phytochemical constituents than alcohol that has less polarity. This verified why water is mostly used as solvent by traditional medical practitioner and individuals in preparation of dosage forms (Ajazuddin and Shailendra, 2010).

Throughout the world, there is increasing interest in the importance of dietary minerals in the prevention of several diseases (Jeremiah *et al.,* 2019). Minerals are of critical importance in the diet, even though they compromise only 4-6 % of the human body. However, lack of full understanding of the amount and type of elements found in medicinal plants can cause a lot of danger to consumers as some of these plants may contain toxic elements in high quantities (Jeremiah *et al.,* 2019). Again, proper dose rate of many of these medicinal plants is not established and makes it difficult for users to take them appropriately. The probability of taking overdose to facilitate healing processes is high and these can cause serious problems for users because they are ignorant of the dangers involved (Yazdinezhad *et al.,* 2016). Thus, the elemental composition of *L. inermis* was screened in the present study. The mineral element concentrations in milligram per kilogram of the leaf powder of *L. inermis* revealed that it contains both the macro and micro elements (Fe, Zn, Mn among others). Iron content (1.916ppm) was observed to be the element with the highest concentration. Iron (1220 ppm) is an essential component of respiratory pigments haemoglobin and myoglobin and also of various enzyme systems including the cytochromes, catalases, peroxidases, and the enzymes xanthine and aldehyde oxidase, and succinic dehydrogenase. As a component of the respiratory pigments and enzymes concerned in tissue oxidation, iron is essential for oxygen and electron transport within the body (Jeremiah *et al.,* 2019). Iron deficiency is the most prevalent nutritional deficiency in humans (Lokhande *et al.,* 2010; Jeremiah *et al.,* 2019).The recommended daily in-take for iron is 13.7 - 6.5 mg/day for children and 19.3 - 20.5 day for adults (CRDR, 2010). The results showed the presence of various elements detected with different concentrations and its medicinal importance with regards to life

processes and the probable significant roles of the plant in metabolism in the human body.

The result of this study reveals the efficacy of aqueous and methanolic extracts of leaves of *L. inermis* based on *in vitro* evaluation of antimicrobial activity of the extracts on clinical isolates of *Staphylococcus aureus, Escherichia coli, Klebsiella*, *Pseudomonas aeruginosa* and *Candida albicans*. The methanolic extract of leaves recorded the highest antibacterial activity on the clinical isolates than the aqueous extracts. Although methanol is polar, but it also has the ability to attract non-polar molecules. It has very low toxicity, completely miscible in water, volatile and easily removed from plant material at low temperature (Roy *et al.*, 2014).

The phytochemical screening of *Lawsonia inermis* leaves extracts recorded the presence of compounds carbohydrates, cardiac glycosides, triterpenes, flavonoids, saponins and alkaloids present in all extracts and responsible for antimicrobial activities as reported in similar studies (Hamid *et al*., 2011).A study conducted by Sharall *et al*., (2013) reported the presence of fatty acids and terpenes responsible for bactericidal activity. Hamid *et al.* (2011) also reported that phenolics, flavonoids, saponins and phorbol esters as antimicrobial compounds in *L. inermis.*

Generally, the aqueous extracts contained mainly acetic acid, furfural, pyrogallol, and saponins. Pyrogallol has been reported to be an effective antimicrobial agent and its bacterial toxicity is attributed to the three hydroxyl groups present in its structure (Kocacaliskan *et al.,* 2006). The result disagrees with most studies that showed activity of *L. inermis* on Gram negative organisms, which may be attributed to the fact that cells of Gram negative bacteria are less viable thus less affected by the extracts inhibitory activity when compared to those of Gram positive bacteria .Gram negative bacteria also possess efflux system that extrude antibacterial agents out of the cell (Tenovar, 2006). Arekemase *et al*. (2011) recorded high activity of the plant extract on *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus* spp. So also does Sammy *et al*., (1998). Similar result was also reported by Kalimathu *et al.* (2010) on *Pseudomonas aeruginosa* and *Staphylococcus aureus* with leaf extract of *Lawsonia inermis.* This study recorded high antibacterial activity on Gram positive *S. aureus*. While high activity on gram negative *E. coli* and *Pseudomonas aeruginosa* which might be attributed to their difference in cell wall composition.

The antibacterial effects of *L. inermis* had been previously studied and reported that the extract and latex displayed potent antimicrobial activity against *S. aureus, P. aeruginosa, E. coli* and host of other bacteria and fungi, giving inhibitory concentration as low as 0.5ml (Arekemase *et al.,* 2011) which confirms the potency of this plant in treating human infections. The MIC and MBC assay procedures are frequently used to evaluate some diverse agents such as antibiotics, antiseptics, disinfectants and chemotherapeutic agents (Andrews, 2001). In this study, the MIC and MBC values of both clinical isolates with both aqueous and methanolic extracts of *L. inermis* indicates significant bactericidal activities. This implies the strong efficacy of the extracts as stated by Arekemase (2011) that the constituents of the leaves of *L. inermis* contains phenols, flavonoids and some secondary metabolites that are very useful in antimicrobial activity. The MIC and MBC effects observed with different concentrations of various extracts against susceptible isolates in this study could be attributed to the presence of organic and fatty acids detected in the extracts. The interactions of these hydrocarbons with the hydrophobic structures of bacteria had been reported to result in antimicrobial activity (Zulfiker *et al*., 2011).

Conclusion

In conclusion, the results obtained in this study clearly demonstrated the effectiveness of aqueous and methanolic extracts of *L. inermis* as well as the control (Ampicillin and ketoconazole) on Gram positive and Gram negative bacteria and *Candida* of clinical origin. It also revealed the probable

chemical compounds in the crude extracts by phytochemical analysis which are responsible for the antibacterial activities. Based on the result of the analysis therefore, the work can be considered as part of an effort to validate the use of *L. inermis* in traditional medicine as well as source of future discovery of antimicrobial drugs.

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