

Full length Research Paper

Phytochemical screening and antibacterial activity of the aqueous extracts and fractions of ethanolic extracts of *Lawsonia inermis* leaf

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Accepted 05 December, 2011

The *Lawsonia inermis* leaves were extracted using water and ethanol. The ethanol extract was fractionated using petroleum-ether, chloroform, ethyl acetate and methanol. The aqueous extract, the fractions and the fractionation residue were subjected into phytochemical screening and antibacterial activity tests using standard methods. The phytochemical screening revealed the presence of alkaloids, carbohydrates, resins, saponins, sterols and tannins in different composition in the aqueous extract, fractions of ethanol extract and fractionation residue of the leaves. *In-vitro* antibacterial activities of the aqueous extract, fractions of ethanol extract and fractionation residue of the leaves were investigated against *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Salmonella typhi* and *Shigella dysenteriae* using agar-disc diffusion method. The aqueous extract, the fractions and the fractionation residues all showed antibacterial activities against the test isolates. The methanol, ethyl acetate, petroleum-ether fractions and the fractionation residues leaf exhibited MIC and MBC against majority of the test bacterial isolates within the range of 5mg/ml to 40mg/ml. The overall results of the study suggested that the *Lawsonia inermis* leaf could be a good source of antibacterial compounds.

Keywords: *Lawsonia inermis*, leaf extracts, phytochemical screening, antibacterial activities.

INTRODUCTION

The frequent emergence of antibiotic resistance strains of pathogenic bacteria has led to the need of finding alternative treatment using among others, plant extracts singly or in combinations. Plants have served as the basis of traditional medicine systems for thousands of years in countries such as China, India and in Africa (Mukhtar and Okafor, 2002). The need for new antimicrobial agents is closely associated with the problems of the emergence of strains that are resistant to most conventional antibiotics (Finland *et al.*, 1966). The use of herbal drugs in traditional medicine needs to be evaluated by using current scientific approaches with the view to giving the patient an appropriate dosage of the medication as against the most practiced unquantifiable

approach by the native healers (WHO, 1991). *Lawsonia inermis* is of family *Lythraceae* commonly found in savanna and deciduous forest. It is a glabrous, much branched shrub or quite a small tree with grayish brown bark. Leaves are opposite, sub-sessile, elliptic or broadly lanceolate entire acute or obtuse, 2-3cm long and 1-2cm wide. Flowers are numerous, small, white or raised coloured, fragrant, stamens 4-8. The fruits are globular, about 5mm in diameter. *Lawsonia inermis* leaves have been used traditionally in northern Nigeria as a remedy against diarrhea, dysentery and other related diseases, which are caused by *Corynaebacterium* spp., *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Shigella dysenteriae* (Aliyu, 2006). The use of herbal drugs in traditional medicine needs to be evaluated by using current scientific approaches with the view to giving the patient an appropriate dosage of the medication as against the most practiced unquantifiable

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approach by the native healers (WHO, 1991). The present study was therefore aimed at investigating the phytochemical constituents and antibacterial activities of the aqueous extracts and ethanolic fractions of *L. inermis* leaf extracts.

MATERIALS AND METHODS

Collection and identification of the research plants

The plant material was collected from Dawakin Tofa Local Government Area of Kano State. When collected the plants was identified in the Botany section of the Department of Biological Sciences, Bayero University, Kano with the aid of botanical keys (Arber, 1972).

Extraction of the *Lawsonia inermis* leaves

The *Lawsonia inermis* leaves were extracted in accordance with the procedure described by Fatope *et al* (1993) using distilled water and ethanol. Here, 100 grams of the powdered, air dried leaves were percolated in a litre of distilled water for one week with occasional shaking. At the end of one week, the extract was filtered using a Whatmans No.1 filter paper and the crude extract was evaporated to dryness using a water bath at 40°C. The dried extracts were weighed and kept in a freezer until required for further analysis. Similar treatment was repeated for the ethanolic extract. However, the crude extract was concentrated to dryness using a rotary evaporator at 40°C.

Fractionation of the crude ethanolic extracts

The crude ethanolic *L. inermis* leaf extract was fractionated by maceration procedure using petroleum-ether, chloroform, ethyl-acetate and methanol. The extract was macerated several times with the individual solvents using a volume ranging between 20 and 40ml until the initial colouration observed when the solvent was first added became very faint and negligible. The fraction recovered was filtered with filter paper and labeled as the fraction of the particular solvent used. The left over extract after final maceration with the last solvent was dried and labeled as the fractionation residue. The other four fractions were evaporated using rotary evaporator at 40 °C. The dried fractions and the fractionation residue were weighed and kept in a freezer until required for further use.

Phytochemical screening of the aqueous extracts and fractions of ethanolic extract of *L. inermis* leaf

Phytochemical analysis was carried out to determine the active ingredients of the aqueous extracts, fraction of ethanolic extract and fractionation residue of the *Lawsonia inermis* leaves. Procedures described by Sofowora (1993) were adopted for the detection of the presence of alkaloids, carbohydrates, flavonoids, glycosides, resins, saponins, sterols and tannins.

Bioassay studies

The test microorganisms

The test organisms were biochemically identified clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgaris*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Shigella dysenteriae*. They were obtained from Aminu Kano Teaching Hospital and some biochemical tests were carried out in the Microbiology laboratory of Bayero University Kano to confirm the authenticity of their identities (Chesebrough, 2002).

Standardization of the inoculum

The bacterial isolate was sub-cultured in nutrient broth for 24 hours. A loopful of the overnight nutrient broth was diluted in normal saline (0.85% NaCl w/v). The turbidity matched with 0.5 McFarland standard, which contained a mean of 3.33×10^6 cfu/ml, which matches with the standard turbidity of 1% (w/v) barium sulphate solution (Mukhtar and Tukur, 2000).

Preparation of extract concentrations

The extract concentrations were prepared in accordance with the dilution method described by Baker *et al* (1993). Concentrations of 400,000µg/ml, 200,000µg/ml, 100,000µg/ml and 50,000µg/ml were prepared using sterile distilled water for the aqueous extracts and DMSO for the fractions and the fractionation residues. Stock solutions were prepared by dissolving 0.8g of the aqueous extract in 2ml of sterilized distilled water and the fractions and the fractionation residue each in 2ml of DMSO. Thus, each stock solution has a concentration of 400mg/ml (400, 000µg per ml). Subsequent test

concentrations were prepared from the stock solutions using the formula demonstrated by Baker *et al* (1993), i.e., $(R \times V)/O$, which gave the volume of the stock solution that was diluted to the final volume required with the distilled water ('R' is the required concentration, 'V' is the total volume of solution required and 'O' is the original concentration of the stock solution).

Preparation of sensitivity discs

The sensitivity discs were prepared by punching a Whatman's No. 1 filter paper using a perforator (6 mm diameter). The discs were sterilized by autoclaving at 121°C for 15 minutes and a 1ml of each of the prepared concentrations for the aqueous extracts, fractions and the fractionation residues was used to impregnate 100 filter paper discs. Thus, the disc potencies of 4000 µg/ml 2000 µg/ml, 1000µg/ml and 500µg/disc were obtained respectively. The impregnated discs were then dried in an oven at 37°C for sixty minutes (Stokes and Ridgesway, 1980).

Sensitivity testing

The sensitivity testing was carried out using disc diffusion method described by Kirby-Bauer (1966). Appropriate sterile agar (nutrient, blood or chocolate) media were prepared depending on the test organism in use and carefully transferred in to sterile Petri dishes. The media were allowed to solidify and the plates were placed in a drier to remove excess moisture. The plates were marked to indicate the organism and the position of four discs of different test concentrations (50,000, 100,000, 200,000 and 400,000µg/ml). From the standard inoculum of each isolate, a loopful of a test bacterial inoculum was taken and streaked over the entire surface of the dried agar. Four discs of different concentrations were placed at the marked positions while one antibiotic disc was placed in the center to act as positive control. The plates were inverted and incubated for 24 hours at 37°C. At the end of this incubation period, the plates were observed for the presence of zones of inhibition as evidence of antibacterial activity. The degree of sensitivity was determined by measuring the diameter of visible zones of inhibition to the nearest millimeters with respect to each isolate and extract concentration.

Determination of MIC

The minimum inhibitory concentrations of the aqueous extract, fractions of ethanolic extract and fractionation residues were determined using tube dilution technique. Solutions of two-fold dilutions were prepared using sterilized distilled water to obtain concentrations of

5mg/ml, 10mg/ml, 20mg/ml and 40mg/ml. Equal volume of the above concentrations were incorporated in nutrient broth in 1:1 ratio and 0.1ml of standard suspension of the test organisms (3.33×10^6 cfu/ml) was added to each of the test tube. The tubes were then incubated aerobically at 37°C for 24 hours. Tubes containing broth and extract without inocula were included to serve as positive control while a tube containing broth and inocula serves as negative control for comparison. The presence of growth (turbidity solution) or absence of growth (clear Solution) at the end of incubation period was recorded. The highest dilution (least concentration) of the extract showing no detectable growth was regarded as the minimal inhibitory concentration (Baker *et al.*, 1993; NCCLS, 1999).

Determination of MBC

The minimum bactericidal concentrations of the aqueous extracts, fractions of ethanol extracts and the fractionation residues were determined by sub culturing 0.1ml from the last MIC test dilution that showed visible growth (turbidity) and all others in which there was no detectable growth on a fresh extract free solid medium and incubated at 37°C for further 24 hours. The highest dilution that shows no single bacterial colony was considered as the minimum bactericidal concentration (Baker *et al.*, 1993; NCCLS, 1999).

RESULTS AND DISCUSSION

Table 1 presents the physical characteristics of the aqueous and ethanol extracts of the *Lawsonia inermis* (leaf). The aqueous extract was brownish in colour, gummy in texture and about 12.6% was recovered after the extraction processes. The ethanol extract was dark green in colour, oily in texture and 32.3% was recovered after the extraction processes. Therefore, it can be observed that the powdered leaf of *Lawsonia inermis* contained chemical constituents that are more soluble in ethanol than water.

Table 2 shows the physical characteristics of petroleum ether, chloroform, ethyl acetate and methanol fractions of ethanol extracts of the *Lawsonia inermis* (leaf). The percentage recovery of the individual fraction was 4.03, 3.55, 1.81 and 87.19 respectively. It can be observed from the table that the ethanol extract of the *Lawsonia inermis* (leaf) was highly soluble in methanol compared to the rest of the solvent. The variation in colour may be due to the differences in the natural colour of the constituents of the plant's parts from which the extracts and fractions were obtained. Muhammad and Muhammad (2005) reported that lawsone is the chief constituent responsible for the dyeing properties of *Lawsonia inermis* leaf. The variation in texture may be due to the differences in the biochemical composition of the different parts of the

Table 1. Physical characteristics of aqueous and ethanolic *L. inermis* leaf extracts

Characteristics	Aqueous extract	Ethanolic extract
Colour	Brownish	Dark green
Texture	Gummy	Oily
Initial weight (g)	100	100
Final weight (g)	12.6	32.3
Recovery (%)	12.6	32.3

Table 2. Physical characteristics of the fractions of ethanolic *L. inermis* leaf extracts

Characteristics	Petroleum-ether fraction	Chloroform fraction	Ethyl-acetate Fraction	Methanol Fraction
Colour	Greenish	Greenish	Brownish	Brownish
Texture	Oily	Gummy	Gummy	Oily
Initial weight (g)	32.30	31.00	29.90	29.36
Final weight (g)	1.30	1.10	0.54	25.60
Recovery (%)	4.03	3.55	1.81	87.19

Table 3. Phytochemical constituents of the aqueous and fractions of ethanolic *L. inermis* leaf extracts

	Aqueous extract	Petroleum-ether fraction	Chloroform fraction	Ethyl-acetate fraction	Methanol fraction
Alkaloids	-	-	+	+	-
Carbohydrates	+	+	+	+	+
Flavonoids	-	-	-	-	-
Glycosides	-	-	-	-	-
Resins	-	+	+	-	-
Saponins	+	+	+	-	+
Sterols	+	-	-	+	+
Tannins	-	+	-	-	+

Key: + = Present, - = Absent

research plants. Some parts may contain some gummy substances, while some may contain oily substances. The variation in the percentage recovery may be due to the differences in the solubility of the metabolites content of the *Lawsonia inermis* leaf in a particular solvent. The methanol fraction of the *Lawsonia inermis* leaf had higher fraction recovery and this may possibly be due to the high polarity of the methanol.

Table 3 shows the phytochemical constituents of the aqueous extract and fractions of ethanolic extract of *Lawsonia inermis* leaf. Carbohydrates were found to be present in the aqueous extract as well as in all the fractions, while flavonoids and glycosides were not detected in the aqueous extract and in all the fractions. In addition to the carbohydrates, the aqueous extract contained saponins and sterols, the petroleum ether fraction contained resins, saponins and tannins, the chloroform fraction contained alkaloids, resins, and

saponins, the ethyl acetate fraction contained alkaloids and sterols while the methanol fraction contained saponins, sterols and tannins.

Table 4 presents the antibacterial activities of the aqueous extract and fractions of ethanolic extracts of *Lawsonia inermis* leaf. The streptomycin (30µg) used as a positive control was found to be active against all the test bacterial isolates with diameter of inhibition zones ranging between 21mm and 25mm. The aqueous extract of the *Lawsonia inermis* leaf was found to be inactive against *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Shigella dysenteriae* at all discs concentration, but was active against the rest of the test bacteria at 2000µg/disc with diameter of zones of inhibition ranging between 8mm to 11mm. The highest inhibition zone of 13mm was shown by *Salmonella typhi* at disc concentration of 4000 µg/disc. The petroleum-ether fraction of ethanol extracts of the *Lawsonia inermis*

Table 4: Antibacterial activities of the aqueous extract and fractions of ethanol extract of *Lawsonia inermis* (leaf)

Organisms	Diameter of zone of inhibition (mm)/ Extract concentration ($\mu\text{g}/\text{disc}$)																				
	Cont. (S)	Aqueous extract				Petroleum-ether				Chloroform				Ethyl-acetate				Methanol			
	30 μg	500	1000	2000	4000	500	1000	2000	4000	500	1000	2000	4000	500	1000	2000	4000	500	1000	2000	4000
<i>Staph. aureus</i>	22	-	-	10	12	-	-	-	-	-	-	8	10	-	-	8	10	-	-	9	11
<i>Proteus vulgaris</i>	22	-	-	9	11	-	-	-	9	7	8	10	12	-	9	15	17	-	-	9	10
<i>Streptococcus Pneumoniae</i>	25	-	-	-	-	-	-	-	-	-	-	8	11	-	-	-	12	-	-	-	9
<i>Pseudomonas aeruginosa</i>	22	-	-	9	10	-	-	-	10	-	-	9	11	-	-	-	9	-	-	9	13
<i>Escherichia coli</i>	25	-	-	8	10	-	-	-	-	-	-	8	12	-	8	9	16	-	-	8	13
<i>Klebsiella pneumoniae</i>	21	-	-	10	12	-	-	-	12	7	9	10	12	-	-	13	18	-	-	10	12
<i>Salmonella typhi</i>	14	-	-	11	13	-	-	7	10	-	9	12	14	-	9	11	12	-	-	8	10
<i>Streptococcus pyogenes</i>	23	-	-	-	-	-	-	-	10	-	-	9	11	-	-	-	-	-	-	10	13
<i>Shigella dysenteriae</i>	22	-	-	-	-	-	-	9	11	7	9	10	12	-	10	12	14	8	9	10	12

Key:- = Disc diameter (6mm). S = Streptomycin

leaf was found to be inactive against the entire test bacteria at concentration of 500 μg and 1000 $\mu\text{g}/\text{disc}$. The fraction was also inactive against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Escherichia coli* at all disc concentrations. However, at 2000 $\mu\text{g}/\text{disc}$ it was found to be active against only *Salmonella typhi* and *Shigella dysenteriae* with diameter of inhibition zones of 7mm and 9mm respectively. The rest of the test bacterial isolates were all sensitive to the fraction at concentration of 4000 $\mu\text{g}/\text{disc}$ with diameter of inhibition zone ranging between 9mm and 12mm. The chloroform fraction of ethanol extract of the *Lawsonia inermis* leaf was found to be active against all the test bacteria at various disc concentrations. At

500 $\mu\text{g}/\text{disc}$ the fraction was slightly active against *Proteus vulgaris*, *Klebsiella pneumoniae* and *Shigella dysenteriae* with diameter of inhibition zones of 7mm each. *Salmonella typhi* was found to be sensitive to the fraction at 1000 $\mu\text{g}/\text{disc}$ with 9mm diameter of inhibition zone. At 2000 $\mu\text{g}/\text{disc}$ it was active against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus pyogenes* with diameter of zones of inhibition of 8mm each for *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pneumoniae* and *Escherichia coli* and 9mm each for *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. The highest inhibition zone of 14mm was shown on *Salmonella typhi* at 4000 $\mu\text{g}/\text{disc}$.

The ethyl-acetate fraction of ethanol extract of the *Lawsonia inermis* leaf was found active against all the test bacterial isolates except *Streptococcus pyogenes* which was not sensitive to the fraction at all disc concentrations. At 1000 $\mu\text{g}/\text{disc}$, the fraction showed diameter of inhibition zones of 8mm for *Escherichia coli*, 9mm each for *Proteus vulgaris* and *Salmonella typhi* and 10mm for *Shigella dysenteriae*. It was also found active against *Staphylococcus aureus* and *Klebsiella pneumoniae* at 2000 $\mu\text{g}/\text{disc}$ with diameter of zones of inhibition of 8mm and 13mm respectively. *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* were found sensitive to the fraction only at 4000 $\mu\text{g}/\text{disc}$ with 12mm and 9mm inhibition zone respectively. The highest

Table 5. MIC and MBC of the aqueous and fractions of ethanolic *L. inermis* leaf extracts

Fractions Organisms	Diameter of zone of inhibition (mm)/Extract concentration (µg/disc)									
	Aqueous extract		Petroleum-ether		Chloroform		Ethyl-acetate		Methanol	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>Staph. aureus</i>	40	-	-	40	-	-	20	40	40	-
<i>Proteus vulgaris</i>	-	-	20	40	40	-	5	10	5	10
<i>Strep. pneumoniae</i>	-	-	-	-	40	-	20	40	20	40
<i>Pseudo. aeruginosa</i>	-	-	20	40	-	-	5	10	5	10
<i>Escherichia coli</i>	-	-	-	-	40	-	-	-	20	40
<i>Kleb. pneumoniae</i>	-	-	-	-	-	-	-	-	20	40
<i>Salmonella typhi</i>	-	-	10	20	40	-	-	-	5	10
<i>Strep. pyogenes</i>	-	-	20	40	10	20	-	-	5	10
<i>Shig. dysenteriae</i>	-	-	-	-	-	-	-	-	-	-

Key: - = > 40mg/ml

zone of inhibition of 18mm was shown against *Klebsiella pneumoniae* at 4000µg/disc. The methanol fraction of ethanol extract of the *Lawsonia inermis* leaf showed activity against all the tested bacterial isolates at different disc concentrations. It was active against *Shigella dysenteriae* at 500µg/disc with 8mm inhibition zone, while *Streptococcus pneumoniae* was sensitive to the fraction only at 4000 µg/disc concentration with 9mm inhibition zone. The rest of the bacterial isolates were all sensitive to the fraction at 2000 µg/disc with diameter of zones of inhibition ranging between 8mm and 10mm. The highest inhibition zones of 13mm each was shown against *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus pyogenes* at 4000 µg/disc.

Table 5 presents the results of MIC and MBC of the aqueous and fractions of ethanolic *L. inermis* leaf extracts. The aqueous extract was found to inhibit the growth of *Staphylococcus aureus* at 40mg/ml but does not showed inhibitory or bactericidal effect against the rest of the bacteria. The petroleum ether fraction of *Lawsonia inermis* leaf was found to exert both inhibitory and bactericidal effects against *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* at 20mg/ml and 40mg/ml respectively. It also showed MIC and MBC of 10mg/ml and 20mg/ml against *Salmonella typhi*. The chloroform fraction of *Lawsonia inermis* leaf was showed inhibitory effects against *Proteus vulgaris*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Salmonella typhi* both at 40mg/ml, while the growth of *Streptococcus pyogenes* was inhibited at 10mg/ml and was killed at 20mg/ml. The ethyl acetate fraction of *Lawsonia inermis* leaf was found to shows inhibitory and bactericidal effects against *Staphylococcus aureus* and *Streptococcus pneumoniae* at 20mg/ml and 40mg/ml respectively. It also inhibits the growth of *Proteus vulgaris* and *Pseudomonas aeruginosa* at 5mg/ml while at 10mg/ml the fraction showed a bactericidal effect against the test organisms. The methanol fraction of *Lawsonia inermis* leaf was found to

shows MIC of 5mg/ml and MBC of 10mg/ml against *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Streptococcus pyogenes*, MIC 20mg/ml and MBC of 40mg/ml against *Streptococcus pneumoniae*, *Escherichia coli* and *Klebsiella pneumoniae* and an MIC of 40mg/ml *Staphylococcus aureus*. The bactericidal and growth inhibitory effects of *Lawsonia inermis* leaf fractions of methanol (Table 5), petroleum ether and ethyl acetate might be related to the presence of alkaloids, resins, saponins, sterols, and tannins in the individual fraction. A previous study by Ali *et al* (2001) indicated that the ethyl acetate extract of *Lawsonia inermis* showed broad spectrum of antibacterial activity. Usman *et al* (2009) reported that the presence of aloes, anthraquinones derivatives, cardenolides, cardiac glycosides, flavonoids, resins, saponins and tannins in the partitioned portion of *Bauhinia rufescens* bark were responsible for the antibacterial activity. Report of a study by Isaac and Chinwe (2001) revealed that alkaloids along with tannins and saponins are responsible for antibacterial activity of the extract of *Tetracapidium conophorum*. Onoruvwe and Olorunfemi (1998) also attributed the antibacterial effect of the root extract of *Dichrostachys cinerea* to alkaloids, saponins and flavonoids.

CONCLUSION

The results of the phytochemical screening showed that alkaloids, carbohydrates, resins, saponins, sterols and tannins were present in varied compositions in the aqueous extract and the fractions of ethanolic extract of the *Lawsonia inermis* leaf. The results of the sensitivity test also showed that both the aqueous extract and the fractions of ethanolic extract of the *Lawsonia inermis* leaf had antibacterial activities against the tested bacterial isolates.

RECOMMENDATIONS

Since the finding of this study revealed that both the aqueous extract and fractions of the ethanolic extract of the *Lawsonia inermis* leaf showed antibacterial activities against the test bacterial isolates. Therefore, it was recommended that further studies should be carried out on them to further purify the actual bioactive compounds that have the antibacterial activity and to ascertain their toxicity level before recommending for consumption.

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