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Original Article

Antibacterial Activity of *Lawsonia inermis* (Sudanese Henna) Leaves Extracts against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* among Recurrent Urinary Tract Infection patients in Omdurman Military Hospital

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ABSTRCT

Background: *Lawsonia inermis* (Sudanese Henna) a perennial plant that has the ability to grow in various environments in Sudan and traditionally uses as cosmetically for skin dye and hair and rich in antimicrobial agents that can be used for discovery of new classes of compounds that help in treatment of bacterial resistance strains.
Methods: A total of 100 urine samples were collected from patient with recurrent urinary tract infection and inoculated on to CLED agar. Leaves of *Lawsonia inermis* were extracted by methanol and water and tested for its antibacterial activity using agar diffusion method whilst the phytochemical analysis of *L.inermis* leaves methanol extract using quantitative gas chromatoghraphy method.
Results: Out of 32 isolated bacteria, 4 were *Staphylococcus aureus* (12.5%), 16 *Escherichia coli* (50%) and 3 *Pseudomonas aeruginosa* (9.4%). The reminder 9 (28%) were other bacteria. The studied methanol and water leavse extracts of *L. inermis* displayed various degree antibacterial activities were tested against isolated bacteria with minimum inhibitory concentration (MIC) between (6.25-25mg/ml), whilst water extract displayed no activity against *E. coli* and MDR *E. coli*. Gas chromatography analysis revealed that 51 chemical compounds of *L. inermis* (Henna).
Conclusion: The results of the present study support that *Lawsonia inermis* has antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, MRSA and MDR *E. coli.*

Keywords: *Lawsonia inermis*, recurrent urinary tract infection, minimum inhibitory concentration (MIC), phytochemical compounds.

INTRODUCTION

According to the World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Herbal drugs have found wide spread use in many countries because they are easily available, cheaper and safer than synthetic drugs. [1] Antimicrobial resistance is a major and increasing global health care problem, a large number of bacteria have responded to the use of antibiotics with their ability to evolve and transmit antimicrobial resistance to other species, increased consumption of antimicrobial agents and inappropriate use can accelerate this phenomenon. Also the continuous migrations of people play an important role in acquisition and spread of Multi drug resistant strains. [2] Urinary tract infection causing bacteria become more resistant to available antibiotics, the need to explore new strategies for managing UTIs is clear. [3] Henna has a wide spectrum of antimicrobial activity including antibacterial, antiviral, antimycotic and antiparasitic activities. With the ever increasing resistant strains to the already available and synthesized antibiotic, the naturally available *L. inermis* could be a potential alternative.[4] Out of forty-five species of 29 plant families used in traditional medicine by Iranian people showed antibacterial activities against eleven bacterial species, henna showed strong activity against *Bordetella bronchiseptica*. These findings indicated that *L. inermis* can be used in the treatment of bacterial infections. [5] Henna leaves contain lawsone dye this molecule has an affinity for bonding with protein, and thus has been used to dye skin, hair, silk and wool. [6] Henna has been used cosmetically and medicinally for over 9000

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years. Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments as rheumatoid arthritis, headache, ulcer, diarrhea, leprosy, fever, leucorrhoea, diabetes, cardiac disease, jaundice, hepatoprotective and coloring agent. [7] In addition henna is used as anti-cancer and antioxidant properties. [8] This study aimed to evaluate the antibacterial activity, minimum inhibitory concentration and chemical compounds of *Lawsonia inermis* (henna) extract against some clinical isolates from patients with recurrent urinary tract infections.

MATERIALS AND METHODS

Study design: Descriptive, cross sectional and hospital based study.

Study area: Omdurman Military Hospital.

Study duration: The study was carried out in the period from May to August 2015

Study Population: Patients with recurrent urinary tract infection.

Inclusion criteria: Patients with recurrent urinary tract infection were included.

Exclusion criteria: Community members (free of recurrent urinary tract infection).

Sampling: Non - probability sampling.

Sample size: One hundred urine samples (n=100) were collected as randomized from patients with recurrent urinary tract infection.

Study variables: Screen on recurrent urinary tract infection patients (dependent variable).Age and gender taken as independent variables.

Data collection: The data were collected from records of hospitals.

Ethical considerations: Permission of this study was obtained from the local authorities in the area of study, the objective of the study clearly and simply were explained to all individuals participating in the study, verbal informed consent was obtained.

Sampling method: Mid-stream urine samples were collected in universal wide mouth sterile urine containers. Specimen was carried in ice bag in order to be preserved till reached the laboratory.

Culture: The specimens were inoculated under aseptic conditions on Cystine lysine electrolyte deficient (CLED) (Hi-Media laboratories Pvt, Ltd, India). The inoculated culture media were incubated aerobically at 37 °C overnight for 18-24 hrs and examined for growth and identification done by routine bacteriological methods.

Antimicrobial susceptibility test: The isolated pathogens were sub cultured on nutrient agar to obtain fresh isolated colonies. The antibiotics used in this were Ciprofloxacin (30 mcg), Gentamicin (10 mcg), ceftriaxone (30mcg), penicillin (5mcg), Vancomycin (30cg), Oxacillin (1mcg) and Nalidixic Acid (30mcg) (Hi-Media laboratories Pvt, Ltd, India).

Kirby-Bauer Disk-Diffusion Method: Under aseptic condition the suspension from all growth culture media were prepared by using normal saline, 2-3 colonies were emulsified from each isolate in separate tube and compared with turbidity standard (McFarland standard 0.5=10 cfu/ml) in a good light for adjustment, then using sterile swab immersed in suspension in the surface of the tube to remove the excess. Muller Hinton (Hi-Media laboratories Pvt, Ltd, India) surface was inoculated by swabbing, then application of antimicrobial disc by using sterile forceps to the medium, the distances were at least 24mm between two disc on the inoculated plate and 15mm from the edges of the plate, plates were incubated at 37˚C overnight. [9]

Collection and identification of plant material: *Lawsonia inermis* leaves were collected from Omdurman city. Fresh leaves were washed, dried in shade at room temperature for 24hr and ground into powder using mortar and pestle. Then the leaves were taxonomically identified by Medicinal and Aromatic Plants Research Institute (MAPRI) in Khartoum.

Henna leaves extraction: Extraction was carried out according to method described by [10]

Preparation of the methanol extract: Fifty grams of the plant sample was grinded using mortar and pestle and extracted with methanol using soxhelt extractor apparatus. Extraction carried out for about eight hours till the solvent returned colorless at the last siphoning times. Solvent was evaporated under reduced pressure using rotary evaporator apparatus (40˚C). Finally extract allowed to air in Petri dish till complete dryness and the yield percentage was calculated as followed: Weight of extract obtained / weight of plant sample x 100.

Preparation of the aqueous extract: Fifty grams of the plant sample was soaked in 500 ml hot distilled water, and left till cooled down with continuous stirring at room temperature. Extract was then filtered and freezed. Freezed extract was dried using freeze dryer till powdered extract obtained. Yield percentage was calculated.

Antibacterial susceptibility of henna extracts (Cup diffusion Method): Sterile cotton swab was dipped into the bacterial test suspension matched with 0.5 McFarland standards to inoculate entire surface of Mueller- Hinton agar plate. Wells or cups of 8mm were made with a sterile cork borer in the inoculated agar plates. 100µl volumes of methanol extract from different concentrations were poured directly into the wells. The plates were allowed to stand for 1 hour in refrigerator for diffusion of the extract to take place and incubated at 37˚C for 24 hours, after incubation inhibition zone diameters were measured in millimeter. [11]

Determination of minimum inhibitory concentration (MIC) by agar diffusion dilution method: Determination of inhibition zones and MIC of henna extracts were assessed using Agar diffusion dilution method as described in [12] and [13]. One gram from each extract was dissolved in 10ml 100% methanol for alcohol extract and distilled water for water extract, then serially diluted two fold to an obtain final concentration (50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml), 60 microliters of each prepared concentration were added in to the corresponding well. The plates were left for 1 hour in refrigerator (4°C), and then incubated at 37°C for 24 hours. Inhibition zone around each well were measured using a ruler in millimeter. MIC is the lowest concentration of plant extract that did not permit any visible growth of the inoculated test organism.

Interpretation of Results: After 24 hours incubation antibacterial activity result were expressed in diameters of inhibition zones in millimeter were measured < 9 mm zone was considered as inactive; 9-12mm as partially active while 13-18mm as active and > 18mm as very active. [14]

Quality control procedure

Control of culture media: The performance of culture media was controlled by testing each patch with known strains, and then checked after 24hours incubation for expected characters of growth.

Control susceptibility testing method

Reference strain quality control: The quality control *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC25922 were brought from National Public Health Laboratories, those reference strains were recommended for controlling the susceptibility test as described in NCCLS document M7-A7. The stock culture was stored at -20°C in 10% glycerol broth and sub cultured on to agar plate to obtained fresh colonies. Control strains suspended according to the recommended inoculums preparation procedures.

Batch quality control: Each batch of susceptibility test was tested with the reference strain to determine if zone diameter obtained within the expected rang or not. Also non inoculated agar plate was incubated over night to ensure the medium was sterile.

Phytochemical Screening; Phytochemical screening for the active constituents was carried out for the most effective methanol extract of henna using Gas Chromatographic Mass Spectroscopy (GC-MS). Model: GC-MS. QP. 2010. Made in Japan. In gas chromatography, the moving phase was Helium. The stationary phase was a microscopic layer of liquid or polymer on an insert solid support inside a piece of glass or metal tubing called a column (a homage to the fractionating column used in distillation).

Data analysis: SPSS version 11.5 (One-Way ANOVA: $P < 0.05$) was used for data analysis.

RESULTS

Among of the 100 urine specimens 41(41%) were males and 59(59%) were females (fig 1). Out of 100 investigated samples 32 showed bacterial growth while 68 showed no bacterial growth (Figure 2). In this study percentage of recurrent urinary tract infections more in females than males (Fig 3).

Antibacterial Susceptibility Test: The antibacterial susceptibility test of isolates and standard organisms were determined using standard disk diffusion method. The results showed that all *S. aureus* isolates were resistant to penicillin (100%). Out of 4 positive 2(50%) *S. aureus* and the standard *S. aureus* ATCC29213 were susceptible to oxacillin while 2(50%) were oxacillin resistant. Oxacillin resistant isolate termed to be MRSA (tables 3,4,5,6 and fig 5). *Escherichia coli* susceptibility testing results showed (75%) *E. coli* were resistant to Ciprofloxacin, (69%) Gentamicin, (81%) Ceftriaxone and (88%) Nalidixic Acid termed to be *E. coli* multiple drug resistant (MDR). The reminder 4(25%) and *E. coli* ATCC25922 were susceptible (tables 1&2) and (fig 4).

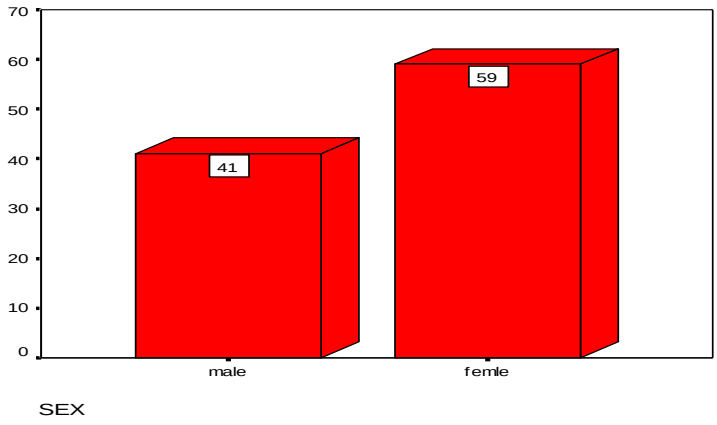

Fig 1: Distribution of samples according to gender

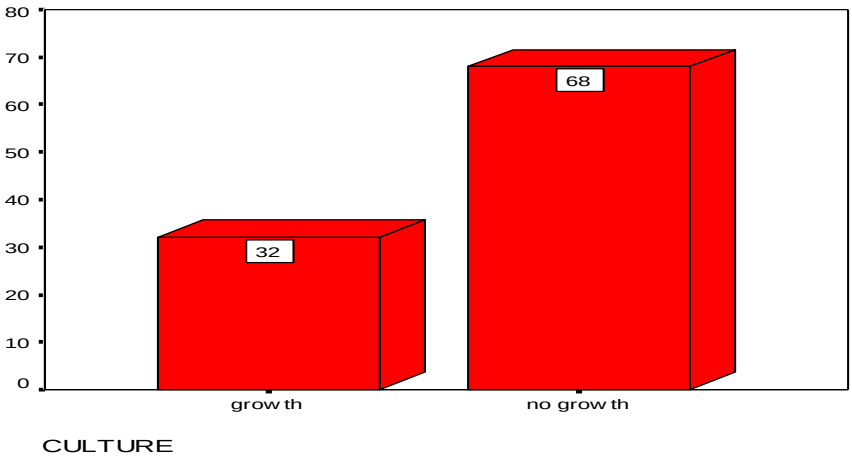

Fig 2: **The percentage of bacterial growth on CLED**

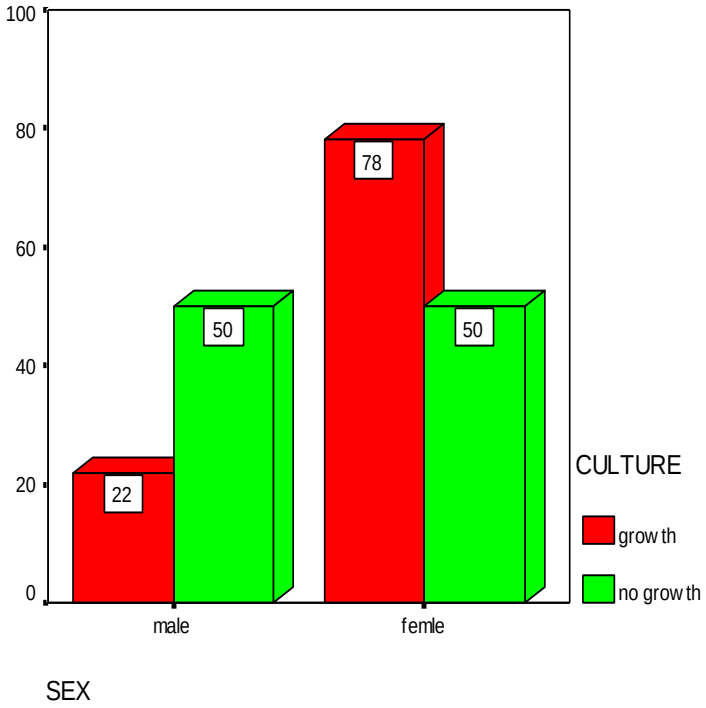

Fig 3: Percentage of growth in two genders

Table1: Antibiotic susceptibility pattern of *E. coli* and *P. areuginosa* isolates from recurrent urinary tract patients.

Bacterial species	T	Ciprofloxacin		Gentamicin		Ceftriaxone		Nalidixic Acid	
		S	R	S	R	S	R	S	R
E. coli	16	4 25%	12 75%	5 31%	11 69%	3 19%	13 81%	2 12%	14 88%
P. areuginosa	3	3 100%	0 0%	1 33%	2 67%	2 67%	1 33%	0 0%	3 100%

*** Key: T: Total; S: Sensitive; R: Resistant; %: Percentage**

Table2: Antibiotic susceptibility pattern of *S. aureus* isolate from recurrent urinary tract patients.

Bacteria species	T	Penicillin		Oxacillin		Ciprofloxacin		Vancomycin	
		S	R	S	R	S	R	S	R
S. aureus	4	0 0%	4 100%	2 50%	2 50%	2 50%	2 50%	4 100%	0 0%

Fig 4: Antimicrobial susceptibility testing of *E. coli* ATCC25922 and *E. coli* (MDR) to ciprofloxacin, Gentamicin, Ceftriaxone and Nalidixic Acid

Antibacterial activity of Henna: Among this study both methanol and water extracts of *Lawsonia inermis* showed antibacterial activity against strains of *S. aureus*, MRSA, *P. areuginosa*, *E. coli*, *E. coli* MDR and control, water extract did not show antibacterial activity against *E. coli*, *E. coli* MDR and ATCC. Results were expressed as mean ± SD. The statistical significance was established at $P < 0.05$ (Table3 & 4).

Minimum inhibitory concentration (MIC) of *Lawsonia inermis* obtained by agar diffusion method: The Minimum inhibitory concentration of each bacterium against the different extracts obtained by agar diffusion method are shown in (table5) and (figures from 5 &6).

Gas chromatography results: GC-MS Chromatogram of methanol leaves extract of *Lawsonia inermis* (figure 8) clearly showed 51 peaks indicating the presence of 51 phytochemical compounds (Table 6), 30 compounds were found as antibacterial effect (Table 7).

Table 3: Mean of inhibition zones of water extract in different concentrations against bacterial isolates and standards (mm).

Water extract concentrations				
Bacterial isolates	**50%**	**25%**	**12.5%**	**6.25%**
S.aureus	15 ± 1.4	11.7 ± 0.5	8.3 ± 0.4	NA
MRSA	12.5 ± 0.7	9.5 ± 0.7	NA	NA
P. aeruginosa	16.1 ± 1	13 ± 1	10 ± 1	NA
E.coli	NA	NA	NA	NA
***E.coli* MDR**	NA	NA	NA	NA
***S.aureus* ATCC29213**	12 ± 1.4	10.5 ± 0.7	NA	NA
***P. areuginosa* ATCC27853**	15.5 ± 0.7	10.8 ± 0.4	9.3 ± 0.4	NA
***E. coli* ATCC25922**	NA	NA	NA	NA

***key: Diameter of inhibition zone include diameter of well (8mm); Values are represented as mean ± SD; P<0.05; MRSA: Methicillin Resistant S.aureus; MDR: Multi-Drug Resistant; NA: Not Affect.**

Table 4: Mean of inhibition zones of methanol extract with different concentrations against bacterial isolates and standards (mm).

Methanol extract concentrations				
Bacterial isolates	**50%**	**25%**	**12.5%**	**6.25%**
S.aureus	23 ± 1.4	19.3 ± 1	12 ± 1.4	8.5 ± 0.7
MRSA	18.3 ± 1.1	13.5 ± 0.7	9.8 ± 1.1	NA
P. aeruginosa	18.8 ± 1.6	14.8 ± 1	10.7 ± 1.5	NA
E.coli	16.4 ± 0.9	12.9 ± 0.9	9.9 ± 0.6	NA
***E.coli* MDR**	14.4 ± 1.1	11.2 ± 0.9	NA	NA
***S.aureus* ATCC29213**	26.5 ± 0.7	22.5 ± 0.7	17 ± 1.4	8.5 ± 0.7
***P. aeruginosa* ATCC27853**	22.5 ± 1.4	16 ± 1.4	11.5 ± 0.7	9 ± 1.4
***E. coli* ATCC25922**	21 ± 1.4	17 ± 1.4	11.5 ± 2	8.5 ± 0.7

*** P < 0.05**

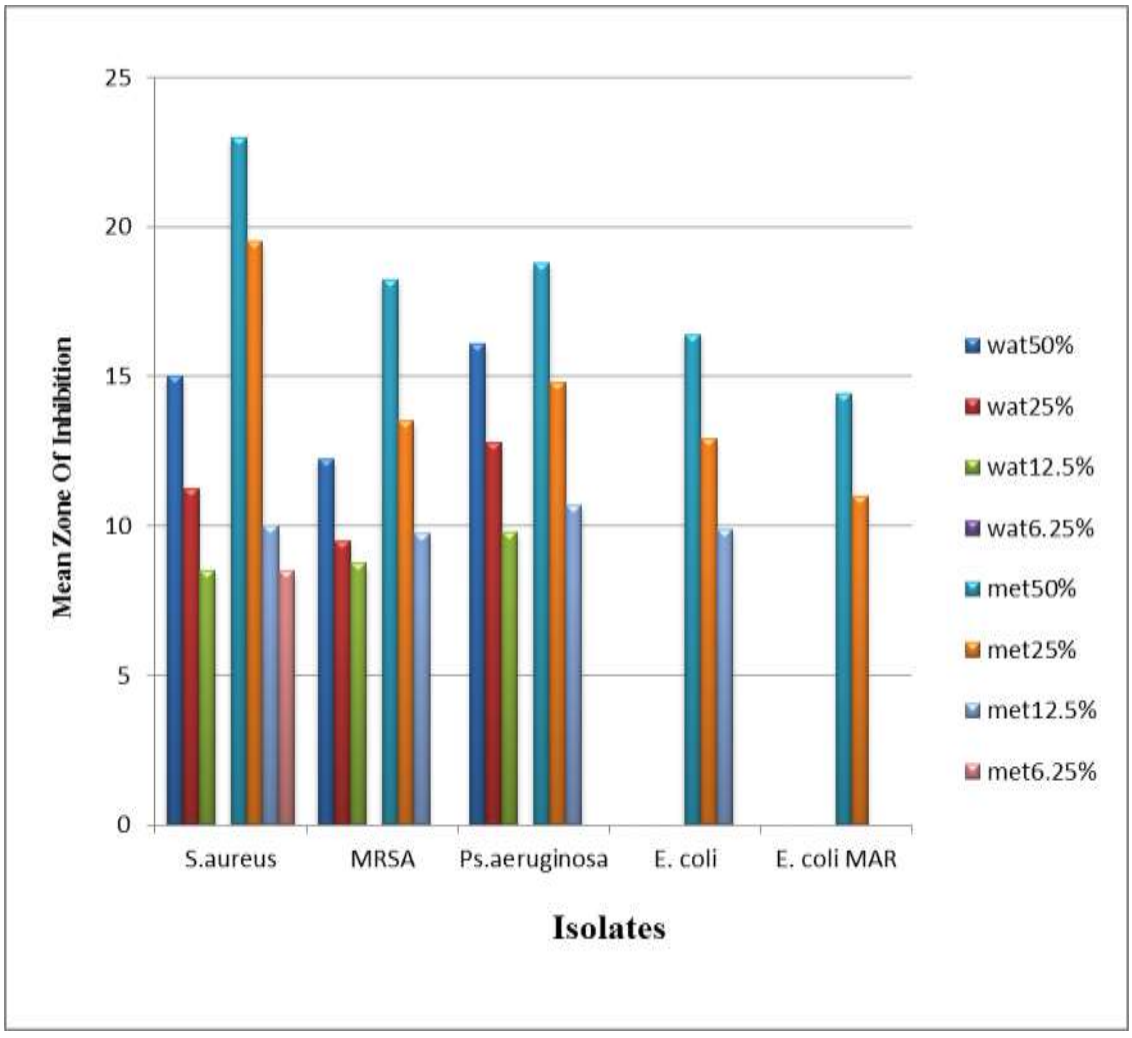

Fig 5: Mean of inhibition zones after in vitro exposure of isolates to henna methanol (Meth) and water (Wat) extracts in different concentrations (Diameter in millimeter).

Table5: Minimum inhibitory concentration of *Lawsonia inermis* methanol and water extracts obtained by agar diffusion method

Bacteria species	Water extracts	Methanol extracts
***S. aureus* ATCC29213**	25mg/ml	6.3mg/ml
S. aureus	25mg/ml	12.5mg/ml
Methicillin-resistant *S. aureus*	25mg/ml	12.5mg/ml
***P. areuginosa*ATCC**27853	12.5mg/ml	6.25mg/ml
P. areuginosa	12.5mg/ml	12.5mg/ml
***E. coli*ATCC**25922	50mg/ml (R)	12.5mg/ml
E. coli	50mg/ml (R)	12.5mg/ml
***E. coli* MAR**	50mg/ml (R)	25mg/ml

P < 0.05

R: Resistant

Fig 6: Activity of *Lawsoni inermis* methanol and water extracts on *S.aureus* ATCC29213 with concentration 5omg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml.

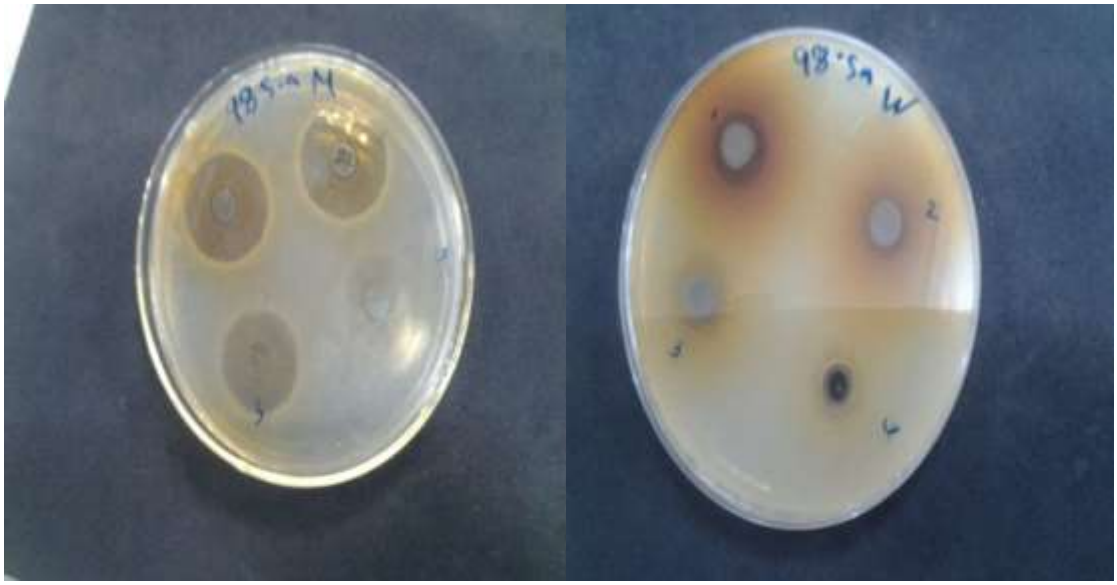

Fig 7: Activity of *L. inermis* methanol and water extracts on *S.aureus* isolate with concentration 5omg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml.

Table 6: Gas chromatography analysis of *Lawsonia inermis* leaves methanol extract

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	3.288	185640	0.52	2-Furanmethanol
2	3.748	886185	2.47	(S)-(+)-2-Amino-3-methyl-1-butanol
3	4.317	113851	0.32	6-Oxa-bicyclo[3.1.0]hexan-3-one
4	5.427	143069	0.40	2-Hydroxy-gamma-butyrolactone
5	5.522	143561	0.40	7-Oxabicyclo[4.1.0]heptan-2-one
6	6.317	141155	0.39	2,5-Piperazinedione
7	6.735	59810	0.17	1,3,2-Dioxaborolan-4-one, 2-ethyl-
8	7.088	378782	1.05	Thymine
9	7.261	78114	0.22	1-Butene, 4-iodo-
10	7.421	87208	0.24	Mequinol
11	8.283	95618	0.27	Ethanamine, N-ethyl-N-nitroso-
12	8.460	738676	2.06	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydrox
13	9.346	899111	2.50	Neopentyl glycol
14	9.578	66614	0.19	Catechol
15	9.950	1115581	3.10	Benzofuran, 2,3-dihydro-
16	10.136	1093712	3.04	5-Hydroxymethylfurfural
17	10.397	153391	0.43	3-Acetoxy-3-hydroxypropionic acid, methy
18	11.577	314758	0.88	3-cis-Methoxy-5-cis-methyl-1R-cyclohexan
19	11.877	543789	1.51	2-Methoxy-4-vinylphenol
20	12.349	85966	0.24	Pentanoic acid, pentyl ester
21	12.585	116165	0.32	Phenol, 2,6-dimethoxy-
22	12.700	131419	0.37	Phenol, 2-methoxy-4-(2-propenyl)-, acetate
23	13.080	587770	1.64	1,2,3-Benzenetriol
24	13.427	483189	1.34	Quinoline, 8-hydrazino-
25	13.756	104619	0.29	1,4-Naphthalenedione
26	15.061	4711503	13.11	.beta.-D-Glucopyranose, 1,6-anhydro-
27	15.634	105350	0.29	Menadione
28	15.863	107096	0.30	2-Propanone, 1-(4-hydroxy-3-methoxypher
29	16.098	1323747	3.68	1,4-Naphthalenedione, 2-hydroxy-
30	19.028	211498	0.59	Benzeneacetic acid, 4-hydroxy-3-methoxy-,
31	19.188	419341	1.17	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyp
32	20.016	3780552	10.52	Ethanone, 1-(2,3,4-trihydroxyphenyl)-
33	20.357	169585	0.47	Naphtho[1,8-de]-1,3,2-dioxaborin, 2-ethyl-
34	20.411	526419	1.47	1,4-Eicosadiene
35	20.944	281280	0.78	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
36	21.466	369194	1.03	Hexadecanoic acid, methyl ester
37	21.860	1797492	5.00	l-(+)-Ascorbic acid 2,6-dihexadecanoate
38	21.999	215388	0.60	Naphtho[1,2-b]furan-4,5-dione, 2-methyl-
39	22.441	138106	0.38	2-Acetylamino-3-amino-1,4-naphthoquinor
40	23.297	175418	0.49	9,12-Octadecadienoic acid (Z,Z)-, methyl e
41	23.370	395649	1.10	11,14,17-Eicosatrienoic acid, methyl ester
42	23.478	242193	0.67	Phytol
43	23.541	196332	0.55	2,3-Dihydro-5-hydroxy-4-methyl-2-oxonap
44	23.674	479238	1.33	9,12-Octadecadienoic acid (Z,Z)-
45	23.749	1601306	4.46	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
46	23.951	297207	0.83	1(3H)-Isobenzofuranone, 5-hydroxy-3-[(4-h
47	24.346	59648	0.17	4,7-Dihydroxy-1,10-phenanthroline
48	24.825	781112	2.17	Benzyl .beta.-d-glucoside
49	28.280	224169	0.62	10,11-Dihydro-10-hydroxy-2,3-dimethoxyd
50	28.457	190446	0.53	Butyl 9,12,15-octadecatrienoate
51	29.361	8382236	23.33	.psi.,.psi.-Carotene, 7,7',8,8',11,11',12,12',1
		35929258	100.00	

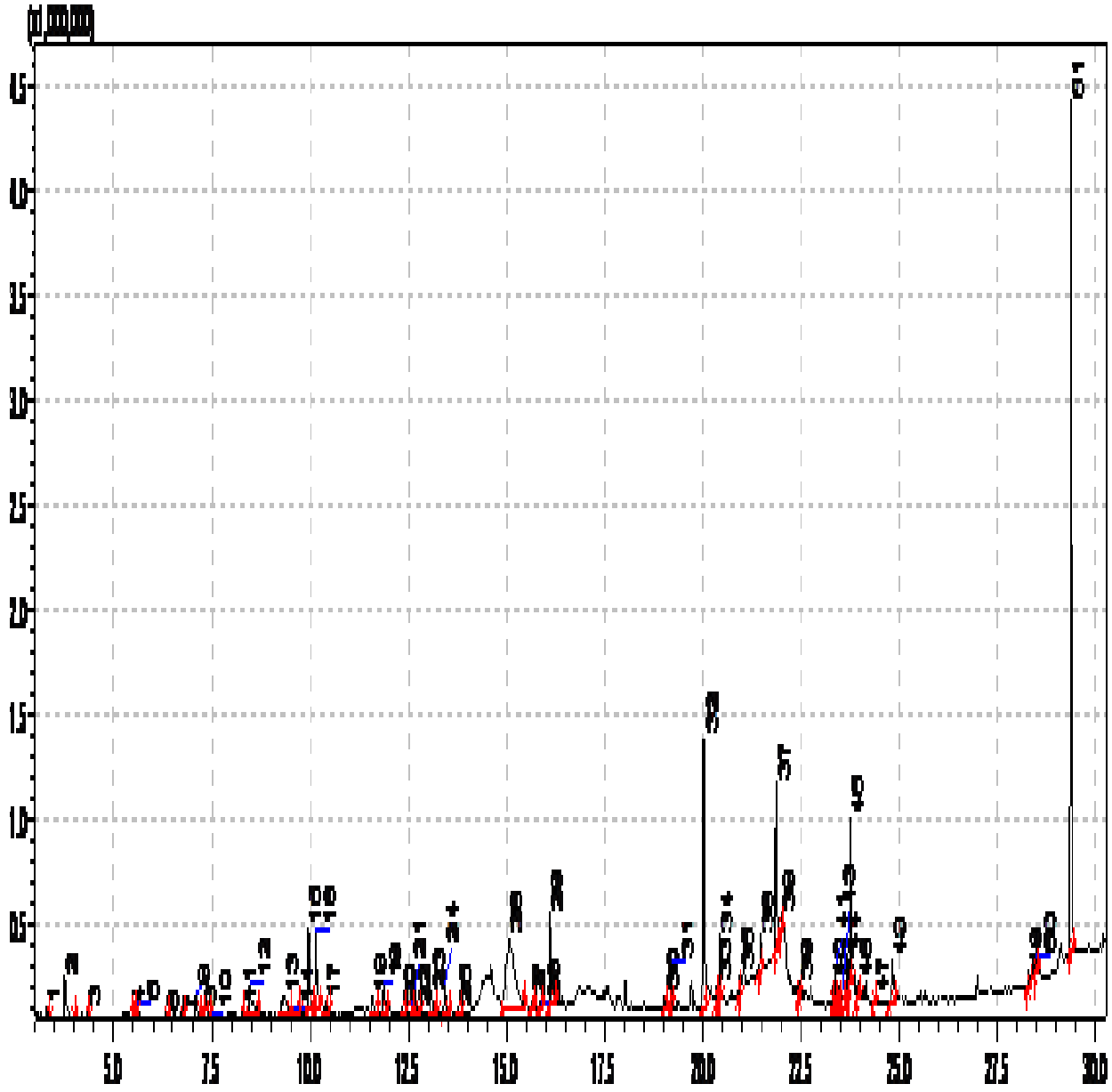

Fig 8: **GC-MS Chromatogram of methanol extract of *Lawsonia inermis* leaves clearly showed 51 peaks**

Table 7: **Active antibacterial compounds in methanol extract of henna .**

NO	Active ingredient compounds	%
1	psi.,.psi-caroten,7,7,8,8,11,11,12,12,1	23,33
2	Ethanone, 1-(2, 3, 4-trihydroxyphenyl)-	10.52
3	l-(+)-Ascorbic acid 2, 6-dihexadecanoate	5.00
4	9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)-	4.46
5	1, 4-Naphthalenedione, 2-hydroxy-	3.68
6	Benzofuran, 2,3 -dihydro-	3.10
7	5-Hydroxymethylfurfural	3.4
8	Neopentyl glycol	2.50
9	Benzyl, beta.-d.glucoside	2.17
10	Quinoline, 8-hydrazino-	1.34
11	9, 12-Octadecadienoic acid (Z, Z)-	1.33
12	Hexadecanoic acid, methyl ester	1.03
13	1(3H)-Isobenzofuranone,5-hydroxy-3-[(4-hydroxyphenyl) methylene]-	0.83
14	3, 7, 11, 1 5-Tetramethyl-2-hexadecen-1 -01	0.78
15	PhytoI	0.67
16	Naphtho[1 ,2-b]furan-4,5-dione, 2-methyl-	0.60
17	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	0.49
18	3-Acetoxy-3-hydroxypropionic acid, methyl ester	0.43
19	2-Hydroxy-gamsna-butyrolactone	0.40
20	2,5-Piperazinedione	0.39
21	2-Acetylamino-3-amino-1, 4-naphthoquinone	0.38
22	Phenol, 2-rnethoxy-4-(2-propenyl)-, acetate	0.37
23	6-Oxa-bicyclo [3. 1 .0] hexan-3-one	0.32
24	Phenol, 2, 6-dimethoxy	0.32
25	1, 4-Naphthalenedione	0.29
26	Ethanamine, N-ethyl-N-nitroso-	0.27
27	Pentanoic acid, pentyl ester	0.24
28	Mequinol	0.24
29	Catechol	0.19
30	4,7-Dihydroxy-1,10-phenanthroline	0.17

DISCUSSION

The present study demonstrated the *in vitro* antibacterial activity of *Lawsonia inermis* methanol and water extracts against *S. aureus*, *P. aeruginosa* and *E. coli* isolates from patient with recurrent urinary tract infections also against standards *S. aureus* ATCC29213, *E. coli* ATCC25922 and *P. aeruginosa* ATCC2785.

Among this study recurrent urinary tract infection was more in females (78%) than male (22%) and the most frequently isolated bacteria was *E. coli* 16 (50%) followed by *S. aureus* (12.5) and *P. aeruginosa* (9.4%) of the total growth, this study is in agreement with study of Irving [15] who reported that urinary tract infection was more in females and most infections caused by *Escherichia coli*, also agreed with the study of Kebira [16] in Kenya.

Escherichia coli showed high rate of resistance to antibiotic used in this study (75% MDR), this in agreement with study of WHO (2015) [17] reported that resistance to urinary antibiotic most common by *Escherichia coli*, also in agreement with Niranjan [18] who reported that 76.51% of *Escherichia coli* isolated from urinary tract infection patients were multi drug resistance (MDR).

The antibacterial of *Lawsonia inermis* leaves extracts has been evaluated *in vitro* against isolates and standards. Study revealed that methanol extract of henna performance inhibition of bacterial growth, the maximum inhibition zone in high concentration was observed against *S. aureus* ATCC29213 (26.5±O.7mm) followed by *S. aureus* (23±1.4mm), *P. aeruginosa* ATCC27853 (22.5±1.4mm), *E. coli* ATCC25922 (21±1.4mm), *P. aeruginosa* (18.8± 1.6 mm), and *E. coli* (16.4± 0.9mm) respectively, with MIC (6.3, 12.5, 6.3, 12.5, 12.5 and 12.5) mg/ml respectively this in agreement with the study of Arun [19] which found that methanol extract have shown maximum activity against *S. aureus*, *P. aeruginosa* and *E. coli* (zone of inhibition 21- 24). Also methanol extract was highly active against MRSA (inhibition zone 18.3 ± 1.1 mm and MIC 12.5mg/ml) this in agreement with Jain [20], Ali [21] and Gull [22], who reported that methanol extract of *L. inermis* leaves highly effective than water extract. Variation in MIC and zone of inhibition may be due to the variation in method of antibacterial activity or the nature and combination of phytochemical compounds present in extract due to environment or type of soil.

Water extract was effective against *S. aureus*, *S. aureus* ATCC29213 *P. aeruginosa* and *P. aeruginosa* ATCC27853 with inhibition zone (15 ± 1.4, 112±104, 16.1 ± 1 and 15.5±0.7) mm and MIC (25, 25, 12.5and 12.5) mg/ml respectively and had no effect against *E. coli*, *E. coli* MDR and *E. coli* ATCC25922 this result is in agreement with Kannahi [23] and Hussein [24] who determine water extract was not effective against *E. coli*, this may be due to the difference of solvent properties. However, it was disagreed with Saadabi [25] in Sudan who reported the water extract most effective one followed by methanol extract was used agar disc diffusion method. Ababutain [26] in Sudia Arabia were found that the aqueous extract had the best inhibitory zone on 8 out of 9 tested bacteria include *S. aureus*, *P. aeruginosa* and *E. coli*. The variation in the results of previous and present study may be due to the variation in the method of antibacterial activity of henna, extraction method and the difference of environment and soil.

Moreover our result showed that the *E. coli* multi drug resistance was susceptible to methanol extract with inhibition zone 14.4 ± 1.1mm and MIC 25mg/ml. Antibacterial activity may be due to numerous free hydroxyls that have the capability combine with the carbohydrates and proteins in the bacterial cell wall and get attached to enzyme site rendering them in active.

The alcoholic extract showed the lowest MIC compared to water extracts and this may be due to the large quantity of active substances that were precipitated in methanol more than water during the extraction process. Furthermore phytochemical compounds of *Lawsonia inermis* (Sudanese henna) methanol leaves extract was determined by gas chromatography showed 51compounds (Table 6). Some of these (30 compounds) (Table 7) were detected in form of groups by other methods including tannic acid, naphthaquinone, flavonoid, mucilage, glycoside, protein, carbohydrate, tannins, quinones, fatty acid and phenol compounds [27], [28], [21], [25].

More research work is required to validate these results and to determine the role of the other remaining compounds using advanced techniques.

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