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Sudan University of Science and Technology

College of Graduate Studies

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

نشاط مستخلص أوراق الحناء كمضاد بكتيري على المكورات العنقودية الذهبية، الاشكريشيا القولونية والزائفة الزنجارية المعزولة من عدوى المسالك البولية المتكررة

A thesis submitted in partial fulfillment for requirment MSc in Medical Laboratory Science (Microbiology)

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الآيـة

قال تعالى:

(وَهُوَ الَّذِيَ أنزَلَ مِنَ السَّمَاء مَاء فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُّخْرِجُ مِنْهُ حَبًّا مُّتَرَاكِبًا وَمِنَ النَّخْلِ مِن طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِّنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَابِهٍ انظُرُواْ إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي ذَلِكُمْ لَآيَاتٍ لِّقَوْمٍ يُؤْمِنُونَ)

صدق الله العظيم

سورة الأنعام الأيه 99

Dedication

To the soul of my lovely sister,

To my parents,

To my brothers,

To my sisters,

To my family

And

To my best friends

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Firstly thanks to ALMIGHTY **ALLAH** for giving me patience and strength to complete this work.

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Abstract

This was a descriptive and cross sectional study conducted during the period from May to August 2015 to determine the antibacterial activity of *Lawsonia inermis* leaves extract against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* among recurrent urinary tract infection patients in Omdurman Military Hospital. A total of 100 urine samples were collected from patient with recurrent urinary tract infection. These specimens were inoculated onto Cystine Lactose Electrolyte Deficiency (CLED) media and incubated aerobically at 37˚C for 24 hours. The isolates were then identified using conventional method. 32(32%) out of 100 investigated samples showed bacterial growth. Eight of the patients were males and 24 were females with age rang between 35-70 years (mean = 50.41±10.324). 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 antibiotic susceptibility testing was performed using standard disk diffusion method. The results showed that all *S. aureus* isolates were resistant to penicillin (100%). 2(50%) out of 4 positive *S. aureus* were susceptible to oxacillin while 2(50%) were oxacillin resistance. *S. aureus* ATCC29213 was also susceptible to oxacillin. *E. coli* susceptibility results revealed 12(75%) *E. coli* showed high resistance to Naldixic Acid (88%) followed by Ceftriaxone (81%), Ciprofloxacin (75%) and Gentamicin (69%). The reminder 4(25%) and *E. coli* ATCC25922 were susceptible.

P. aeruginosa susceptibility results showed high resistance to Naldixic Acid (100%) followed by Gentamicin (67%), Ceftriaxone (33%) and was susceptible to Ciprofloxacin.

The antibacterial activity of *Lawsonia inermis* water and methanol leaves extract against *Staphylococcus aureus, MRSA, S. aureus* ATCC29213, *Escherichia coli*, *E. coli* ATCC25922, *Pseudomonas aeruginosa* and *P. aeruginosa* ATCC27853 was performed at different concentrations using the agar dilution method. Methanol extract of *Lawsonia inermis* showed antibacterial activity against *Staphylococcus aureus, S. aureus* ATCC29213, *Escherichia coli*, *E. coli* ATCC25922, *P. aeruginosa* and *P. aeruginosa* ATCC27853 also water extract showed antibacterial activity against all strains except *Escherichia coli* and *E. coli* ATCC25922. The MIC of henna methanol and water extracts obtained by agar diffusion method for *S. aureus* isolates was 12.5mg/ml / 25mg/ml, *P. aeruginosa* isolates was 6.25mg/ml and 12.5mg/ml respectively. Also *E. coli* isolates was 25mg/ml in methanol and resistance to water extract.

Gas chromatography analysis revealed that 51 chemical compound of *L. inermis* (Henna) which identified qualitatively by retention time and quantitatively by the area under curve. 30 active antibacterial compounds were recorded.

مستخلص الاطروحة

أجريت هذه الدراسة الوصفية المسحية فى الفترة من مايو حتى اغسطس 2015 فى ولاية الخرطوم لدراسة نشاط **مستخلص الحناء** على **المكورات العنقودية الذهيبية، الاشكريشيا القولونية والزائفة الزنجارية المعزولة من عدوى المسالك البولية المتكررة**. تم جمع مئة عينة بول من مستشفى السلاح الطبى امدرمان.

تم تزريع هذه العينات فى وسط الستستين الشحنات ناقصة اللاكتوز والتحضين الهوائي فى درجة حراره 37 درجة مئوية لمدة 24 ساعة ثم أجريت اختبارات التعريف للبكتريات.

أظهرت النتيجة ان(32%) من 100 عينة تم عزلها من الجنسين(8 زكور و 24 إناث) تتراوح اعمارهم بين35-70 سنه (متوسط 50,41 ± 10,324). تم قياس الحساسية للبكتريات المعزوله لبعض المضادات البكتيريا بواسطة طريقة الانتشار الطبقى القياسي. كل المكورات العنقودية الذهيبية المعزوله مقاومه للبنسلين،2 (50%) لم تقاوم الاوكساسلين منها 2 (50%) مقاومه للاوكساسلين.12(75%) من الاشكريشيا القولونية عالية المقاومة للمضادات الحيوية للنالدكسيك اسيد بنسبة (88%) يليه سفترياكسون بنسبة (81%) ، سيبروفلوكساسين بنسبة (75%) وللجنتمايسن بنسبة (69%)، و4(25%) لم تقاوم. نسبة مقاومة الزائفة الزنجارية المعزولة للمضادات الحيوية اقله للنالدكسيك اسيد بنسبة (100%) يليه جنتمايسن بنسبة (67%)، سفترياكسون بنسبة (33%) ولم تقاوم السيبروفلوكساسين.

ايضا تضمنت هذه الدراسة نشاط المضاد للبكتيريا لتراكيز مختلفه للمستخلصات الماء والميثانول لنبات الحناء باستخدام طريقة اختبار الانتشار الطبقى للاجار. أظهرت الدراسة ان مستخلصات الحناء ميثانول له فعالية ضد كل المكورات العنقودية الذهيبية، كل الاشكريشيا القولونية و الزائفة الزنجارية المعزولة ايضا مستخلص ماء الحناء ماعدا مع كل الاشكريشيا القولونية لم يظهر فعالية.

أظهرت الدراسة ايضا التركيز المسبط الادنى لمستخلصات الحناء للمكورات العنقودية الذهيبية(ماء 25 ملغ/مل وميثانول12.5 ملغ/مل)، الاشكريشيا القولونية (ميثانول25ملغ/مل) والزائفة الزنجارية (ماء12.5ملغ/مل والميثانول 6.25 ملغ/مل).

أظهر التحليل اللونى للغاز 51 مركبا" للحناء وحددت هذه المركبات نوعيا" بواسطة الوقت المحتفظ وكميا" بواسطة المنطقة تحت المنحنى وجدت منهم ثلاثون مركبا" لهم نشاط مضاد للبكتيريا.

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CHAPTER ONE
1. INTRODUCTION

1.1. 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 (Retnam and Britto, 2007). 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 (Nerino *et al*., 2013).

Urinary tract infection causing bacteria become more resistant to available antibiotics, the need to explore new strategies for managing UTIs is clear (Foxman, 2003).

The development of resistance in microorganisms to antibiotics and emergence of new infectious diseases create urgent need to discover novel, safe and effective antimicrobial compounds (Rojas *et al*., 2003). In modern pharmaceutical industries, natural sources and semi synthetic derivatives of natural products play a key role for the production of novel drugs (Sudisha *et al*., 2009).

Plants derived compounds are likely to provide a valuable source of new antimicrobial agents. Several plants have ability to treat the multiple drug resistance strains (Carvalho and Ferreira, 2001).

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 (Bonjar, 2004). The leaves of *L. inermis* are non toxic and are used to cure boils, burns, bruises and other skin infection (Rout *et al*., 2001).

Henna contains lawsone dye this molecule has an affinity for bonding with protein, and thus has been used to dye skin, hair, fingernails, leather, silk and wool. The dye molecule, lawsone, is primarily concentrated in the leaves. Products sold as "black henna" or "neutral henna" is not made from henna, but may be derived from indigo (in the plant *Indigofera tinctoria*) or *Cassia obovata*, and may contain unlisted dyes and chemicals (Singh *et al*., 2005). Henna has been used cosmetically and medicinally for over 9000 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 (Chaudhary *et al*., 2010). In addition henna is used as anti-cancer and antioxidant properties (Kamal and Jawaid, 2010).

1.2. Rationale

Henna is a perennial plant that has the ability to grow in various environments in Sudan. Its leaves are collected, dried and ground to produce fine powder which is mainly used as cosmetics for Sudanese females as a part of ancient traditions. Lately an antimicrobial activity has been noticed in henna extracts, and researches have been conducted to assess the effectiveness of henna as an alternative natural herbal antimicrobial to chemical antibiotics. As a raised percentage of resistance and multi drug resistance to regular antibiotics, herbal compounds such as henna could be the solution.

Despite the existence of potent antimicrobial agents, resistant or multi-resistant strains are continuously emerging, imposing the need for a continuous search and development of new drugs (Barbour *et al*., 2004).

1.3. Objectives

1.3.1. General objective

To study antibacterial activities of *Lawsonia inermis* (henna) extract against some clinical bacterial isolates.

1.3.2. Specific objectives

1- To isolate and identify the bacteria from patients with recurrent urinary tract infection.

2- To determine the frequencies of resistance bacteria and evaluated *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeuroginosa*.

3- To evaluate the antibacterial activity of methanolic and aqueous extract of *Lawsonia inermis* against some bacteria isolated in patients with recurrent urinary tract infection.

4- To determine the minimum inhibitory concentration (MIC) of methanolic and aqueous extract of *Lawsonia inermis* against some bacteria isolated from patients with recurrent urinary tract infection.

5- To identify the major chemical compounds of *Lawsonia inermis* as analyzed by gas chromatography.

CHAPTER TWO
2. LITERATURE REVIEW

2.1. Plant extracts as antimicrobial agent

Plant extracts from more than 157 plant families have been described which have potential antimicrobial properties (Narayan *et al*., 2010). In United States of America (USA) about $1/4^{th}$ to $1/2^{th}$ of the pharmaceutical have their origin of higher plants (Cowan, 1999).

The Sudan Atlas of medicinal plants record the scientific name of more than 2000 medicinal herbs collected from different parts of the country. All of these herbs are in current use in traditional medicine (WHO, 2001).

Aqueous extract of *Acacia nilotica* fruit collected from central Sudan showed activity against *C.albicans*, both Gram positive and Gram negative bacteria (Abd El-nabi *et al*., 1992).

Several studies have been done in Sudan for different plant extracts to render the importance of medicinal plants such as Kheir *et al* (2014) on *Moringa oleifera* and Abd alfatah *et al* (2013) on four plants species in west of Sudan.

The most important bioactive compounds of plant are alkaloids, tannins and phenolic compounds (Edeoga *et al*., 2005

2.2. Nature and pharmacological properties of *Lawsonia inermis*

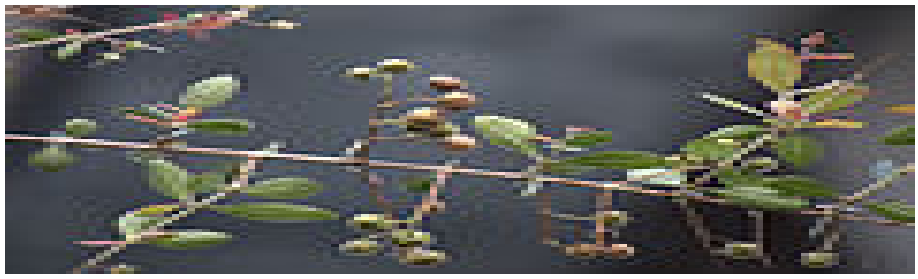

Figure 1: Henna leaves

2.2.1. Origins and nature of henna

Henna is a flowering plant, having a height of 5 meters, natal to subtropical and tropical regions of world including South Asia, Africa, oases of Sahara Dessert and even in northern regions of Australia. Leaves of henna plant are entire, opposite, sub-sessile, oval-shaped and smooth (Ashnagar and Shiri 2011). Henna flowers have four sepals and a 2 mm calyx tube, with 3 mm spread lobes. Its petals are obvate, with white or red stamens found in pairs on the rim of the calyx tube. The ovary is four-celled, 5 mm long, and erect. Henna fruits are small, brownish capsules, 4–8 mm in diameter, with 32–49 seeds per fruit, and open irregularly into four splits (Kumar *et al*., 2005).

2.2.2. Classification

Kingdom: Plantae

Division: Angiospermae

Class : Dicotyledoneae

Order : Myrtales

Family : Lythraceae

Genus : Lawsonia

Species : inermis (Singh and Singh, 2001).

2.2.3. Phytochemical constituent of *Lawsonia inermis*

The leaves of *Lawsonia inermis* contain 2-Hydroxy-1, 4-naptho-quinone, 1,2 -dihydroxy-glucoyloxynaphthalene,2hydroxy1,4diglucosyloxy naphthalene, Flavonoids (luteolins, apigenin, and their glycosides). Coumarins (esculetin, fraxetin, scopletin) and Steroids (β-sitosterol), also reported to contain soluble matter tannin, gallic acid, glucose, mannitol, fat, resin and mucilage. Bark contains naptho-quinone, isoplumbagin, triterpenoids-Hennadiol, aliphatics (3-methyl-nonacosan-1-ol). Flowers on steam distillation gave an essential oil (0.02 %) rich in ionones (90 %) in which β-ionones predominated (Amit *et al*., 2011).

2.2.4. Previous studies of in vitro antimicrobial activity of *L. inermis* extracts

Henna has awid 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 **(**Babu and Subhasree, 2009).

2.2.4.1. Antibacterial Activity

Ethanol extracts of 20 plants species used by Yemeni traditional healers to treat infectious diseases were screened for their antibacterial activity against both gram positive and gram negative bacteria. The ethyl acetate extract of *L. inermis* was found to be the most active against all the bacteria in the test system (Abulyazid *et al*., 2010, Nadjib *et al*., 2013).

In Sudan study done by Saadabi (2007) showed effects of water , methanol and chloroform extracts of *L. inermis* using disc diffusion method against *Staphylococcus aureus , Escherichia coli , Bacillus subtilis* , *P. aeuroginosa* and some pathogenic fungi isolated from different sources. *L. inermis*

showed antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeuroginosa* with inhibition zone (16, 19, 16 and 18 respectively) using water extract, 14, 16, 17 and 16 respectively using methanol extract and 14, 13, 14 and 15 respectively using chloroform.

Phytochemical screened of leaves showed the presence of tannic acid, naphaquinone, crysophanic acid, anthraquinone and mucilage in high level.

Also study conducted by (Kannhi and Vinotha , 2013) in India. Henna leaves were collected and selected for antimicrobial activity against some human pathogens isolated from soil such as *S. aureus*, *Streptococcus mutans, P. aeruginosa, Aspergills niger, Aspergills flaves* and *Fusarium,* henna leaves were extracted with methanol, ethanol and aqueous. The maximum activity was showed in methanol extraction against all isolated human pathogens, then ethanol extraction. There was no activity in aqueous extract.

In study investigated Phytochemical, toxicological and antimicrobial evaluation of *lawsonia inermis*, methanol, chloroform, acetone and water leaves extracts against *E. coli, Salmonella typhi, Klebsiella spp, Shigella sonnei, Bacillus subtilis, S. aureus* and *Staphylococcus epidermidis*, using disc diffusion method. The results revealed that all extracts exhibited antimicrobial activity against all bacterial strains. The minimum value of MIC for different bacterial strains ranged from 2.31 mg/ml to 9.27 mg/ml.

No sign of toxidrome were observed during *in vivo* toxicity evaluation in mice at 300 mg/kg concentration (Gull *et al*., 2013).

In Iraq, (Ali *et al*., 2013) were used aqueous, ethanolic and methanolic leaves extracts of *L. inermis* against *S. aureus,* MRSA isolated from milk

and standerd bacteria (*E. coli* ATCC25922 and *P. aeruginosa* ATCC 27853 using disk diffusion method. They showed that the antimicrobial activity of methanolic extract high potency with inhibition zone (14.3± 1.8) follwed by ethanol (12.95± 2.0) then aqueous extracts (11.63± 2.24). The MIC of *L. inermis* aqueous, ethanolic and methanolic leaves extracts against *S. aureus* (3, 3 and 1.5) mg/disc, *P. aeruginosa* were (3, 3, 1.5) mg/disc and *E. coli* were (12, 6 and 3) mg/disc respectively. Qualitative phytochemical analysis of *L. inermis* extracts reveal that the presence of tannins, flavonoids, phenolic compound and glycoside.

The antibacterial activity of the water chloroform and methanol extracts of *Lawsonia inermis* against *Escherichia coli, Proteus* sp and *Pseudomonos* sp in Egypt was investigated by agar well diffusion method, four different concentrations were prepared 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml. The chloroform extract most effective one followed by methanol extract while water extract had just little effect against *Proteus* sp and no effect against *Escherichia coli* and *Pseudomonos* sp (Hussein, 2010).

Also in Nigeria demonstrated the aqueous leaves extract (cold or hot) when oxidized with potassium permanganate can be substitute to the usual counter stains used in Gram staining reactions (Hafiz *et al*., 2012).

2.2.4.2. Antifungal studies of henna

In Sudan Suleiman and Mohamed (2014) investigate antifungal activity of *Lowsonia inermis* ethanol and petroleum leaves extract against tested fungi. MIC of 5, 7.5 and 10mg/ml was found to inhibit the growth of tested dermatophytes.

2.2.4.3. Antiviral studies of henna

Henna definitely has anti-viral effect that became clear by it is action on warts, whitlow and herpes simplex, it dried the vesicles at the site early, prevent ulceration and crust formation. This antiviral effect of henna should be explored further; it could be used as treatment of AIDS. It looks to have no side effect even when taken by oral route (Hussain, 2010).

2.3. Urinary tract infection (UTI)

A urinary tract infection is an infection in the urinary tract caused by microbes including bacteria, fungi and viruses. Bacteria are the most common causes of UTIs.

The urinary tract includes two kidneys, two ureters, a bladder and a urethra.

Bacteriuria is the multiplication of bacteria in urine with in renal tract a concentration of greater than 10^5 organism/ ml.

Urinary tract infections are common infections, increase with age and are more common in females. The most infections caused by *Escherichia coli* and a minority caused by *Klebsiella* species, *Proteus* species, *Enterococcus faecalis* and *Staphylococcus saprophyticus* and *Staphylococcus aureus*.

Risk factors include structural abnormalities of the urinary tract, urinary catheter, urological surgery, diabetes and immunosuppresion (Irving *et al*., 2006).

2.3.1. Recurrent urinary tract infection

Recurrent UTI occur due to bacterial reinfection or bacterial persistence. Persistence involves the same bacteria not being eradicated in the urine 2 weeks after sensitivity-adjusted treatment. A reinfection is a recurrence with

a different organism, the same organism in more than 2 weeks, or a sterile intervening culture (Shawn *et al*., 2011).

2.3.1.1. Incidence

- Women have a lifetime risk of UTI of 1 in 3, and men 1 in 20.
- It accounts for 5% of women each year presenting with frequency and dysuria.
- Up to 20% of non-pregnant women with cystitis will have a recurrence and most are due to re-infection.
- UTI is rare in men aged 20-50 years and uncommon in young boys and elderly men **(**National Institute for Health and Care, 2007).

2.3.1.2. Risk factors

There is evidence to suggest that deregulation of candidate genes in humans may predispose patients to recurrent UTI, diabetes is also a predisposing factor (Gorter *et al*., 2010).

In women

Atrophic urethritis and vaginitis (postmenopausal), abnormalities of urinary tracts (indwelling catheter, neuropathic bladder, vesico-ureteric reflux (VUR), outflow obstruction, anatomical anomalies), incomplete bladder emptying (dysfunctional urination), contraception- diaphragm, spermicide-coated condoms, history of urinary tract surgery and Immune compromise (Schols *et al*., 2005).

In men

Abnormalities of urinary tract function, incomplete bladder emptying (prostatic enlargement, chronic indwelling catheter), previous urinary tract surgery, immunocompromised state and anal intercourse (European Association of Urology 2013).

WHO has reported Resistance to one of the most widely used antibacterial drugs for the oral treatment of urinary tract infections caused by *E.coli* – fluoroquinolones – is very widespread (WHO, 2015).

2.4. Most common bacteria that cause recurrent urinary tract infections

2.4.1. *Escherichia coli*

Escherichia coli are a Gram negative usually motile rod, minorities of strains are capsulate, aerobic and facultative an aerobic, optimum temperature for growth is 36-37°C. It's naturally found in the intestinal tract, soil and water. *E. coli* is the commonest pathogen isolated from patients with cystitis (Cheesbrough, 2006).

Recurrent infections are common in women, infections of wounds, peritonitis, sepsis and endotoxin induced shock. *E. coli* capsular type K1 is associated with neonatal meningitis, infantile gastroenteritis, traveler's diarrhea, dysentery and hemorrhagic diarrhea which my progress to hemolytic uremic syndrome (Cheesbrough, 2006).

2.4.2. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram negative rod, obligate aerobe, non - sporing and motile, some strains are capsulate. It is usually recognized by the pigments produces including pyocyanin a blue – green pigment and pyoverdin a yellow – green fluorescent pigment. *P. aeruginosa* can be found in the intestinal tract, water, soil and sewage. It frequently found in moist enviroments in hospitals and able to grow in some eye drops, saline and aqueous solution. Many infections with *P. aeruginosa* are opportunistic hospital – acquired and often difficult to eradicate due to *P. aeruginosa* being resistant to many antimicrobials. Infections caused by *P. aeruginosa* include: Skine infections, Septicaemia, Urinary tract infections, Respiratory

tract infections, External ear infection and eye infections (Cheesbrough, 2006).

2.4.3. *Staphylococcus aureus*

Staphylococcus aureus one species of *Staphylococci* are Gram positive cocci arranged in irregular grape like clusters non motile, non–spore forming and catalase positive. *Staphylococcus aureus* found as normal flora on human skin and mucosal surfaces can survive on dry surfaces, MRSA now the most common cause of community-acquired skin and soft tissue infections Species characterized by the presence of coagulase, protein A and species-specific ribitol teichoic acid with N-acetylglucosamine residues ("polysaccharideA"). Virulence factors include structural components that facilitate adherence to host tissues and avoid phagocytosis and a variety of toxins and hydrolytic enzymes. Diseases include: toxin-mediated diseases (food poisoning and toxic shock scalded skin syndrome), pyogenic diseases (impetigo, folliculitis, furuncles, carbuncles, and wound infections), urinary tract infection and other systemic diseases. Hospital- and community-acquired infections with Methacillin Resistant *S.aureus* are a significant worldwide problem (Parrick *et al*., 2009).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study design

Descriptive, cross sectional and hospital based study.

3.2. Study area

Omdurman Military Hospital.

3.3. Study population and duration

Patients with recurrent urinary tract infection.

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

3.3.1. Inclusion criteria

Patients with recurrent urinary tract infection were included.

3.3.2. Exclusion criteria

Community members (free of recurrent urinary tract infection).

3.4. Sampling

Non - probability sampling.

3.4.1. Sample size

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

3.5. Study variables

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

3.6. Data collection

The data were collected from records of hospitals.

3.7. 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.

3.8. 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.

3.9. Culture

The specimens were inoculated under aseptic conditions on Cystine lysine electrolye 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.

3.10. Identifications technique

3.10.1. Colonial Morphology

The cultures morphologically examined for size, color, fermentation of lactose on cystein lactose electrolyte deficient agar.

3.10.2. Gram's Stain

From cultured growth pure single colony was selected to prepare smear on slide using sterile loop, air dried and fixed by flame, the smear was covered with crystal violet for 30-60 seconds then washed by clean tape water and covered with Lugol's iodin for 30-60 seconds, washed by tape water and decolorized by alcohol for 20-30 seconds, finally the smear was covered by saffranine for 2minutes, then washed by clean tape water, dried by blotting on a filter paper and examined by using oil immersion lens (Cheesbrough, 2006).

3.10.3. Biochemical test

3.10.3.1. Kligler Iron Agar (KIA)

By using of sterile straight wire the KIA media (HiMedia laboratories Pvt, Ltd, India) was inoculated with organism under test. First the butt was stabbed, then the slope was streaked and the incubation was done at 37^{o}C overnight. A yellow butt (acid production) and red-pink slope indicates the fermentation of glucose and lactose. Blackening along the stab line or throughout the media indicates hydrogen sulphide (H_2S) (Cheesbrough, 2006).

3.10.3.2. Indole Test

Under aseptic condition the tested organisms were inoculated in the test tube containing 3ml of sterile tryptone water (Hi-Media laboratories Pvt, Ltd, India) then incubated aerobically at 37˚C for 18-24 hours. Indole production was detected by Kovac's or Ehrlich's reagent which contains 4(p)-dimethylaminobenzaldehyde, this reacts with the indole to produce a red colored compound; indol test positive, no change in color; negative (Cheesbrough, 2006).

3.10.3.3. Citrate Utilization Test

The test was done by inoculating organism on Simmon's citrate agar (Hi-Media laboratories Pvt, Ltd, India) under aseptic condition then incubated aerobically at 37˚C for 18-24 hours. Bright blue (in the presence Bromothymole blue) indicate ability of organism to utilize sodium citrate to obtain carbon for energy; citrat test positive, no change in color; negative (Cheesbrough, 2006).

3.10.3.4. Urease test

The test were done by inoculating the urea agar (Hi-Media laboratories Pvt, Ltd, India) with tested organism and incubated aerobically at 37˚C for 18-24 hours. Color changed to pink indicte organism produce urease enzyme; positive, no change color; negative (Cheesbrough, 2006).

3.10.3.5. Oxidase test

Oxidase disk were placed inside the Petri dish, small inoculums were taken by using wooden stick and smeared on the disk. Blue purple color; oxidase test positive, no change color; negative (Cheesbrough, 2006).

3.10.3.6. Catalase Test

Organisms were tested for catalase production by brining it into contact with hydrogen peroxide use wooden stick. Active air bubbles of oxygen are relased indicate; positive catalase test, no air bubbles; negative (Cheesbrough, 2006).

3.10.3.7. Coagulase Test

On clean slide a drop of distilled water was placed and emulsified a colony of tested organism, then a loopful of plasma was added to the suspension and mixed gently for 10 seconds. Clumbing with in 10 seconds indicate; positive, no clumbing in more than 10 seconds; negative coagulase test (Cheesbrough, 2006).

3.10.3.8. Deoxyribonuclease test (DNase test)

The tested organisms were cultured on media which contains DNA (Hi-Media laboratories Pvt, Ltd, India). After overnight incubation at 37˚C, the colonies were tested for DNase production by flooding the plate with a weak hydrochloric acid solution to precipitate the unhydrolyzed DNA in the media

and waited for minutes until clear zone appear around colonies which considered as; positive result, no clear zone; negative (Cheesbrough, 2006).

3.10.3.9. Mannitol fermentation test

The tested organisms were streaking on MSA media (Hi-Media laboratories Pvt, Ltd, India) after overnight incubation at 37˚C observed the change of the color to yellow (mannitol fermenter colonies); MSA test positive, red colonies (non manitol fermenter colonies); MSA test negative (Cheesbrough, 2006).

3.11. Storage

Isolated organisms were kept in nutrient agar slope at 4˚C for subsequent susceptibility tests. Nutrient glycerol broth used for long stage preservation of isolates at -20˚C refrigerator.

3.12. 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).

3.12.1. 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 (Cheesbrough, 2006).

3.13. 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.

3.13.1. Henna leaves extraction

Extraction was carried out according to method descried by (Sukhdev *et al.*, 2008).

3.13.2. 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.

3.13.3. 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.

3.13.4. 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 methanolic 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 (Aneja and Joshi, 2009).

3.13.5. 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 (NCCLS, 2000) and (Al Waili & Sloom, 1999).

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.

3.13.6. 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 (Mukhtar and Ghori, 2012).

3.14. Quality control procedure

3.14.1. 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.

3.14.3. Control susceptibility testing method

3.14.4. Reference strain quality control

The quality control *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC25922 were brough from National Public Health Laoratories, 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.

3.14.4. Batch quality control

Each batch of susceptibility test was tested with the reference strain to determine if zone diameter obtained with in the expected rang or not.

Also uninoculated agar plate was incubated over night to ensure the medium was sterail.

3.15. 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 Hellium. The stationary phase was a microscopic layer of liquid or polymer on an insert solid support inside apiece of glass or metal tubing called a column (a homage to the fractionating column used in distillation).

3.16. Data analysis

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

CHAPTER FOUR

4- RESULTS

Among of the 100 urine specimens 41(41%) were males and 59(59%) were females (fig2).

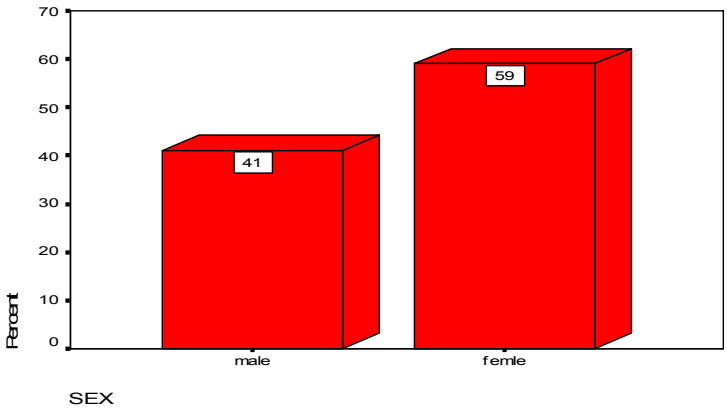

Fig2: Distribution of samples according to gender

Out of 100 investigated samples 32 showed bacterial growth while 68 showed no bacterial growth (Figure 3).

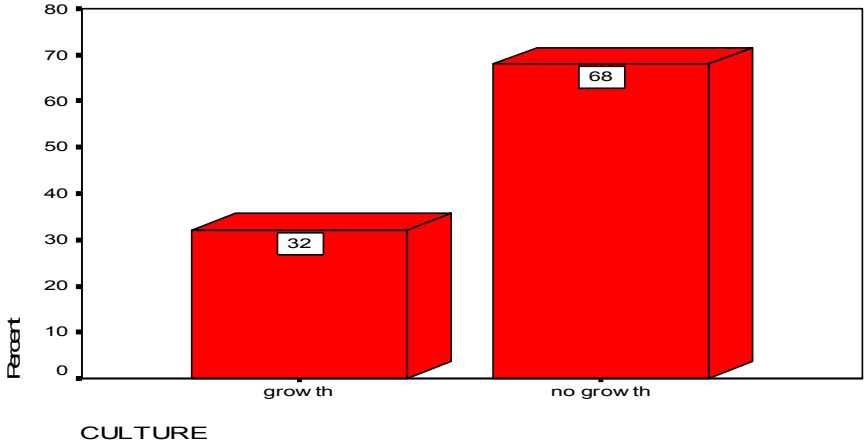

Fig3: **The percentage of bacterial growth on CLED**

In this study percentage of recurrent urinary tract infections more in females than males (Fig4)

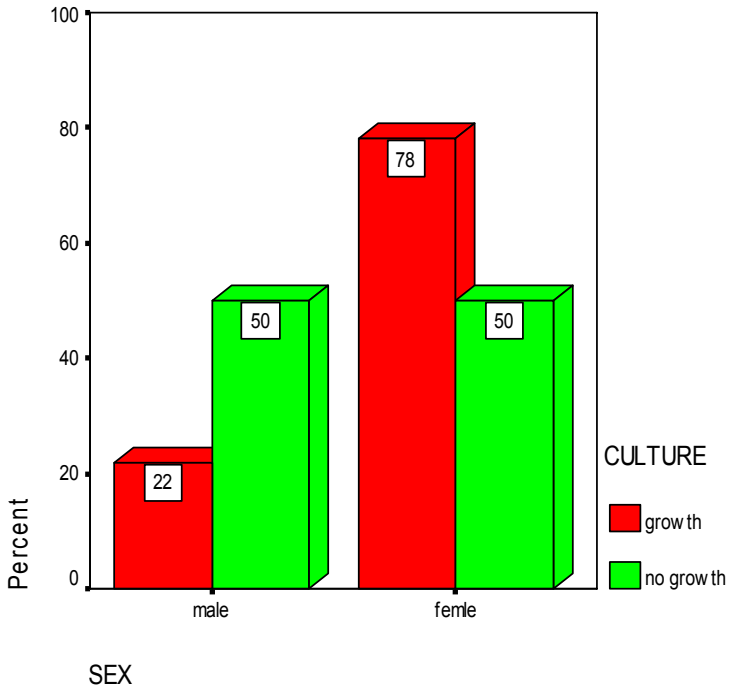

Fig4: Percentage of growth in two genders

4.1. Culture

On CLED some isolated showed yellow fermented colonies while others none fermented organisms due to the presence of bromothimol blue indicator (Appendix 2).

4.2. Gram Stain

Showed Gram positive cocci in clusters (violate color) and Gram negative rod (red color).

4.3. Biochemical tests of different bacterial isolates

Table -1 summarizes the biochemical properties of various Gram negative bacteria, while table -2 show biochemical properties of Gram positive bacteria isolated from patients with recurrent urinary tract infection.

Table1: Biochemical characteristics of isolated Gram negative bacteria from recurrent urinary tract infection patients.

Isolated bacteria	Biochemical tests							No. of Isolation	%
	Indole	Urease	Citrate	KIA					
				Slope	Butt	Gas	H2S		
P. areuginosa	- ve	- ve	+ve	R	R	- ve	- ve	3	9.4
K. pneumoniae	- ve	+ve	+ve	Y	Y	- ve	- ve	4	12.5
P. vulgaris	+ve	+ve	+ve	R	Y	+ve	+ve	2	6.25
E. coli	+ve	- ve	- ve	Y	Y	+ve	- ve	16	50

Key:

R: red

Y: yellow

+ve: positive

-ve: negative.

Table2: Biochemical characteristics of isolated Gram positive bacteria from recurrent urinary tract infection patients.

Isolated bacteria	Catalase	Bile esculin hydrolysis	Manitol fermentation	Dnase	No. Of Isolation	%
S. aureus	+ve	- ve	+ve	+ve	4	12.5
E. faecalis	- ve	+ve	- ve	- ve	2	6.25
S. epidermidis	+ve	- ve	- ve	- ve	1	3.1

4.4. Antibacterial Susceptibility Test

The antibacterial susceptibility test of isolates and standerd 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 standerd *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%) Naldixic Acid termed to be *E. coli* multiple drug resistant (MDR). The reminder 4(25%) and *E. coli* ATCC25922 were susceptible (tables 3/4/5and 6) and (fig 5, Appindix2).

Table3: Antibacterial susceptibility test of control strains and isolates of Gram negative bacteria against the corresponding standard antibiotics.

Bacterial species	Ciprofloxacin	Gentamicin	Ceftriaxone	Naldixic Acid
P. areuginosa **ATCC**27853	Sensitive 33 mm	Sensitive 20 mm	Sensitive 25 mm	Resistant 3mm
P. areuginosa	Sensitive 30-32 mm	Sensitive 16-19 mm	Resistant 0-17 mm	Resistant 0-10
E. coli **ATCC**25922	Sensitive 33 mm	Sensitive 21mm	Sensitive 29 mm	Sensitive 22 mm
E. coli	Sensitive 21-30mm	Sensitive 16-22mm	Intermediate 19-21mm	Resistant 0-7mm

***Key: zone of inhibition in millimeters**

Ciprofloxacin: Resistant < or =15mm sensitive > 21mm

Gentamicin: Resistant < or = 6mm sensitive > 10mm

Ceftriaxone: Resistant < or = 19 mm sensitive > 23mm

Nalidixic Acid: Resistant < or = 13mm sensitive > 19 mm

Table4: 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

%: Percentag

Table5: Antibacterial Susceptibility Test of control strain and isolates of *Staphylococcus aureus* against the corresponding standard antibiotics.

Bacteria species	Penicillin	Oxacillin	Ciprofloxacin	Vancomycin
S. aureus **ATCC**29213	Resistant (14mm)	Sensitive (32mm)	Sensitive (36mm)	Sensitive (19mm)
S. aureus	Resistant (0-2mm)	Sensitive (18-19mm)	Sensitive (25-29mm)	Sensitive (18-22mm)

***Key:**

Zone of inhibition in millimeters

Penicillin: Resistant < or = 28 mm sensitive > 29 mm

Oxacillin: Resistant or = 10 mm sensitive > 21 mm

Ciprofloxacin: Resistant or= 15mm sensitive > 21mm

Vancomycin: Resistant or= 15mm sensitive > 19 mm

Table6: **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%

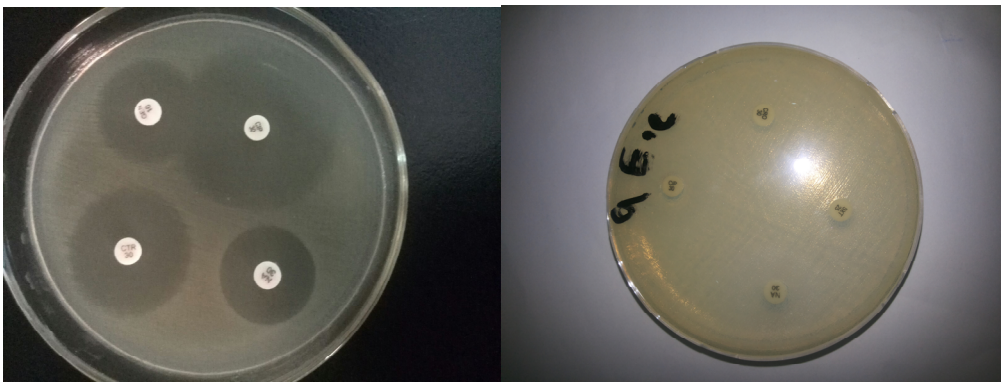

Fig5: **Antimicrobial susceptibility testing of *E. coli* ATCC25922 and *E. coli* (MDR) to ciprofloxacin, Gentamicin, Ceftriaxone and Nalidixic Acid**

4.5. Antibacterial activity of Henna

Table7: **Weight and yield percentage of extracts by methanol and water**

Weight of Sample	Methanol		Aqueous	
	Weight of extract	Yield %	Weight of extract	Yield %
50 g	12.04 g	24.08 %	7.41 g	14.82 %

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 $P < 0.05$ (Table8 &9).

Table8: **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.

Table9: 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. areuginosa* 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**

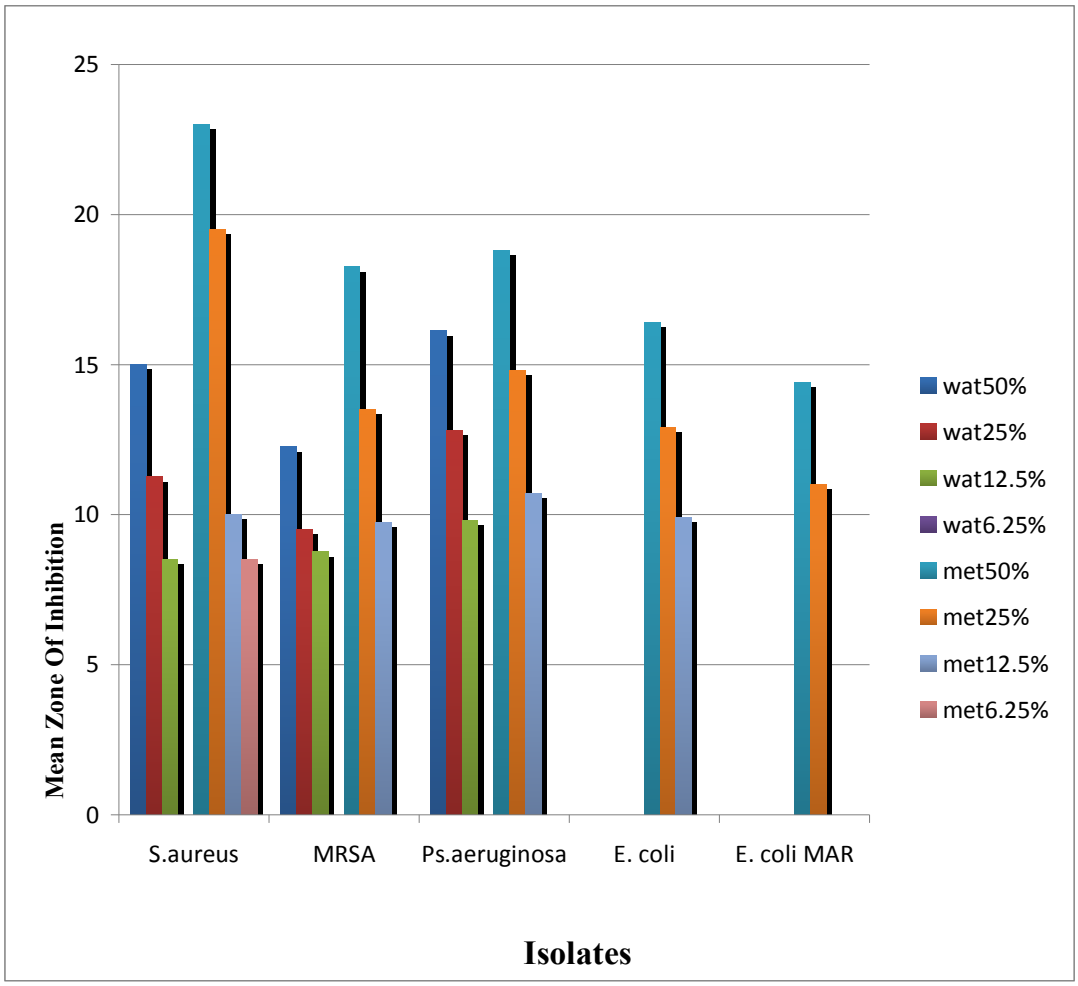

Fig 6: Mean of inhibition zones after in vitro exposure of isolates to henna methanol (Meth) and water (Wat) extracts in different concentrations (Diameter in millimeter).

4.6. Minimum inhibitory concentration (MIC) of *Lawsonia inermis* obtained by agar diffusion method

The Minimum inhibitory concentration of each bacteria against the different extracts obtained by agar diffusion method are shown in (table10) and (figures from 6 /7and appendix 2).

Table10: 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

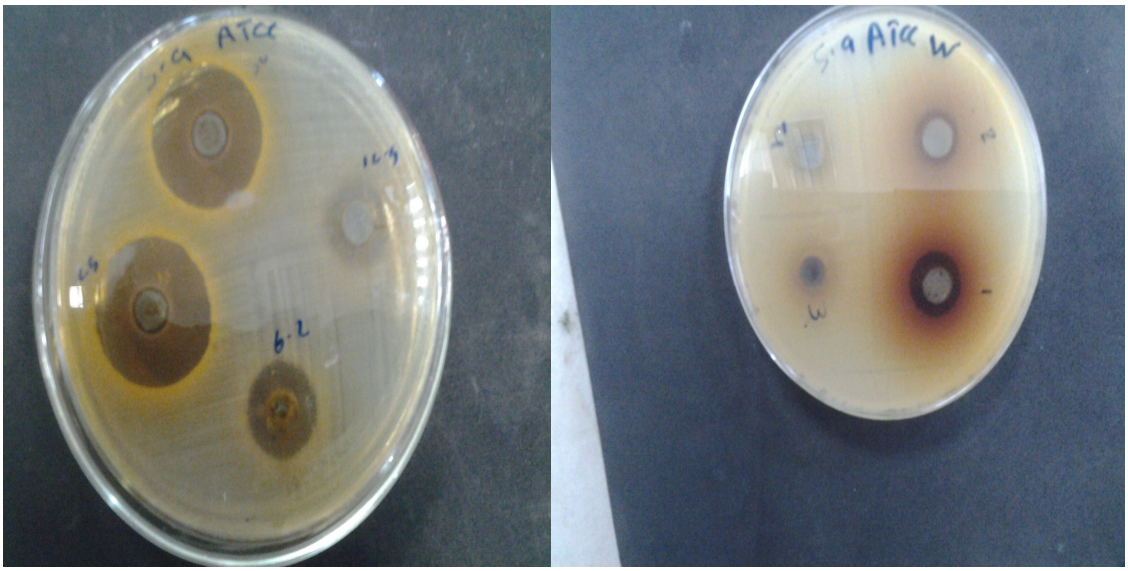

Fig 7: Activity of *Lawsoni inermis* methanol and water extracts on *S.aureus* ATCC29213 with concentration 5omg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml.

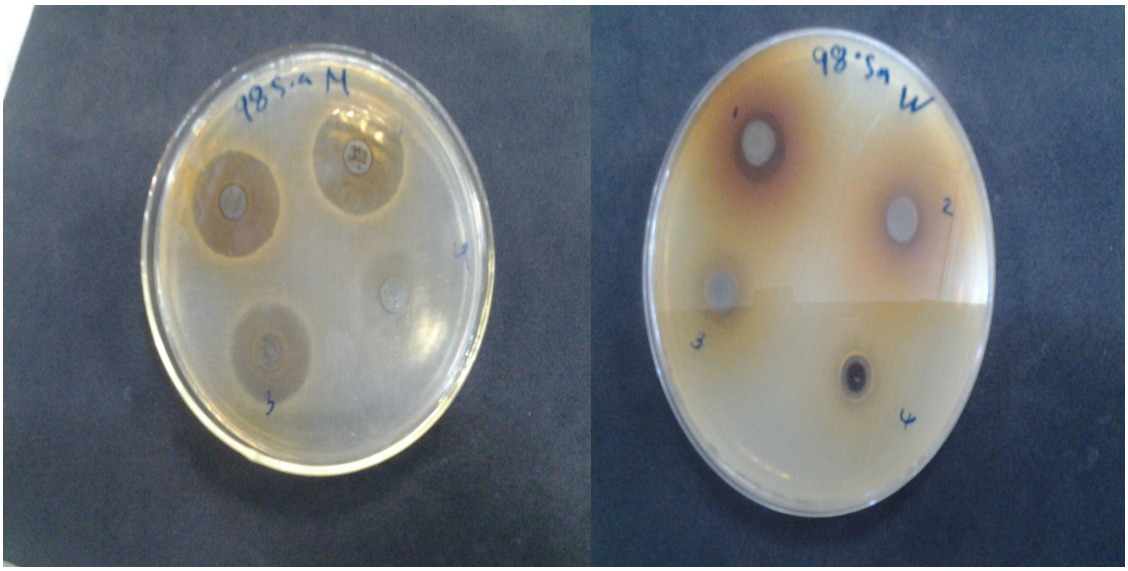

Fig 8: Activity of *L. inermis* methanol and water extracts on *S.aureus* isolate with concentration 5omg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml.

4.7. Gas chromatography results

GC-MS Chromatogram of methanol leaves extract of *Lawsonia inermis* (figure9) clearly showed 51 peaks indicating the presence of 51 phytochemical compounds (Table11) and (information about 51 compounds mention in Appendix1).

Table11: 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-methoxyphen
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	

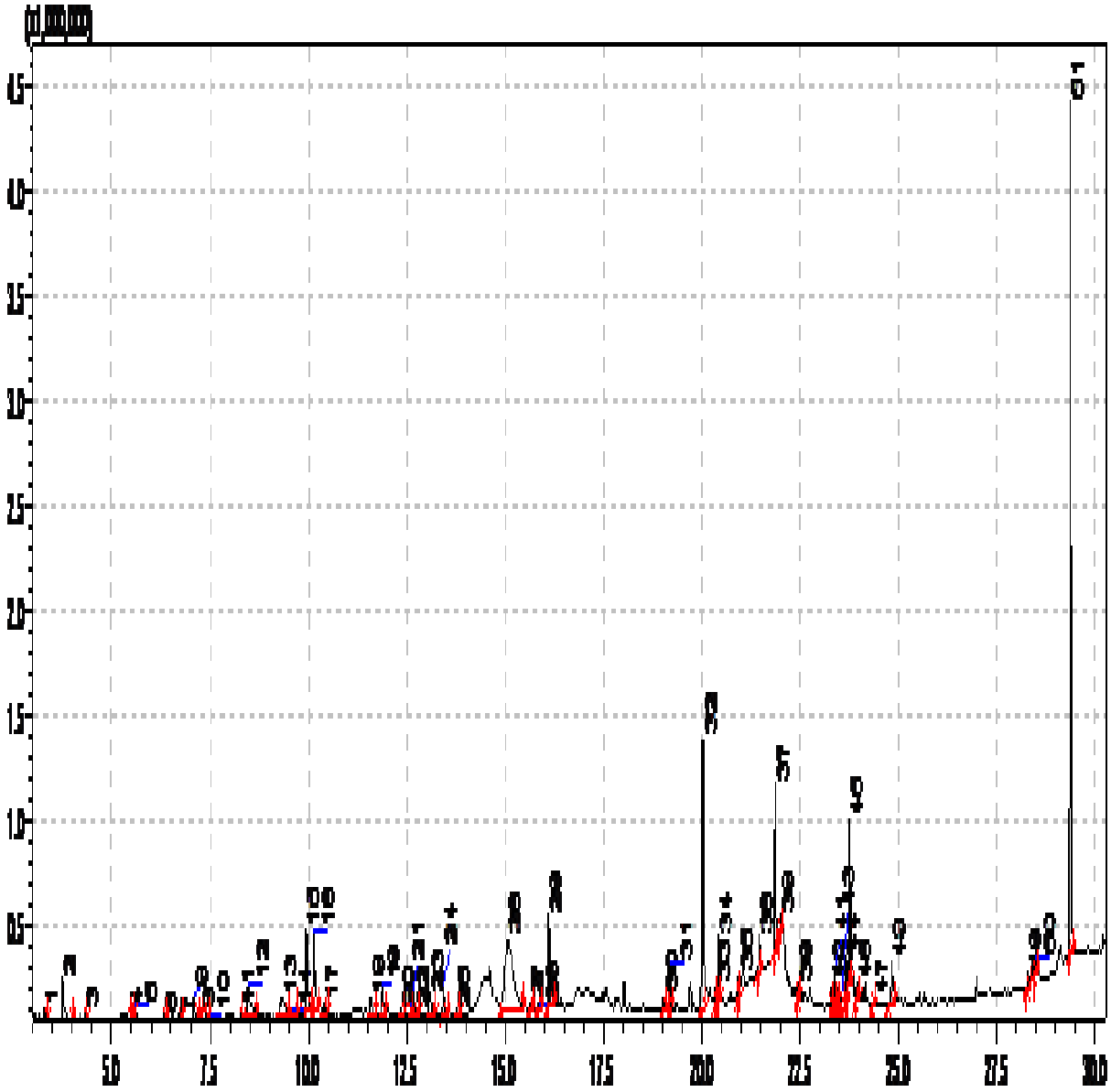

Fig 9: **GC-MS Chromatogram of methanol extract of *Lawsonia inermis* leaves clearly showed 51 peaks**

CHAPTER FIVE
5. 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 standerds *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 *et al* (2006) who reported that urinary tract infection was more in females and most infections caused by *Escherichia coli*, also agreed with the study of Kebira *et al* (2009) 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) reported that resistance to urinary antibiotic most common by *Escherichia coli*, also in agreement with Niranjan and Malini (2014) 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 standerds. 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 *et al* (2010) which found that methanol extract have shown maximum activity against *S. aureus, P. aeruginosa* and *E. coli* (zone of inhibition 21- 24). Also methanolic extract was highly active against MRSA (inhibition zone 18.3 ± 1.1 mm and MIC 12.5mg/ml) this in agreement with Jain *et al* (2010), Ali *et al* (2013) and Iram *et al* (2013), who reported that methanol extract of *L. inermis* leaves highly effective than water extract. Variation in MIC and zone of inhibition my be due to the variation in method of antibacterial activity or the nature and combination of phytocompounds 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 inhibtion 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 and Vinotha (2013) and Hussein (2010) who determine water extract was not effective against *E. coli*, this my be due to the difference of solvent properties. However, it was disagreed with Saadabi (2007) in Sudan who reported the water extract most effective one followed by methanol extract was used agar disc diffusion method. Ababutain (2014) 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 neumerous 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.

Further more phytochemical compounds of *Lawsonia inermis* (Sudanese henna) methanol leaves extract was determined by gas chromatography showed 51compounds (Table11). Some of these (30 compounds) (Appendix3) were detected in form of groups by other methods including tannic acid, naphaquinone, flavonoid, mucilage, glycoside, protein, carbohydrate, tannins, quinones, fatty acid and phenol compounds (Singh *et al.,* 2014, Nasir *et al.,* 2014, Ali *et al*., 2013 and Saadabi, 2007).

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

5.2. Conclusion

Increase the prevalence of MDR *E. coli* among recurrent urinary tract Infection patients was found to be 75%. MRSA among recurrent urinary tract Infection patients was found to be 6.25%.

Sudanese Henna possesses high antibacterial activities against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* in RUTI patients and standered organisms. Methanol extract was highly potent than water extract.

The more effective concentration 50% methanol and 50% water, zone of inhibtion was increased with the increase of concentration of extracts ($P<0.05$).

Staphylococcus aureus, Pseudomonas aeruginosa isolates were more susceptible to Henna methanol and water extract compared to *Escherichia coli,* water extract gave no antibacterial activity against *Escherichia coli*, *E. coli* MDR and *E. coli* ATCC25922.

MIC methanol and water extracts of isolates range from (6.3% - 25%) and (12.5% - 25%).

Gas chromatography analysis of henna methanol leaves extract showed 51 compounds, 30 active antibacterial compounds were found.

5.3. Recommendation

1- Further work in Sudanese Henna from different locations and more studies to be done in active ingredients compounds responsible for the antibacterial activity.

2- Study of antibacterial activity and determine MIC of Henna against other organisms using different solvents.

3- Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

4- Henna dyes extracts can be used as stains for laboratories purposes.

5- Pharmacological, toxicological studies should be carried out to assess their safety, therapeutic efficiency and potential for commercial utilizations.

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APPENDICES

Appendix 1: Compound information of henaa extract analysis by Gas chromatography

1/ 2-Furanmethanol

Formula: C5F16O2 CAS: 98-00-0 MolWeight: 98 Retlndex: 885

Compound names: 2-Furanmethanol $$ Furfuryl alcohol $$ .alpha.-Furfuryalcohol alpha.-Furanmethanol $$ Furfuralcarbolsol $$ Furfuryl alcohol $$ Furfuryl alcarbinol $$

2/ (S)-(+)-2-Arnino-3-methyl-1-butanol

Formula: C5H1 3NO CAS: 2026-48-4 MolWeight: 103 Retlndex: 876

Compound names: (S)-(+)-2-Arnino-3-methyl-1-butanol $$ L-Valinol $$ (S)-2-Amino-3-methylbutanol $$ 1-Butanol, 2-amino-3-methyl-, (S) - $$ 2-Amino-3-meth3

3/ 6-Oxa-bicyclo [3. 1 .0] hexan-3-one

Formula: C5H6O2 CAS: 7401 7-10-0 MolWeight: 98 Retlndex: 782

Compound name: 6-Oxa-bicyclo [3. 1 .0] hexan-3-one

4/ 2-Hydroxy-gamsna-butyrolactone

Formula: C4H6O3 CAS: 19444-84-9 MolWeight: 102 Retlndex: 1013

Compound names: 2-Hydroxy-gamsna-butyrolactone $$ 3-Hydroxydihydro-2(3H)-furanone # $$.

5/ 7-Oxabicyelo [4. 1 .O] heplan-2-one

Formula: C6H8O2 CAS: 6705-49-3 MolWeight: 112 Retlndex: 902

Compound names: 7-Oxabicyelo [4. 1 .O] heplan-2-one $$ Cyclohexanone, 2,3- epoxy- $$ 2,3-Epoxycyclohexanone $$

6/ 2,5-Piperazinedione

Formula: C4H6N2O2 CAS: 106-57-0 MolWeight: 114 Retlndex: 1046

Compound names: 2,5 -Piperazinedione $$ Cylo (glycylglycyl) $$ Cyclic(glycylglycyl) $$ .alpha.,.gamma.-Diacipiperazine $$ Cycdliglycine $$ Cycloglycyiglycin.

7/ I, 3, 2-Dioxaborolan-4-one, 2-ethyl

Formula: C4H7BO3 CAS: 74646-l 2-i MoiWeight:1 14 RetIndex:0

Compound names: I, 3, 2-Dioxaborolan-4-one, 2-ethyl- $$

2-Ethyl-i,3,2-dioxaboroian-4-one # $$

8/ Thymine

Formula: C5H6N2O2 CAS: 65-71 -4 MolWeight: 126 Retlndex: 1118

Compound names: Thymine $$ 2,4 (1H,3H)-Pyrimidinedione, 5-methyl- $$ Thymin $$ 24-Dihydroxy-5-methylpyriniidine $S 5-Methyluracil $$

5-Methyl-2,4- diox.

9/ 1 -Butene, 4-iodo-

Formula: C4H71 CAS: 7766-5 1-0 MolWeight: 182 Retlndex: 823

Compound names: 1 -Butene, 4-iodo- $$ 4-Iodo-1-butene # $$

10/ Mequinol

Fornsula: C71-1802 CAS: 150-76-5 MolWeightl24 Retlndex: 1090

Compound names: Mequinol $$ Phenol, 4-methoxy- $$ Phenol, p-methoxy- $$ p-Guaiacol $$ p-Hydroxyanisole $$ p-Methoxyphenol $$ Hydroquinone methyl eth

11/ Ethanamine, N-ethyl-N-nitroso-

Formula: C4HION2O CAS: 55-18-5 MolWeight: 102 Retlndex: 877

Compound names: Ethanamine, N-ethyl-N-nitroso- $S Diethylamine, N-nitroso- $$ Diethylnitrosamine $$ DENA $$ N-Nitroso-N,N-Diethylamine $S

N- Nitrosodie.

12/ 4H-Pyran-4-one,2,3 -dihydro-3,5-dihydroxy

Formula: C61-1804 CAS: 28564-83-2 MolWeight: 144 Retlndex: 1269

Compound names: 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-rnethyl- $$ 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one $$ 2,3-dihydro-3,5-dihydroxy-.

13/ Neopentyl glycol

Formula: C5H12O2 CAS: 126-30-7 MoiWeight: 104 Retlndex: 919

Compound names: Neopentyl glycol $$ 1,3-Propanediol, 2,2-dimethyl- $$ Dimethyloipropane $$ Neopentanediol $$ Neopentylene glycol $$ 2,2-Dimethyl-1,3-pro.

14/ Catechol

Formula: C6H6O2 CAS: 120-80-9 MolWeiglst: 1 10 Retlndex:1 122

Compound Names: Catechol $ 1,2-Benzenediol $$ Pyrocatechol $$ o-Benzenediol $$ o-Dihydroybenzene $$ o-Dioxybenzene $$ o-Hydrmcyphenol $$ o-Phenyh.

15/ Benzofuran, 2,3 -dihydro-

Formula: C8H8O CAS: 496-1 6-2 MolWeight: 120 Retlndex: 1036

CompName: Benzofuran, 2,3- dihydro- $$ Coumaran $$ Dihydrobenzofuran $8 Dihydrocoumarone $$ Kumaran $$ 2,3-Dihydrobenzofuran $$ 2,3-Dihydro-.

16/ 5-Hydroxymethylfurfural

Formula: C6H6O3 CAS: 67-47-0 MolWeight: 126 Retlndex: 1163

Compound names: 5-Hydroxymethylfurfural $$ 2-Furancarboxaldehyde, 5-(hydroxymethyl) - $$ 2-Furaldehyde, 5-(hydroxymethyl) - $$ HMF $$ 5-(Hyddroxymeth).

17/ 3-**Acetoxy-3-hydroxypropionic acid, methyl ester**

Formula: C6H1005 CAS: 0-00-0 MolWeight: 162 Retlndex: 1115

Compound names: 3-Acetoxy-3-hydroxypropionic acid, methyl ester $S Methyl 3-(acetyloxy)-3-hydroxypropanoate 8 $$.

18/ 3-cis-Methoxy-5-cis-methyl-1R-cyclohexra

Formula: C8H1602 CAS: 5901 3-92-2 MolWeight: 144 Retlndex: 1106

Compound names: 3-cis-Methoxy-5-cis-methyl-1R-cyclohexra $$ 3-cis-Methoxy-5-cis-methyl-1(R)-cyclohexanol $$ 3(Z)-Methoxy-5(Z)-methylcyclohexanol $8.

19/ 2-Methoxy-4-vinylphenol

Formula: C9H10O2 MolWeight: 150 Retlndex: 1293

Compound names: 2-Methoxy-4-vinylphenol $$ phenol, 4-ethenyl-2-methoxy-$$ phenol, 2-methoxy-4-vinyl-$$ 4-Hydroxy-3-methoxystyrene $$ p-Vinylguaiacol.

20/ Pentanoic acid, pentyl ester

Formula: C10H20O2 CAS: 2173-56-0 MolWeight: 172 Retlndex: 1183

Compound names: Pentanoic acid, pentyl ester $$ Valeric acid, pentyl ester $$ Amyl valerate $$ Amyl valerianate $$ Pentyl pentanoate $$ Pentyl valerate $$ 1-Pen.

21/ Phenol, 2, 6-dimethoxy

Formula: C8H10O3 CAS: 91-1 0-1 MolWeight: 154 Retlndex: 1279

Compound names: Phenol, 2, 6-dimethoxy- $$ Pyrogallol 1, 3-dimethyl ether $$ Syringol $$ 1, 3-Dimethoxy-2-hydroxybenzene $$ 2-Hydroxy-1, 3-dimethoxybenzer.

22/ Phenol, 2-rnethoxy-4-(2-propenyl)-, acetate

Formula: C12H14O3 CAS: 93-28-7 MolWeight: 206 Retlndex: 1552

Compound names: Phenol, 2-rnethoxy-4-(2-propenyl)-, acetate $$ Phenol, 4-aIlyl-2-methoxy-, acetate $$ Aceteugenol $$ Acetyleugenol $$ Eugenol acetate $$ Eug.

23/ 1, 2, 3-Benzenetriol

Formula: C6H6O3 CAS: 87-66-I MolWeight: 126 Retlndex: 1342

Compound names: 1,2,3-Benzenetriol $$ Pyrogallol $$ C.I. Oxidation Base 32 $S C.I. 76515 $$ Fouramine Brown AP $$ Fourrine PG $$ Fourrine 85 $$ Pyrogalli.

24/ Quinoline

Formula: C9H9N3 CAS: 14148-42-6 MolWeight: 159 Retlndex: 1681

Compound names: Quinoline, 8-hydrazino- $$ 8-Hydrazinoquinoline # $$

25/ 1, 4-Naphthalenedione

Formula: C10H6O2 CAS: 130-1 5-4 MolWeight: 158 Retlndex: 1491

Compound names: 1, 4-Naphthalenedione $$ 1, 4-Naphthoquinone $$ .alpha.-Naphthoquinone $$ p-Naphthoquinone $$ 1, 4-Dihydro-1, 4-diketonaphthalene $$ 1, 4-.

26/ beta.-D-Glucopyranose, 1, 6-anhydro-

Formula: C6H10O5 CAS: 498-07-7 MolWeight: 162 Retlndex: 1404

Compound names: beta.-D-Glucopyranose, 1, 6-anhydro- $$ Anhydro-d-mannosan $$ Levoglucosan $$ I, 6-Anhydro-.beta.-D-glucopyranose $$ 1, 6-Anhydro-beta.

27/ Menadione

Formula: C11H8O2 CAS: 58-27-5 MolWeight: 172 Retlndex: 1581

Compound names: Menadione $$ I, 4-Naphthalenedione, 2-methyl- $S 1 ,4-Naphthoquinone, 2-methyl- $$ Aquakay $$ Aquinone $$ Hemodal $$ K-Thrombyl $$ K.

28/ 2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-

Formula: C10H12O3 CAS: 2503-46-0 MoiWeight: 180 Retlndex: 1538

Compound names: 2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)- $$ Guaiacylacetone $$ Vanillyl methyl ketone $$ 4-Hydroxy-3-methoxyphenyl acetone $$ 2-Prc.

29/ 1, 4-Naphthalenedione, 2-hydroxy-

Formula: C10H6O3 CAS: 83-72-7 MolWeight: 174 Retlndex: 1621

Compound name: 1, 4-Naphthalenedione, 2-hydroxy- $$ Henna $S 1, 4-Naphthoquinone, 2-hydroxy- $$ C.I. Natural Orange 6 $$ C.I. 75480 $$ Flower of Paradise.

30/ Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester

Formula: C10H12O4 CAS: l5964-80-4 MolWeigbt 196 Retlndex: 1569

Compound names: Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester $$ Acetic acid, (4-hydroxy-3-methoxyphenyl)-, methyl ester $$ Homovanillic acid metl.

31/ 4-((I E)-3-Hydroxy-1-propenyl)-2-methoxyphenol

Formula: C10H12O3 CAS: 0-00-0 MolWeight: 180 Retlndex: 1653

Compound name: 4-((I E)-3-Hydroxy-1-propenyl)-2-methoxyphenol.

32/ Ethanone, 1-(2, 3, 4-trihydroxyphenyl)-

Formula: C8H8O4 CAS: 528-2 1-2 MoiWeight: 168 Retlndex: 1691

Compound names: Ethanone, 1-(2, 3, 4-trihydroxyphenyl)- $$ Gallacetophienone $$ Acetophenone, 2,3,4-trihydroxy- $$ Alizarin Yellow C $$ Alizarine Yellow C S.

33/ Naphtho[1,8-de]1 ,3,2-dioxaborin, 2-ethyl

Formnula: CI2H13BO2 CAS: 125452-19-9 MolWeight: 198 Retindex: 0

Compound narnes: Naphtho[1,8-de]1 ,3,2-dioxaborin, 2-ethyl SS 2-Ethytnaphtho[1 ,8-de][1,3,2]dioxaborinine # $$.

34/ 1, 4-Eicosadiene

FormuIa: C20H38 CAS: 0-00-0 MolWeight: 278 Retlndex: 2007

Compound names: 1, 4-Eicosadiene $$ (4E)-1, 4-Icosadiene # $$.

35/ 3, 7, 11, 1 5-Tetramethyl-2-hexadecen-1 -01

Formula: C20H40O CAS: 102608-53-7 MolWeight: 296 Retlndex: 2045

Compound name: 3, 7, 11, 1 5-Tetramethyl-2-hexadecen-1 -01 $$ 2-Hexadecen-1 -01, 3, 7, 11, 15-tetramethyl $$.

36/ Hexadecanoic acid, methyl ester

Formula: C17H34O2 CAS: 112-39-0 MolWeight: 270 Retlndex: 1878

Compound names: Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester $$ n-Hexadecanoic acid methyl ester $$ Metholene 2216 $$ Methyl hexadecano.

37/ l-(+)-Ascorbic acid 2, 6-dihexadecanoate

Formula: C38H68O8 CAS: 28474-90-0 MolWeight: 652 Retlndex: 4765

Cornpound name: l-(+)-Ascorbic acid 2, 6-dihexadecanoate.

38/ Naphtho[1 ,2-b]furan-4,5-dione, 2-methyl-

Formula: C13H8O3 CAS: 17112-93-5 MolWeight: 2 12 Retlndex: 1871

Compound name: Naphtho[1 ,2-b]furan-4,5-dione, 2-methyl- $$ 2-Methylnaphtho[1 ,2-b]furan-4,5-dione 6 $$.

39/ 2-Acetylamino-3-amino-1, 4-naphthoquinone

Formula: C12H10N2O3 CAS: 1 3755-96-9 MolWeight: 230 Retldex: 2313

Compound names: 2-Acetylamino-3-amino-1, 4-naphthoquinone $$ N-(3-Amino-1, 4-dioxo-1, 4-dihydro-2-naphthalenyl) acetamide # $$.

40/ 912-Octadecadienoic acid (Z, Z)-, methyl ester

Formsila: C19H34O2 CAS: 1 12-63-0 MolWeight.294 Retlndex: 2093

Compound names: 912-Octadecadienoic acid (Z,Z)-, methyl ester $$ Linoleic acid, methyl ester 8$ Methyl cis,cis-9,12-octadecadienoate $$ Methyl linoleate $$ M.

41/ 11, 1 4, 17-Eicosatrienoic acid, methyl ester

Formula: C21 H36O2 CAS: 55682-88-7 MolWeight: 320 Retlndex: 2300

Compound names: 11, 1 4, 17-Eicosatrienoic acid, methyl ester $$ Methyl 11,14,1 7-icosatrieoate $$ Methyl 11, 14, 17-eicosatrienoate $$.

42/ PhytoI

Formula: C20H40O CAS: 150-86-7 MolWeight: 296 Retlndex: 2045

Compound names: PhytoI $$ 2-Hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-, [R[R*,R*(E)JJ $$ trans-Phytol $$ 3,7,11,1S-Tetramethyl-2-hexadecen-1-ol-, (2E,7R,11F).

43/ 2, 3-Dihydro-5-hydroxy-4-methyl-2-oxonaphtho(1 ,2-b)furan

Formula: C13H10O3 CAS: 25932-78-9 MolWeight: 2 14 Retlndex: 2 157

Compound names: 2, 3-Dihydro-5-hydroxy-4-methyl-2-oxonaphtho(1 ,2-b)furan $$ 5-Hydroxy-4-methylnaphtho[1 ,2-b]furan-2(3H)-one #$$.

44/ 9, 12-Octadecadienoic acid (Z, Z)-

Formula: C18H32O2 CAS: 60-33-3 MolWeight: 280 Retlndex: 2183

Compound names: 9, 12-Octadecadienoic acid (Z, Z)- $$ cis-9,cis-12-Octadecsdienoic acid $$ cis,cis-Linoleic acid $$ Grape seed oil $$ Linoleic $$ Linoleic acid $

45/ 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)-

Formula: C18H30O2 CAS: 463-40-l MolWeight:278 Retlndex:2 191

Compound names: 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)- $$ Linolenic acid $$ .alpha.-Linolenic acid $$ All-cis-9,12,15-Octadecalrienoic acid $$ cis,cis,cis-9,12,].

46/1(3H)-Isobenzofuranone,5-hydroxy-3-[(4-hydroxyphenyl) methylene]-

Formula: C15H10O4 CAS: 56783-95-0 MolWeight:254 Retlndex:247 1

Compound names: 1(3H)-Isobenzofuranone, 5-hydroxy-3-[(4-hydroxyphenyl) methylene]- $$ (3Z)-5-Hydroxy-3-(4-hydroxybenzylidene)-2-benzofuran-1 (3H)-one.

47/ 4,7-Dihydroxy-1,10-phenanthroline

FormuIa: C12H8N2O2 CAS: 3922-40-5 MolWeight: 212 Retlndex:22 11

Coinpound names: 4,7-Dihydroxy-1,10-phenanthroline $$ 4,7-Dihydroy-1,l0-phenanthroIine hydrochloride $$ 1,10-Phenanthroline-4,7-diol $$.

48/ Benzyl, beta.-d.glucoside

Formula: C13H18O6 CAS: 0-00-0 MolWeiglst: 270 Retlndex:2461

Compound names: Benzyl, beta.-d.glucoside $$ 1 -Deoxy-1-phenylhept-2-ulopyranose # $$.

49/ 10, 11-Dihydro-10-hydroxy-2, 3dimethoxydibenz(b, f)oxepin

Formula: C16H16O4 CAS: 23396-52-3 MolWeight: 272 Retlndex: 2289

Compoundnames:10,11-Dihydro-10-hydroxy2,3dimethoxydibenz(b,f)oxepin $$ 2,3-Dimethoxy-10,11-dihydrodibenzo[b,f]oxepin-I0-oI $$.

50/ Butyl 9, 12, 15-octadecatrienoate

Formula: C22H38O2 CAS: 0-00-0 MolWeight: 334 RetIndex: 2399

Compound name: Butyl 9, 12, 15-octadecatrienoate

51/ psi.,.psi-caroten,7,7,8,8,11,11,12,12,1

Formula: C40H56

Compound names: psi.,.psi-caroten,7,7,8,8,11,11,12,12,1, Beta caroten, provitaminA, Beta carotene, cartenoids, dry alpha carotene.

Appindix2

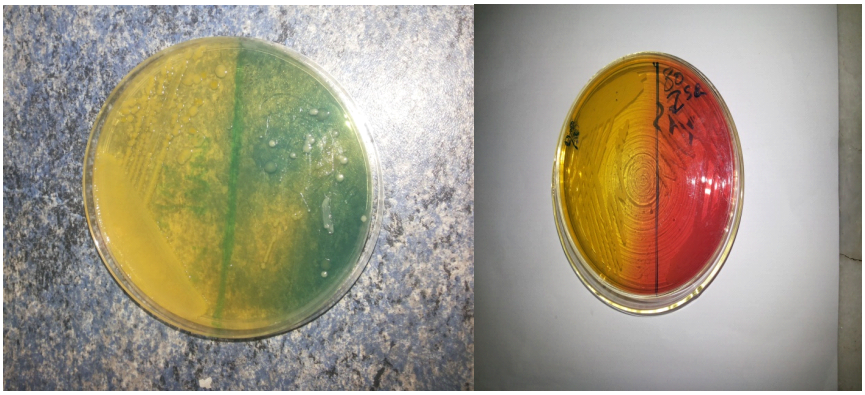

Figure10: Growth on CLED and MSA show yellow ferment colonies

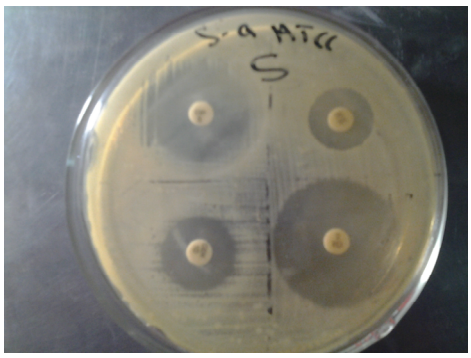

Figure11: Antimicrobial susceptibility testing of *S. aureus* ATCC29213 to penicillin, oxacillin, ciprofloxacin and vancomycin

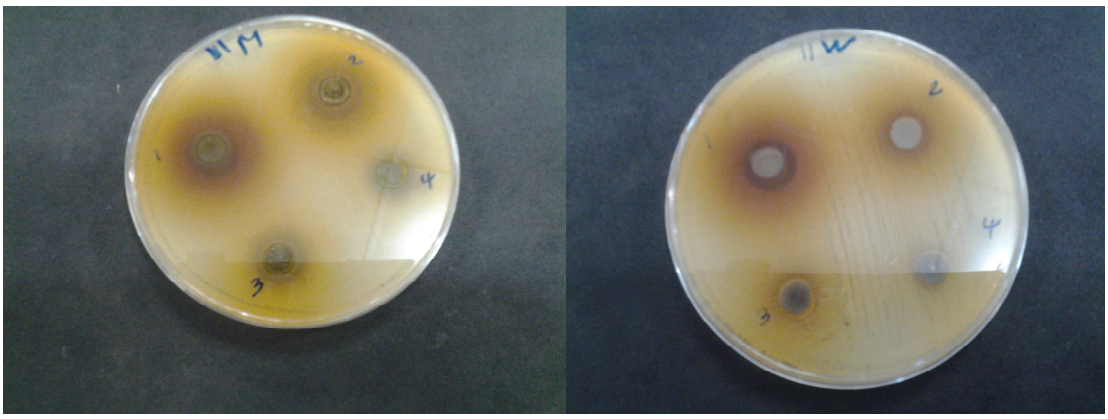

Figure12: Activity of *Lawsonia inermis* methanol and water extracts on MRSA isolate with concentration 5omg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml.

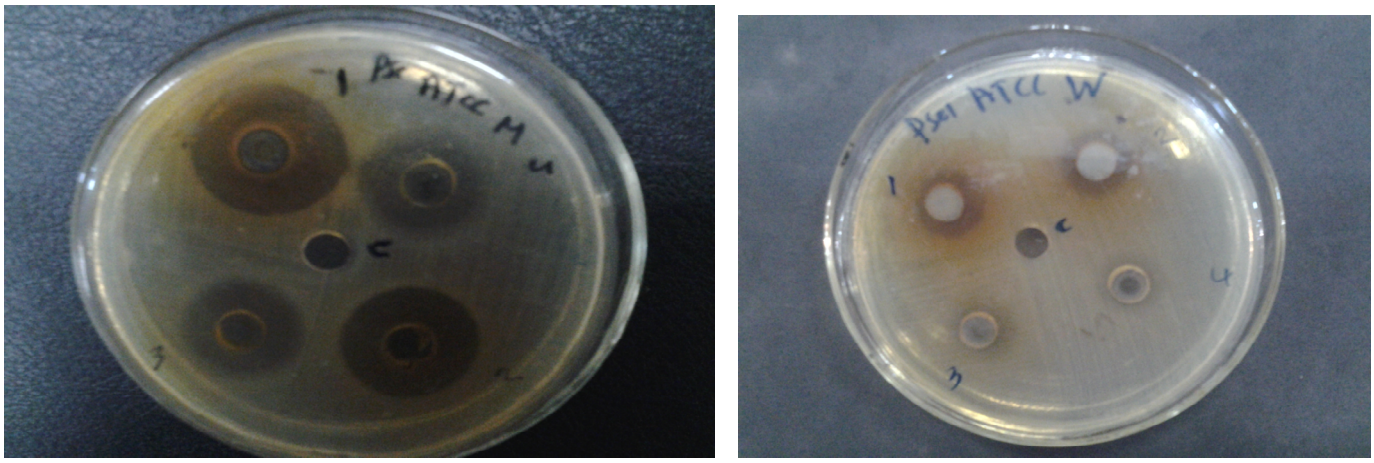

Figure13: Antibacterial activity of *L. inermis* methanol and water extract on *P. areuginosa*ATCC27853 with concentration 5omg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. C: methanol and water as control negative.

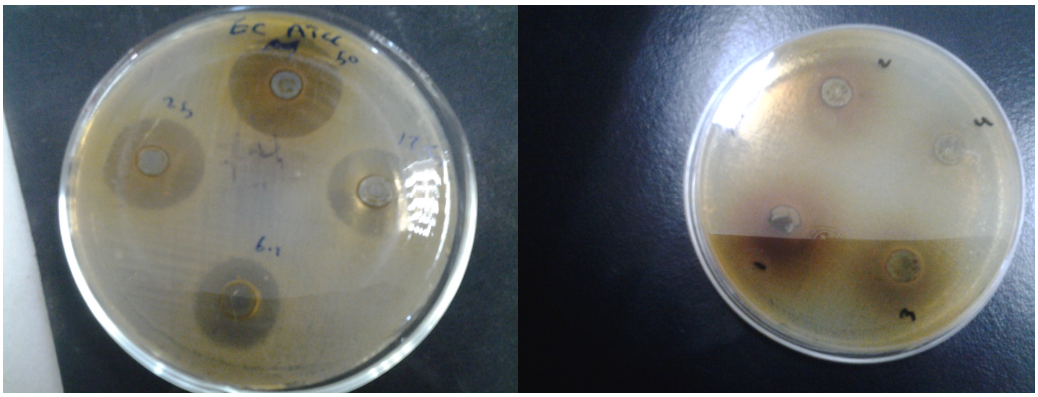

Figure14: Antibacterial activity of *L. inermis* methanol and water extracts on *E. coli* ATCC25922 with concentration 5omg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml.

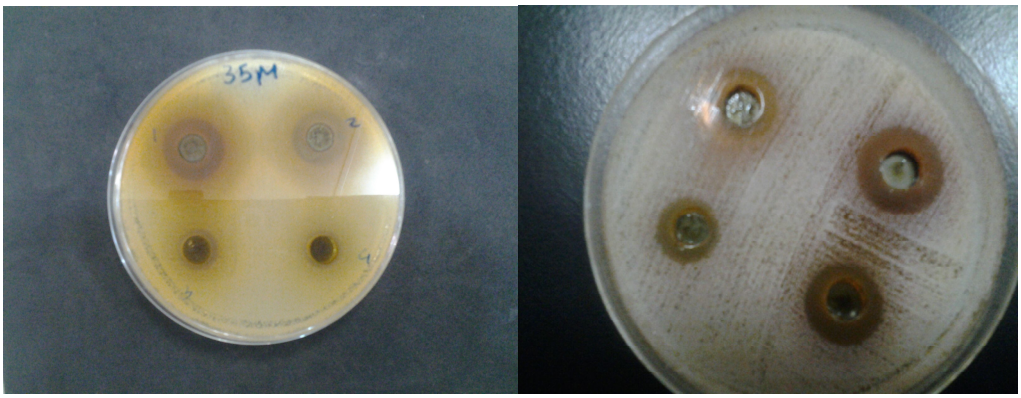

Figure15: Antibacterial activity of *L. inermis* methanol extract on *E. coli* isolate with concentration 5omg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml.

Appendix3: **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