Sudan University of Science and Technology

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Antimicrobial Activity of *Azadirachta Indica* (Neem) Against Bacteria Isolated from Urinary Tract Infected Patients in Khartoum State

نشاط مستخلص اوراق النيم على البكتيريا المعزولة من عدوى المسالك البولية من مرضى في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirement of M.Sc. in Medical Laboratory Science (Microbiology)

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الآيـــــــــــة

قَالَ تَعَالَىٰ:

﴿ ٱللَّهُ لَآ إِلَٰهَ إِلَّا هُوَ ٱلۡحَيُّ ٱلۡقَيُّومُۚ لَا تَأۡخُذُهُۥ سِنَةٞ وَلَا نَوۡمٞۚ لَّهُۥ مَا فِي ٱلسَّمَٰوَٰتِ وَمَا فِي ٱلۡأَرۡضِۗ مَن ذَا ٱلَّذِي يَشۡفَعُ عِندَهُۥٓ إِلَّا بِإِذۡنِهِۦۚ يَعۡلَمُ مَا بَيۡنَ أَيۡدِيهِمۡ وَمَا خَلۡفَهُمۡۖ وَلَا يُحِيطُونَ بِشَيۡءٖ مِّنۡ عِلۡمِهِۦٓ إِلَّا بِمَا شَآءَۚ وَسِعَ كُرۡسِيُّهُ ٱلسَّمَٰوَٰتِ وَٱلۡأَرۡضَۖ وَلَا يَـُٔودُهُۥ حِفۡظُهُمَاۚ وَهُوَ ٱلۡعَلِيُّ ٱلۡعَظِيمُ ﴾

سورة البقرة الآية :255

DEDICATION

To

My father

MY mother

My brothers

My sister

And to all my dear friends

Acknowledgments

First of all grateful ALLAH great blessing. My sincere thanks and gratitude go to my Supervisor **Dr: Hisham Nouraldayem Altayeb** for his advice, interest and leadership throughout this study. Deep thanks to my colleague **Hanna Abdalla** and Sudan Microbiology Laboratory staff for their help and patience. Finally my thanks go to every one who contributed in the thesis.

Abstract

This was analytical study conducted during the period from January to April 2017, to determine the antimicrobial activity of *Azadirachta indica* leaves methanolic extract against different bacteria isolated from urinary tract infection. A total of 130 urine samples were collected from patients with Urinary tract infection. These specimens were inoculated into Cystine Lactose Electrolyte Deficiency (CLED) media and incubated aerobically at 37 ° C for 24 hrs. The clinical isolates were then identified using conventional method. Ninety seven (75%) samples showed bacterial growth. The antibiotic susceptibility testing was performed using standard disk diffusion method. The results showed that Ceftazidime had higher resistant rates 70.6% followed by Ciprofloxacin 64.7%, Cotrimoxazole 62.7%, Gentamycin 54.9% and Imipenem 15.7 %. *A. indica* leaves methanolic extract showed antibacterial activity against all pathogenic and reference strains, 50 (% w/v) concentration was the most effective one, but 100 (% w/v) didn’t produce any effect. Gram negative bacteria were more susceptible to *A. indica* leaves methanolic extract than Gram positive bacteria. MIC of Neem leaves methanolic extract for all tested bacteria was 3.125 (% w/v) except for *E. coli* ATCC 25922 which was 12.5 (% w/v). Gas chromatography analysis revealed that 45 chemical active compounds of *A. indica* (Neem), 29 of them have antibacterial activity.

مستخلص الأطروحة

أجريت هذه الدراسة التحليلية في الفترة من يناير حتى أبريل 2017 في ولاية الخرطوم لدراسة نشاط مستخلص النيم على أنواع مختلفة من البكتيريا المعزولة من عدوى المسالك البولية. تم جمع 130 عينة بول من مرضى مصابون بعدوى المسالك البولية. تم تزريع هذه العينات في وسط CLED وتم تحضينها هوائياً في درجة حرارة 37 درجة مئوية لمدة 24 ساعة ثم تم التعرف على البكتريا باستخدام الاختبارت البيوكيميائية. أعطت 97 (75%) عينة نمؤ في الاوساط الزراعية. ثم تم قياس حساسية البكتريا المعزولة لبعض المضادات الحيوية للبكتيريا بواسطة طريقة الانتشار الطبقي القياسي. وجدت الدراسة أن السيفتازيديم لديه أعلى درجة مقاومة 70.6%, يليه السبروفلوكساسين 64.7%, كوترايموكزازول 62.7%, جينتامايسين 54.9% و الإمبينيم 15.7%. وايضاً وجدت الدراسة أن مستخلص أوراق النيم له فعالية على البكتيريا الممرضة والبكتريا المرجعية. كان التركيز 50 (w/v %) أكثر فعالية مقارنة بالتركيز 100 (w/v %) الذي لم يحدث أي تاثير على البكتيريا. مستخلص أوراق النيم له فعالية أكثر على البكتيريا سالبة الغرام مقارنة بالبكتيريا موجبة الغرام. أظهرت الدراسة ايضاً أن التركيز المثبط الادنى لمستخلص النيم هو 3.125 (w/v %) لكل انواع البكتريا المختبرة ما عدا الاشريشيا القولونية كان التركيز12.5 (%w/v). تحليل المركبات الموجودة في اوراق النيم عن طريق جهاز التحليل اللوني للغاز اظهر ان هنالك 45 مركباً من بينهم تسعة وعشرون مركباً له فعالية مضادة للبكتيريا.

Topic	Page NO
الأيــــــــــة	I
Dedication	II
Acknowledgement	III
Abstract (English)	IV
Abstract (Arabic)	V
Table of content	VI
List of figure	VII
List of table	VIII
CHAPTER ONE: INTRODUCTION	
1.1. Introduction	1-2
1.2. Rational	3
1.3. Objectives	4
1.3.1. General objective	4
1.3.2. Specific objectives	4
CHAPTER TOW : LITERATURE REVIEW	
2. Literature review	5
2.1. *Azadirachta indica* (Neem)	5
2. 2. Classification of *A. indica*	5
2. 3. Origin of the tree	6-7
2. 4. Botanical description	7
2. 5. Neem general features	8
2. 6. Secondary metabolites	8-9
2. 7. Extraction of the secondary metabolites	9
2. 8. Medicinal usage	10-11
2. 9. Melluscicidal effect	12
2. 10. Other uses	13
2. 11. Dosing	13
2. 12. Neem benefits and sides effects	13

2. 13. Toxicity 13
2. 14. Urinary tract infections (UTIs) 14
2. 15. Recurrent urinary tract infection 14
2. 16. Incidence 14-15
2. 17. Risk factors 15
2. 18. Most common bacteria that cause urinary tract infections 15
2. 18. 1. *Escherichia coli* (*E. coli*) 15-16
2. 18.2. *Pseudomonas aeruginosa* (*P. aeruginosa*) 16
2. 18. 3. *Klebsiellae pneumoniae* (*K. pneumoniae*) 17
2. 18. 4. *Proteus* species 17
2. 18. 5. *Citrobacter* species 17
2. 18. 6. *Enterococcus feacalis* (*E. faecalis*) 18
2. 18. 7. *Staphylococcus aureus* (*S. aureus*) 18
2. 19. Multidrug resistant (MDR) pathogens 19
2. 20. Classification of MDR pathogens 19
2. 20. 1. Primary Resistance 19
2. 20. 2. Secondary resistance 19-20
2. 20. 3. Clinical resistance 20
Chapter three: material and method
3.1. Study design 21
3.2. Study area 21
3.3. Study population 21
3.4. Study period 21
3.5. Sample size 21
3. 6. Collection and proceeding of urine samples 21-22
3.7. Identification of the clinical isolates 22
3. 7. 1. Colonial morphology 22
3. 7. 2. Preservation and storage of isolated organisms 22

3.7. 3. Gram stain 22-23
3. 7. 4. Biochemical tests 23-24
3. 8. Antibacterial susceptibility testing 24
3. 9. Collection and identification of plant material 24
3. 10. Preparation of *A. indica* extract 24-25
3.11. Antibacterial activity of *A.indica* 25
3. 11. 1. Cup plate method 25
3.11. 2. Determination of minimum inhibitory concentration (MIC) 0f Neem extract by agar diffusion method 25
3. 12. Quality control 25-26
3. 12. 1. Control of culture media 26
3. 12. 2. Control susceptibility testing method 26
3. 12. 3. Batch quality control 26
3. 13. Photochemical screening of *A. indica* 27
3. 13. 1. Method of analysis 27
3. 14. Statistical Analysis 27
3. 15. Ethical consideration 27

CHAPTER FOUR : RESULTS

4. 1. Gender 28
4. 2. Bacterial growth 29
4. 3. Growth of bacteria on culture media 30
4. 4. Gram stain 30
4. 5. Biochemical tests of different bacterial isolates 30-31
4. 6. Antibacterial Susceptibility 32
4. 7. Antibacterial activity of *A.* indica 36
4. 8. Interpretation of results 36
4. 9. Minimum Inhibitory Concentration (MIC) of *A. indica* methanol extract obtained by agar diffusion method 38
4.10. Gas chromatography results 40

CHAPTER FIVE: DISCUSSION

5.1 Discussion 42-44

5.2 Conclusion 45

5.4 Recommendations 46

List of Figure

1. *Azadirachta indica* tree 6

2. Distribution of samples according to gender 28

3. The percentage of bacterial growth on CLED 29

4. Percentage of growth in two genders 29

5. Antimicrobial susceptibility of *E. coli* ATCC 25922 and *E. coli* MDR to Gentamycin, Cotrimoxazole, Cefatzidime, Imipenem and Ciprofloxacin 35

6. Mean of inhibition zones after in vitro exposure of isolates to *A. indica* methanol extract in different concentrations (diameter in millimeter) 37

7. Activity of *A. indica* leaf methanolic extract on *E. coli* ATCC 25922 with concentrations 50 (%w/v), 25 (%w/v), 12.5 (%w/v), 6.25 (%w/v) and 3.125 (%w/v). 39

8. Activity of *A. indica* leaf methanolic extract on *E. coli* MDR and *P. aeruginosa* with concentrations 50 (%w/v), 25 (%w/v), 12.5 (%w/v), 6.25 (%w/v) and 3.125 (%w/v). 39

9. Collection of *A. indica* and drying in shadow and ground by using mortar and pestle 68

10. Growth of bacteria on CLED; yellow color indicate lactose ferment while blue color indicate non-lactose ferment 68

11. Antimicrobial susceptibility of *P. aeruginosa* to Gentamycin, Cotrimoxazole, Imipenem, Cefatzidime and Ciprofloxacin 69

12. G C-MS Chromatogram of methanol extract of *A. indica* leaves clearly showed 45 peaks 69

List of Table

Table1 Biochemical properties of various Gram negative bacteria isolated from patient with urinary tract infection 31

Table 2 Biochemical properties of Gram positive bacteria isolated from patient with urinary tract infection 31

Table 3 Comparison the activity of *A. indica* leaves methanolic extract with activity of commonly used antimicrobial against clinical isolates 33

Table 4 Comparison the activity of *A. indica* leaves methanolic extract with activity of commonly used antimicrobial against control strain 34

Table 5 Showed (MIC) of *A. indica* methanolic extract 38

Table 6 Active antibacterial compounds in methanolic extract of Neem 41

Table 7 The weight and yield of *A. indica* extract by methanol extraction method 70

Table 8 Gas chromatography analysis of *A. indica* leaves methanolic extract 71

CHAPTER ONE

1. INTRODUCTION

1. 1. Introduction

According to the World Health Organization (WHO) more than 80% of the world population relies on traditional medicine for their primary healthcare (WHO, 2015). Herbal drugs have found wide spread use in many countries because they are easily, available, cheaper and safer than synthetics drug (Retnam *et al*., 2007 and Prusti *et al.,* 2008). Antimicrobial resistance is a major and increasing global problem, a large number of bacteria have responded to the use of antibiotics with their ability to evolved and transmit antibacterial resistance to other species. The increased consumption of antimicrobial agents and inappropriate use accelerates this phenomenon. Also the continuous migration of people plays an important role in acquisition and spread of multidrug resistant strains (Nerino *et al.*, 2013). Urinary tract infection causing bacteria become more resistant to available antibiotics, there is urgent to explore new strategies for managing UTIs (Foxman, 2003). The development of resistance in microorganisms to antibiotics and emergence of new infectious disease create urgent need to discover novel safe and effective antimicrobial compounds (Rojas *et al*., 2003). Plants derived compounds are likely to provide a valuable source of new antimicrobial agents. Several plants have ability to treat the multiple-drug resistant strains (Carvalho *et al*., 2001).

Azadirachta indica (*A. indica)* belongs to the botanic family Meliaceae, commonly known as Neem. It is used in traditional medicine as source of many therapeutic agents. *A. indica* (leaf, bark and seeds) are known to contain antibacterial and antifungal (Natarajan *et al*., 2003) activities against different pathogenic microorganisms. The biological activities are attributed to the presence of many bioactive compounds in its different parts (Orhue *et al*., 2014). Aqueous extract of Neem leaf extract has a good therapeutic potentials antihyperglycaemic agent in insulin-dependent and non-insulin dependent diabetes mellitus (Sonia and Srinivaszn, 1999). Furthermore, Neem leaves may be used for treatment of various diseases including eczema, ringworm, acne, inflammation, hyperglycemia, chronic wound infections, diabetic foot and gas gangrene. They may also remove toxins from the body, neutralize the free radicals present in the body and purify blood. Recently they were reported to act as anticancer agents and they were shown to have hepato-renal protective activity and hypolipidemic effects (Kumar *et al.,* 2002).

1.2. Rationale

A.indica is a perennial plant that has ability to grow in various environments in Sudan. Lately an antimicrobial activity has been noticed in *A. indica* leaves extracts and researches have been conducted to assess the effectiveness of *A. indica* as alternative natural herbal antimicrobial to chemical antibiotics. Despite the existence of potent antimicrobial agents, resistant or multi-drug resistant strains are continuously emerging, imposing the need for a continuous search and development of new drugs (Barbour *et al*., 2004). As a raised percentage of resistance and multi-drug resistance to regular antibiotics, herbal compounds such as *A.indica* could be the solution (Orhue *et al*., 2014). There is previous study in Sudan focus on antimicrobial activity of *A. indica* leaves extract but with limited research so this study conducted to assess antimicrobial activity of *A. indica* leaves methanolic extract with more research. The aim of this study to assess the antimicrobial activity of *A. indica* leaves methanolic extract against urinary tract infections pathogens and control strains with gas chromatography to identify the active antimicrobial compounds.

1.3. OBJECTIVES

1.3.1. General objective

To study antimicrobial activity of *Azadirachta Indica* (Neem) against bacteria isolated from urinary tract infected patients in Khartoum state.

1. 3. 2. Specific objectives

1. To isolate and identify the bacteria from patients with urinary tract infection in Khartoum state and to assess the antimicrobial activity of commonly use antibiotics against UTI pathogens.
2. To determine the antimicrobial activity and Minimum Inhibition Concentration (MIC) of methanolic extract of *A. indica* (Neem) leaves against bacteria isolated from patients with urinary tract infection in Khartoum state.
3. To compare the activity of *A. indica* leaves methanolic extract with the activity of commonly used antimicrobial used in the treatment of UTI.
4. To identify chemical compounds of Sudanese *A.indica* by using gas chromatography.

CHAPTER TWO

2. LITERATURE REVIEW

2. 1. *Azadirachta indica* (Neem)

A.indica a tree in the mahogany family Meliacae and also known as Neem, Nimtree and popularly known as Indian Neem (margosa tree) or Indian lilac and the latter as the Persian lilac (Parrotta and Chaturvedi, 1994; Biswas *et al*., 2002).

2. 2. Classification of *A.indica*

Common name: Neem.

Kingdom: *Planate.*

Division: *Magnoliophyta.*

Class: *Magnoliopsidia.*

Order: *Rutales.*

Suborder: *Rotinae.*

Genus: *Azadirachta.*

Species: *A.indica*.

Family: *Meliacae* (mohogangfamily).

Subfamily: *Melioideae*.

Tribe: *Meieae.*

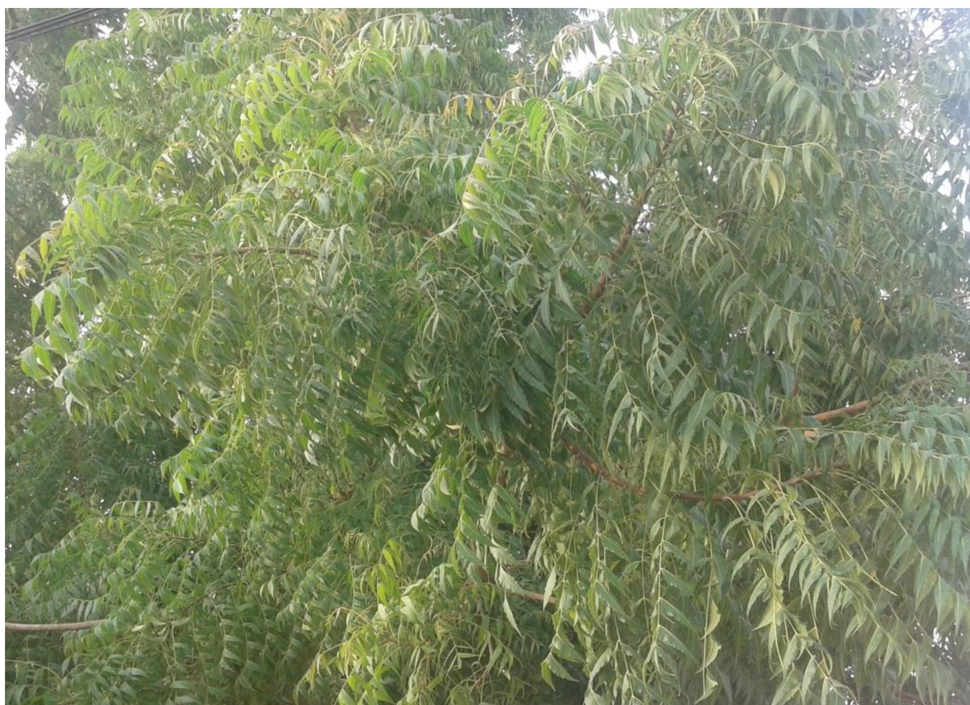

Fig1: *A. indica* tree (Algazira, Alkamleen city)

2. 3. Origin of the tree

The Neem is believed to have originated in Assam and Bunma of south Asia, but other reports suggest that various areas of Pakistan, Sir Lanka, Thailand, Malaysia and Indonesia. The tree also grows in tropical and subtropical areas around the world (Verker and Wright1993). Neem trees have successfully been established in Australia, Haiti, and West Africa. The Dominican Republic, Ecuador, Puerto Rico, the Virigin Islands and in the continental United state in Floride, Oklahoma and Arizona (Jacobson, 1990). *A. indica* is well known in India and its neighboring countries as one of the most versatile medicinal plants having a wide spectrum of biological activity. Neem is an evergreen tree, cultivated in various parts of the Indian subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various human diseases, from past several pharmacological activities and medicinal applications of various

parts of Neem have been documented in the ancient literature. Recently, biological activities and medicinal properties of Neem have been extensively reviewed by (Biswas *et al.,* 2002). Biological activity of Neem is reported with the crude extracts and their different fractions from leaf, bark, root, seed and oil. However, only crude extract of different parts of Neem has been used as traditional medicine for the treatment of various diseases. Neem has been extensively used in Ayurveda, Unani, Homoeopathic and Siddha medicine and has become a cynosure of modern medicine (Varma, 1976). In the present study we have evaluated the antimicrobial potential of *A. indica*.

2. 4. Botanical description

A. indica is the member of the family Meliaceae. It is noted for its dryness resistance. Normally it thrives in area with sub-arid to sub-humid conditions. It is broad-leaved evergreen tree which can reach heights of 30 meters with a trunk girth of 2.5 meters and can lives for over two centuries. Its deep root system is well adapted to retrieving water and nutrients from the soil profile, but this deep root system is very sensitive to water logging. The Neem tree thrives in hot, dry climates where shade temperatures often reach 5°C and annual rainfall ranges from 400-1.200 mm. It can grow in many different types of soil, but it thrives best on well drained deep and sandy soil. The tree can withstand many environmental adversities including drought, infertile, stony, shadow and acidic soils. The Neem produces ellipsoidal drupes, which are about two centimeters in Length, borne on axillary clusters. This fruits contains kernels that have high concentration of secondary metabolites (Schmutterer, 1990a)

2. 5. Neem general features

The chemicals have pesticidal activity can most efficiently be extracted from Neem seed kernels. Neem trees begin their reproductive stage at about three to five years of age but don't become a fully reproductive until they are ten years old. From this time on, the tree yield an average of about 20.5 kilograms of fruit per year, with maximum production reaching 50 kilograms per year (Ibtihag, 2008). Of the fruit yield, only about ten percent is attributed to seed kernels and desired biologically active compounds comprise only ten grams per kilogram of kernel weight. This means that adult Neem tree will only produce about 20 grams of pesticidal compounds in a season (Schmutterer, 1990b).

2. 6. Secondary metabolites

Many biologically active compounds can be extracted from Neem, including triterpenoids, phenolic compounds, carotenoids, steroids and ketones. The tetranortriterpenoid azadirachtin has received the most attention as a pesticide because it is relatively abundant in Neem kernels and has shown biological activity on a wide range of insects. *Azadirachtin* is actually a mixture of seven isomeric compounds labeled as *azadirachtin -A* to *azadirachtin -G* with *azadirachtin-A* begin present in the highest quantity and *azadirachtin -E* regarded as the most effective insect growth regulator(Verker and Wright,1993). Many other compounds have been isolated and they showed antecedent activity as well as growth regulating activity on insects. Polar and non-polar extraction yield about 24 compounds other than *azadirachtin* that have at least some biological activity (Jacobson, 1990). This cocktail of compounds significantly reduces the chances of tolerance or resistance developing in any of the effected organisms. However, only four of the compounds in Neem have been shown to be highly effective in their activity as pesticides:

Azadirachtin, salannin, meliantriol and nimbin (Jacobson, 1990). Neem tissue culture the term tissue cultures generally refer to artificial cultivation of plant tissue (Smith *et al.*, 1999). For production of commercial amounts of secondary metabolites, for different utilization, constant availability with standardized quality is one of the important requirements and therefore in vitro cultures production could more be feasible. Therefore, in order to obtain constant amounts of standardized quality of secondary metabolites, it will be appropriate to employ tissue culture techniques for its production. Various growth factors might have effect in Neem tissue culture processes and might also affect the productivity of the secondary metabolites. It is well known that, establishment of continuous in vitro cultures facilitate production of secondary metabolites even in quantities that allow economically feasible production (Antjet, 1998). While Neem tree product has some shortcomings as conventional alternative, they fit in well as tool to be used in integrated pest management systems. As more and more synthetic chemicals are being pulled from the market, Neem is an environmental benign alternative. It has significant effect on pests without harming beneficial organisms.

2. 7. Extraction of the secondary metabolites

The secondary metabolites can be extracted by many methods and leaching with water is the oldest method. On the other hand, more than one non-polar solvent are use to obtain more varied mixture of chemicals. Hexane, ethanol, methanol, ester and chloromethane are used in extractions as well as mixture of this solvent with water. Once metabolites extracted several separation techniques such as HPLC fractionation, IR spectrum, analysis and 13°C and 111 NMR spectrums are used in analysis and identification of the isolated compounds (Lee *et al.*, 1988).

2. 8. Medicinal usage

Neem fruits, oil, leaves, roots and bark have been used in the traditional medicine. Thus over thousands of years, millions of Asians have used Neem medicinally. In addition, in places where the trees has been introduced in recent time such as tropical America and Africa it has also established a reputation as useful care for various diseases. Today the best established and most widely recognized uses are based on its merits as a general antiseptic. Information relating to the antifungal activities of compounds from Neem is limited (Locke, 1995). Neem has proved effective against certain fungi that infect the human body such as *Aspesrgillus flavus* which cause increasing problem that difficult to be controlled by synthetic fungicides (Bhatnar *et al*., 1990). Different parts of Neem (leaf, bark, and seeds) have been shown to exhibit wide pharmacological activities such as antioxidant, antimalarial (Orhue *et al*., 2014), antimutagenic, anticarcinogenic, anti-inflammatory (Orhue *et al*., 2014), antihyperglycaemic (Bhat *et al*., 2011;Talwar *et al*., 1997 and Sudha *et al*., 2011), antiulcer (Orhue *et al*., 2014) and antiviral activity against *Vaccinia*, *Chikungunya*, *Measles* and *Coxsackie B* viruses (Biswas *et al*., 2002; Singh and Singh, 1979; Xu *et al.,* 2014 ; Badam *et al*., 1990; Mulla and Su, 1999; Parida *et al*., 2002; Du *et al*., 2008; Xu *et al*., 2010). In Sudan there is study for assess the bioactivity of Neem conducted by Ibtihag (2008) that showed there is antifungal activity of *A. indica* against *D. rostrata*, *F. oxysporum* and *A. alternate* and ithas effect on *Biomphlaria snails (B. glabrata)*. Hala *et al.* (2015) studied the antimicrobial activity of ethanolic extract of *A. indica* leaf in Sudan against pathogenic bacteria (*E. coli*, *S. aureus*, *K. pneumoniae, P. mirabilis* and *E. faecalis*), the study found that *A. indica* leaf (Neem) extract showed strong antimicrobial activity against all bacterial species studied at all the concentration.

Another study in neighboring countries, conducted in Nigeria (2014) by Orhue *et al.* reveal that methanolic extract of *A. indica* showed antimicrobial activity against *E. coli, Salmonella* species and *Staphylococcus*. Rajasekaran *et al.* (2008) in India studied antimicrobial activity of *A. indica* leaf (Neem) extract on eight strain of Gram-positive bacteria *Micrococcus glutamicus, Lactobacilli bulgaris, S. aeueus, E. feacalis, Bacillus sterothermophilus, Streptoccocus pyogen, Microccus luteus and Bacillus cereus* and two strain of Gram-negative bacteria *E. coli* and *P. aeruginosa* the Neem extract limited the growth of both Gram-positive and Gram-negative species tested. In China Xu *et al.* (2014) studied antiviral activity of Neem against *Plague virus* that result in virus inactivation. In Many preparations of Neem extracts are reportedly efficacious against a variety of skin disease, septic sores and infected burns. The leaves applied in the form poultices or decoctions, are also recommended for boils, ulcers and eczema. The oil is used for skin disease such as scrofula, indolem ulcers, and ringworm and creams as well as toothpaste, soap, shampoo. Small branches of Neem used as toothbrush. Cures for many more diseases have been claimed but have been independently confirmed by trials under controlled conditions. Neem insecticides are potent insect growth regulators against mosquito larvae: Neem oil and other derivatives can be effective personal repellents against biting adult mosquitoes and certain Neem fraction have anti-malarial action (Kumar *et al.,* 2002).

2. 9. Melluscicidal effect

Snails are the small creatures that attract great attention through their association with snail borne disease such as Shistosomiasis which associated with *Biomphalarea spp*. Eradication of the snails is the hope of eliminating the most parasitic disease of man, animals, birds and fishes. The optimal chemical molluscicied is not been developed and must meet certain restriction (Belding, 1964). Therefore, the use of natural products of plant origin emerged as substitute for snail control. Mollusciecides of plant origin are cheap, safe and easily available and field applicable with simple techniques (Shoeb and Hassan, 1984; Schorder, 1992) Since the product of Neem tree (*A. indica*) was used as multipurpose plant in agriculture and for pest control (Grant and Schmutterer, 1987) extract from different Neem parts could also be tested to assess if they possess a molluscicide activity. The mode of action of Neem extracts is not understood very well. It is quite possible that the different chemicals or different ratios of chemicals found in Neem tree have varied effects on insects (Anderson and Ley, 1990). The precise effect of the various Neem tree extracts on a given species is often difficult to pinpoint. Neem's complexity of ingredients and its mixed modes of action vastly complicate clarification. Moreover, most of the studies up to date are hard to compare because they have used differing test insect, dosage and formulations. Further, the materials used in various tests have often been handled and stored differently, taken from different parts of the tree, or produced under different environment conditions. Although Neem's effects on pestiferous insects are by far the best known, the tree's various products can influence other pest organisms as well. In the long run these may prove important value (Devakumar *et al*., 1984).

2. 10. Other uses

Neem products are cheap and non-toxic to higher animals and most beneficial insects, they are well-suited for pest control in rural areas. Neem tree is of great importance for its anti-desertification properties and possibly as good carbon dioxide sinks. Neem is considered as useful tree in rehabilitating the waste land areas. Neem seed pulp is useful for methane gas production. It is useful as carbohydrate which is rich base for other industrial fermentations. Neem bark contains tannis which are used in tanning and dyeing. Its wood is used to make furniture. The bark of yields and the fiber that is woven into ropes (Review of natural product, 2009).

2. 11. Dosing

The appropriate dose of Neem depends on several factors such as user's age, health, and several other conditions. There are inadequate clinical trials to support specific therapeutic doses of Neem (Review of natural product, 2009).

2. 12. Neem benefits and sides effects

As with any other herbs and plants the use of Neem should follow yours doctors advice. In primates, the use of Neem (*A.indica*) seed extracts (praneem) given orally caused abortion of pregnancy. In the time following treatment, the animals regained their normal cyclicity in the cycles subsequent to praneem treatment (Review of natural product, 2009).

2. 13. Toxicity

Toxicology studies have indicated it to be quite safe to mammals (Schmutterer, 1990b). But the seed of the Neem are poisonous in large dose. Sever poisoning in infants from Neem oil have been reported (Review of natural product, 2009).

2. 14. Urinary tract infections (UTIs)

Urinary tract infections causing by different microorganisms, including fungi and viruses, bacteria are the major causative organisms and are responsible for more than 95% of UTIs cases (Bonadio *et al*., 2001). *E. coli* predominates (>80% of infections), followed by *staphylococci* (8-10%) with the remaining pathogens found in only 1–5% of infections. Within hospitals, the proportion of infections due to *E.coli* is reduced (40–60%), with more infections due to *Klebsiella,* other coliforms, *Pseudomonas* and *Enterococc*i (Will *et al., 2006*). Risk factor includes structural abnormalities of urinary tract, urinary catheter, urological surgery, diabetes and immunosuppresion (Irving *et al*., 2006). The concentrations of these organisms in the urine have been the source of some disagreement in the past. While quantitative cultures usually show a large number of organisms present (> 10^5/mL), about 25% to 30% UTIs will have fewer organisms (> 10^3/mL) (Puro *et al*., 2003). In general, hospital bacteria causing UTIs have higher levels of antibiotic resistance, either through innate resistance (*Enterococc*i, *Pseudomonas*) or acquired resistance (*E.coli, Klebsiella,* other coliforms). The local microbiological epidemiology of UTIs and associated antibiotic resistance rates are important to establish within different healthcare settings, to allow for appropriate empirical antibiotic therapy of UTIs (Will *et al., 2006*).

2. 15. Recurrent urinary tract infection

Recurrence of UTI is a common problem with rates reported as high as 44% at one year (Weinstock *et al*., 1999).

2. 16. Incidence

Women have a lifetime risk of UTI of 1 in 3 in 20. It accounts for 5% of woman 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 (NICE, 2007).

2. 17. Risk factors

There is evidence to suggest that deregulation of Candidate genes in humans may predispose patient to recurrent UTI, diabetes is also 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 empting (dysfunctional urination), contraception diaphragm, spermicide-coated condoms. Also history of urinary tract surgery and Immune compromise are considered risk factors (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 (Grabe *et al*., 2013).

2. 18. Most common bacteria that cause urinary tract infections

2. 18.1. *Escherichia coli* (*E. coli*)

E.coli are a Gram negative usually motile rod, minorities of strains are capsulated, aerobic and facultative anaerobic. Optimum temperature for growth is 36–37 ºC. It naturally found in the intestinal tract, soil and water. *E. coli* is the commonest pathogen isolated from patients with cystitis (Cheesbrough, 2006). *E. coli* is the most common cause of urinary tract infection and gram-negative rod sepsis. It is one of the two important

causes of neonatal meningitis and the agent most frequently associated with "traveler's diarrhea," a watery diarrhea. Some strains of *E. coli* are enterohemorrhagic and cause bloody diarrhea (Levinson, 2012). Treatment of *E. coli* infection has been increasingly complicated by the emergence of resistance to most first-line antimicrobial agents (Sabate *et al*., 2008). Antibiotic resistance rates in *E. coli* are rapidly rising, especially with regard to flouroquinolones and third and fourth generation cephalosporins and extended-spectrum β-lactmas (Laupland *et al*., 2008 and Mesa *et al*., 2006).

2. 18.2. *Pseudomonas aeruginosa* (*P.aeruginosa*)

P. aeruginosa is Gram negative rod, obligate aerobe, non-sporing and motile, some strains are capsulated. It is usually recognize by pigment production 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 environments in hospital 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. *P. aeruginosa* cause Skin infections, Septicemia, urinary tract infection, respiratory tract infection and eye infection (Cheesbrough, 2006). Its intrinsic resistant mechanism considered a problem (Nicolle, 2005). It has become increasingly clear that resistant development in *P. aeruginosa* is multifactorial, with mutations in genes encoding protein, efflux pumps, benicillin-binding protein and chromosomal β-lactmase, all contributing to resistance to β-lactams, carbapenems, aminoglycosides and fluoroquinolones (Ozer *et al*., 2009).

2. 18.3. *Klebsiella pneumoniae* (*K. pneumoniae*)

K. pneumoniae are Gram negative, non-motile, usually capsulated rods aerobes and facultative anaerobes and non motile**.** *K. pneumoniae* causes chest infections and occasionally severe bronchopneumonia with lung abscesses and urinary tract infection. Infections are often opportunistic, occurring in those with existing chest disease or diabetes mellitus, or in malnourished persons (Cheesbrough, 2006). Before 2003 most ESBLs reported in *Klebsiella* spp were mutant TEM and SHV penicillinase. They occurred mainly in specialist units and were often hospital acquired (Livermore, 2007). *K. pneumoniae* is the most frequently encountered carbapenemase -producing *Enterobacteriacae* (Won *et al*., 2011).

2. 18. 4. *Proteus species*

P. mirabilis and *P. vulgaris* are actively motile, non capsulate, Gram negative pleomorphic rods. Motility is not as easily observed at 35–37 ºC as at room temperature (20–28 ºC). When cultured aerobically, most *Proteus* cultures have a characteristic 'fishy' odor and produce swarming on Blood agar. *Proteus* infected urine has an alkaline reaction. In abdominal and wound infections, *Proteus* is often a secondary invader of ulcers, pressure sores, burns and damaged tissues. Also *Proteus* causes septicemia and occasionally meningitis and chest infections (Cheesbrough, 2006).

2. 18. 5. *Citrobacter species*

Citrobacter are Gram negative motile rods. They are opportunistic pathogens and are occasionally isolated from urine, blood, pus, and other specimens (Cheesbrough, 2006).

2. 18. 6. *Enterococcus faecalis* (*E. faecalis*)

E. feacalis are aerobic organisms capable of growing over a wide temperature range, 10–45 ºC. *E. feacalis* are causing about 95% of *Enterococcal* infections including infections of the urinary tract, biliary tract, ulcers (e.g. bed sores), wounds (particularly abdominal) and occasionally endocarditis or meningitis. It is a normal commensal of the vagina and intestinal tract (Cheesbrough, 2006).

2. 18. 7. *Staphylococcus aureus* (*S. aureus*)

S. aureus is Gram positive cocci grow well aerobically and in a carbon dioxide enriched atmosphere. Most strains also grow anaerobically, but less well. Temperature range for growth is 10–42 ºC, with an optimum of 35–37 ºC. *S. aureus* causes boils, styes, pustules, impetigo, infections of wounds (cross-infections), ulcers, burns, osteomyelitis, mastitis, septicemia, meningitis, pneumonia and pleural empyema. Food-poisoning (rapid onset, no fever), toxic shock syndrome and toxic skin exfoliation (Cheesbrough, 2006). However as early as 1942, penicillin-resistant *Staphylocoocci* were recognized, first in hospitals and subsequently in community (Rammelkamp *et al*., 1942). Methicillin was the first of the semisynthetic penicillinase-resistant penicillins. Its introduction was rapidly followed by reports of methicillin-resistant isolates (Jevons, 1961).

2. 19. Multidrug resistant pathogens (MDR)

During the last few decades, the incidence of microbial infections has increase dramatically. Continuous development of antimicrobial drugs in treating infections has lead to emergence of resistance among the various strains of microorganisms. Multidrug resistance is defined as insensitivity or resistance of microorganism to the administrated of antimicrobial medicine despite earlier sensitivity to it (Singh, 2013 and Popeda *et al*., 2014). According to WHO, these resistant microorganisms (like bacteria, fungi, viruses and parasites) are able to combat attack by antimicrobial drugs, which leads to ineffective treatment resulting in persistence and spreading of infections. Studies from WHO report has shown very high rates of resistant in bacteria such as *E. coli* against antibiotics such as cephalosporin and fluoroquinolones, *K. pneuomoniae* against cephalosporin, *Enterococci* resist vancomycin *S. aureus* against methicillin causing common infections (Nikaido, 2009 and WHO, 2014).

2. 20. Classification of MDR Pathogens

2. 20. 1. Primary Resistance

It occurs when the organism has never encountered the drug of interest in particular host.

2. 20.2. Secondary resistance

Also known as "acquired resistance" these terms are used to describe the resistant that only arises in an organism after exposure to the drug (Loeffler and Stevens, 2003 and Khalilzadeh *et al*., 2006). It may further be classified as follows:

Intrinsic resistance:

It refers to insensitivity of all microorganisms of a single species to certain common first-line drugs, which are used to treat disease based on the clinical evidence of the patient. It is also known multidrug resistance (MDR) (Loeffler and Stevens, 2003).

Extensive resistance:

It defines the ability of microorganism to withstand the inhibitory effects of at least one or two most effective antimicrobial drugs. Also termed as XDR, these seemed to arise in patient after they have undergone treatment with first line drugs (Lee *et al*., 2013 and Marks *et al*., 2014).

2. 20.3. Clinical resistance

Situation in which the infecting organism is inhibited by the concentration of antimicrobial that is associated with a high likelihood of therapeutic failure (Loeffler and Stevens, 2003).

CHAPTER THREE

3. MATERIALS AND METHODS

3. 1. Study design

This was analytical study.

3. 2. Study area

The study was conducted in Khartoum state and Sudan University of science and technology, college of medical laboratory science.

3. 3. Study duration

This study was conducted from January to April, 2017.

3. 4. Study population

Male and female of different age with Urinary Tract Infection.

3. 5. Sample size

One hundred and thirty urine sample (n =130) were collected randomly from patients attending in Ribat University Hospital and East Nile Hospital, in Khartoum state.

3. 6. Collection and proceeding of urine samples

Mid-stream urine samples were collected in a universal, wide mouth, sterile urine container. The samples were carried in ice bag and transported to Sudan University microbiology lab. Macro examination was done to detect the color change, smell and turbidity of the samples. Then microscopic examination was done by wet preparation method, started with immersing a test strip in the urine samples to detect the presence of glucose, protein and ketones. The urine samples were then centrifuged and the deposit was

tested for the presence of pus cell, red blood cell, and yeast cell. Urine samples that contain 10 or more white cells per cubic millimeter in urine specimen, 3 or more white cells per high-power field of unspun urine specimens (Horan *et al*., 2008), also colony count greater than 10^5 colony-forming unit (CFU) per milliliter in voided urine (Cheesbrough, 2006), were inoculated at aseptic condition on Cystine Lysine Electrolyte Deficient (CLED) for isolation and identification of the pathogens. The inoculated culture media were incubated aerobically at 37 ℃ for 18-24 hours and examined for significant growth (Cheesbrough, 2006).

3.7. Identification of the clinical isolates:

The clinical isolates were identified in the lab by standard microbiology procedures including the following steps:

3. 7. 1. Colonial morphology

Colonial morphology used as first identification steps focusing on colony size, color, edge and fermentation of lactose in CLED.

3. 7. 2. Preservation and storage of isolated organisms

Isolated organisms were kept in nutrient agar slope at 4 °C for further identification and susceptibility tests. Glycerol (20 ml) with peptone water (80 ml) (20% v/v) was used for long preservation of the isolates at -20℃ (Cody, 2008).

3.7. 3. Gram stain

Fixed and dried smears were prepared from growth. The smear was stained with Gram stain firstly crystal violet stain was applied for 30-60 seconds, washed with water followed by Lugol's iodine for 30-60 sec, washed again then decolorized rapidly by alcohol, washed immediately with water and

covered with Safranin for 2 min then washed and dried to examined microscopically by oil immersion lens (X100) to detected Gram reaction and arrangement of bacteria (Cheesbrough, 2006).

3. 7. 4. Biochemical tests

Biochemical tests including Kliglar Iron Agar test (KIA), indole, urease, citrate, motility test, esculin hydrolysis, catalase test, DNAse test, as well as inoculation on differential selected media such as Mannitol salt agar (MSA) was used to distinguished between the clinical isolates. The principle of the tests started with indole test, the tested organism was cultured in medium contain tryptophan; indole production is detected by kovac's reagent which contain 4-dimethylaminobenzaldehyde. This reacts with indole to give red color compound (Cheesbrough, 2006). Then urease test was done, the tested organism was cultured in medium contains urea and indicator phenol red. If there is change in color from yellow to pink color was recorded (Cheesbrough, 2006), then citrate test, the tested organism was cultured in a medium contain sodium citrate, ammonia salt and indicator bromo-thymol blue showing turbidity. The change in color of the indicator from green to blue was considered as positive (Cheesbrough, 2006). The motility test also done that depend on the properties of bacteria structure which contain flagella or no, also Bile esculin test was done, the tested organism was cultured in esculin agar (Cheesbrough, 2006). While catalase test was used, detect the break down of hydrogen peroxide to give oxygen and water, indicated by production of air bubble. Then Kligler Iron Agar test (KIA) was used for detection of H_2S, gases production and sugar fermentation (lactose), to help in identification of *Enterobacteriasae* (Cheesbrough, 2006). Finally special media were used for more identification of the isolated organisms. Mannitol salt agar was used to differentiate between the *Staphylococcus* species. DNase test also

was used to differentiate between *Staphylococcus* species, the tested organism was cultured on media contain DNA, after inoculation and incubation for over night HCL was used, which precipitates the un hydrolyzed DNA to give clear zone around the colonies (Cheesbrough, 2006).

3. 8. Antibacterial susceptibility testing

Modified Kirby-Bauer Method

Isolated organisms were tested against different antibiotics by using Kirby-Bauer disk-diffusion method in which 3-5 selected colonies were touched by sterile standard loop then emulsified into sterile normal saline and adjusted to 0.5 McFarland standard (Cheesbrough, 2006). The following antibiotic disks (Hi Media laboratories PV+Ltd, India) were used: Ceftazidime (30 mcg), Imipenem (10mcg), Gentamycin (10mcg), Cotrimoxazole (25mcg) and Ciprofloxacin (5mcg). Plates were left at room temperature to dry and incubated at $37°C$ for 18-24 hrs. Zones of inhibition were measured in mm and the result was interpreted according to standardized chart.

3. 9. Collection and identification of plant material

Fresh leaves were collected from Algazira (Alkamleen city) in central Sudan. The plant was taxonomically identified by taxonomist in Herbian at Medicinal and Aromatic plants and traditional Medicine Research Institute National Center for Khartoum, Sudan.

3. 10. Preparation of *A. indica* extract

Fresh leaves of *A. indica* were collected and air dried in shade. The *A. indica* leaf extract was then prepared by grounding 50 g of leaves using mortar and pestle (Appendix 2) then soaked in 80% methanol for about

seventy two hours with daily filtration and evaporation. The Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus (Sukhdev *et al*., 2008).

3.11. Antibacterial activity of *A.indica*:

3. 11. 1. Cup plate method

The agar well diffusion method was done on Muller Hinton Agar (MHA) medium for the assay of the antimicrobial activity of *A. indica* (Neem) leaves methanolic extracts against the isolated pathogens, 3 colonies with the same characteristics were emulsified in 1 ml normal saline and adjusted to 0.5 McFarland turbidity standard. A sterile cotton swab was inserted into the bacterial suspension, rotated and then compressed against wall of the test tube to expel any excess fluid. The swab was then streaked on the surface of MHA plate. To ensure a uniform, confluent growth, the swab was streaked three times over the entire plate surface (Cheesbrough, 2006). A sterile cork borer was then used to make wells (6mm diameter) on MHA medium. Under aseptic conditions 100 µl of five concentrations of *A.indica* extracts (50 (%w/v), 25 (%w/v), 12.5(%w/v), 6.25 (%w/v) and 3.125 (%w/v)) were introduced into the wells. The plates were allowed to stand for 1hour in the refrigerator for diffusion of the extract to take place and incubated at 37°C for 24 hrs. Methanol was used as negative control. Zone of inhibition were measured (in mm) and the mean were calculated (Aneja and Joshi, 2009).

3. 11. 2. Determination of minimum inhibitory concentration (MIC) of Neem extract by agar diffusion method

Determination of inhibition zones and MIC of *A. indica* (Neem) extract were assessed using agar diffusion method as described by NCCLS (2000) and AlWaili &Sloom (1999). One gram from extract was dissolved in 10

ml (100%) methanol and then serially diluted two fold to obtained final concentrations of (50 (%w/v), 25 (%w/v), 12.5 (%w/v), 6.25 (%w/v) and 3.125 (%w/v)). Hundred μl of each prepared concentration was added into corresponding well. The plates were left for 1 hour in refrigerator (4° C) for diffusion of effective compounds of Neem in the media and then incubated at 37°C for 24 hours. Inhibition zone around each well were measured using a ruler in millimeters. MIC considered as the lowest concentration of extract that prevent visible bacterial growth (Aneja and Joshi, 2009).

3. 12. Quality control

3. 12. 1. Control of culture media

The performance of culture media was controlled by testing each patch with control strains *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 to check the quality of the media.

3. 12. 2. Control susceptibility testing method

The quality control strain *S. aureus* ATCC 25923 and *E. coli* ATCC25922were used as described by NCCLs document M7-A7 (NCCLS, 2000) to assess the antimicrobials disks efficiency. The control strains were brought from National Public Health Laboratories in Khartoum.

3. 12.3. Batch quality control

Each batch of susceptibility test was tested within reference strains to determine if zone diameter obtained with in the expected range or not and to check the quality of biochemical.

3. 13. Phytochemical screening of *A. indica*:

3. 13.1. Method of analysis:

The qualitative and quantitative analysis of the sample was carried out by using GM/MS technique model (GC/MS-QP2010-Ultra) from Japan 'Simadzu Company, with capillary column (Rtx-5ms-30m×0.25 mm×0.25μm). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.69 ml/min, the temperature program was started from 50°C with rate 7°C/min to 180°C then the rate was changed to 10°C/min reaching 300°C as final temperature degree with 2 minutes as hold time, the injection port temperature was 300 ° C, the ion source temperature was 200 ° C and the interface temperature was 250 ° C.The sample was analyzed by using scan mode in the range of m/z 40-550 charges to ratio and the total run time was 28 minutes. Identification of components of the *A. indica* methanolic extracted sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library of the National Institute of Standards and Technology (NIST), the results were then recorded (Sofowora, 1982).

3. 14. Statistical Analysis

SPSS version 11.5 was used for data analysis (One-Way-Anova: $P<0.05$ and Independent sample T test was used.

3.15. Ethical consideration

Permission to carry out the study was taken from the college of medical laboratory sciences, Sudan University of Science and Technology. All the participate were informed for the purpose of the study before collection of the specimens and consent was taken from them.

CHAPTER FOUR

RESULTS

4. 1. Gender

Among of 130 urine specimens collected 52 (40%) were males and 78 (60%) were females (Fig. 2)

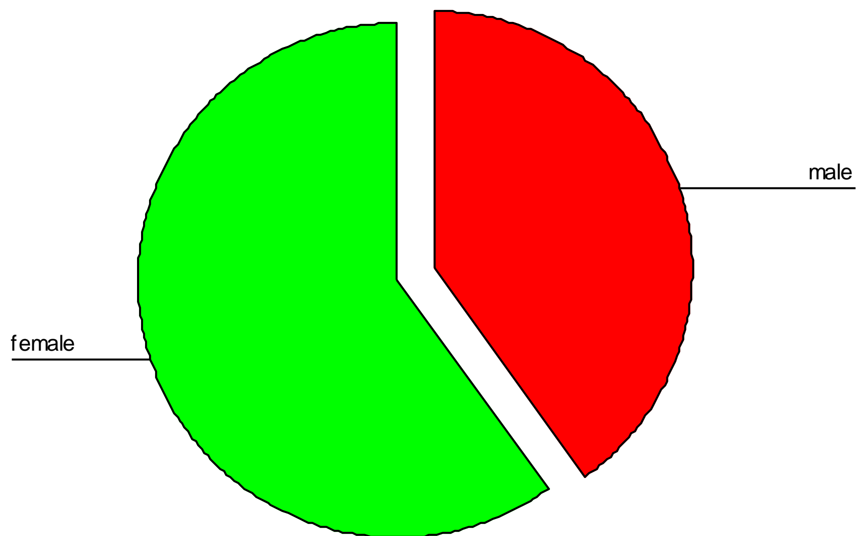

Fig.2: Distribution of urine samples according to gender

4. 2. Bacterial growth

Out of 130 investigated samples 97 showed significant bacterial growth, while 33showed no bacterial growth.

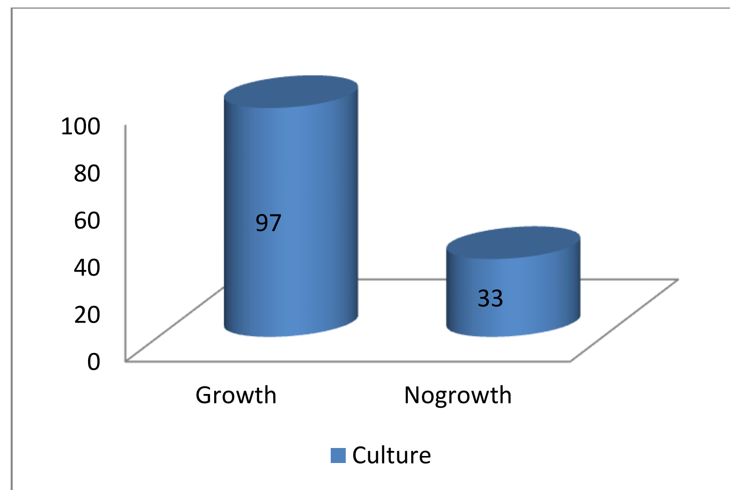

Fig.3: The percentage of bacterial growth on CLED

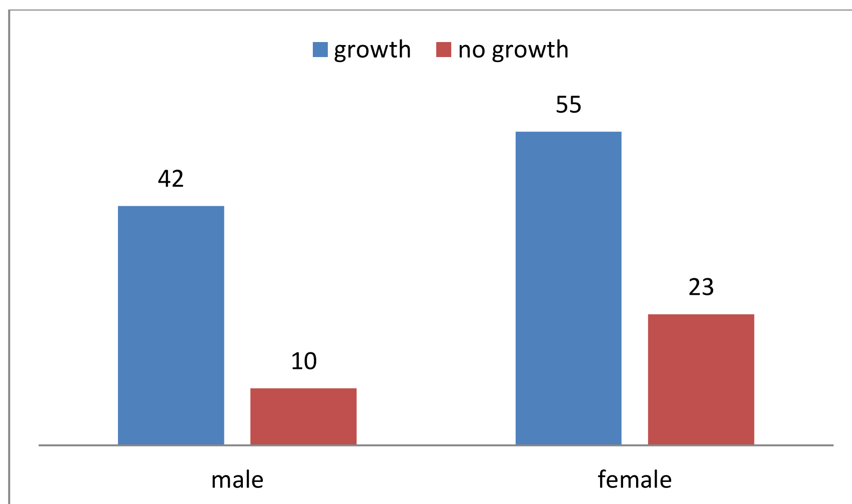

Fig. 4: Percentage of growth in two genders

In this study we found the percentage of UTIs is more in females (55) than males (42) with $P > 0.05$, with age range from 14 days to 90years, and there is significant association between age groups and UTI (p-value<0.05), whom attending at hospital with different wards (out patient, in patient, ICU, HDU and pediatric unit with $P>0.05$).

4. 3. Growth of bacteria on culture media

On CLED some isolated showed yellow fermented colonies (88 bacteria) while others none fermenter organisms (12 bacteria), due to the presence of bromo-thymol blue indicator (Appendix3).

4. 4. Gram stain

From 100 bacteria, 10 were Gram positive cocci (violet color) and 90 were Gram negative rod (red color).

4. 5. Biochemical tests of different bacterial isolates

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

Table1: biochemical properties of various Gram negative bacteria isolated from patients with urinary tract infection.

Isolated bacteria	Biochemical tests							No. of isolates and %	
	Indole	Urease	Citrate	KIA					
				Slope	Butt	Gas	H_2S		
E.coli	+ve	-ve	-ve	Y	Y	+ve	-ve	70	70%
P.aeruginosa	-ve	-ve	+ve	R	R	-ve	-ve	5	5%
P. vulgaris	+ve	+ve	+ve	R	Y	+ve	+ve	3	3%
P. mirabilis	-ve	+ve	+ve	R	Y	+ve	+ve	2	2%
Citrobacter	-ve	+ve	+ve	Y	Y	+ve	-ve	1	1%
K.pneumoniae	-ve	+ve	+ve	Y	Y	+ve	-ve	9	9%

Key: R: red, Y: yellow, +ve: positive, -ve: negative

Table 2: biochemical properties of Gram positive bacteria isolated from patients with urinary tract infection

Isolated bacteria	Catalase	Bile solubility hydrolysis	Mannitol fermentation	DNase	No. of isolates and %	
S. aureus	+ve	-ve	+ve	+ve	1	1%
E. faecalis	-ve	+ve	-ve	-ve	8	8%
S.epidermidis	+ve	-ve	-ve	-ve	1	1%

Key: +ve: positive, -ve: negative

4. 6. Antibacterial Susceptibility tests

The antibacterial susceptibility test of clinical isolates and standard organisms were determined using standard disk diffusion method. The results showed that 81.4% of *E. coli* isolates were resistant to Ciprofloxacin, 62.9% to Gentamycine, 10% to Imipenem, 71.4% to Co-trimoxazole and 75.7% to Cefatzidime. *P. aeruginosa* was resistant to Ciprofloxacin 40%, Gentamycine 40%, Imipenem 0%, Co-trimoxazole 40% and Cefatzidime 80%. *Proteus* species were resistant to Ciprofloxacin 20%, Gentamycin 20%, Imipenem 40%, Co-trimoxazole 60 %and Cefatzidime60 %. *K. pneumoniae* was resistant to Ciprofloxacin 22.2%, Gentamycin 22.2%, Imipenem 22.2%, Co-trimoxazole 55.6% and Cefatzidime 66.7%. *E. faecalis* resistant to Ciprofloxacin 37.5%, Gentamycin 37.5%, Imipenem 37.5%, Co-trimoxazole 25% and Cefatzidime 50%.

Table 3: Comparison the activity of *A. indica* leaves methanolic extract with activity of commonly used antimicrobial against clinical isolates

Bacterial isolates	Ciprofloxacin 5 mg		Gentamycin 10 mg		Cotrimoxazole 25 mg		Ceftazidime 30 mg		Imipenem 10 mg		T	Methanolic extract concentrations (%w/v)					
	S	R	S	R	S	R	S	R	S	R	%	50	25	12.5	6.25	3.125	1.5
E. coli	13 18.6%	57 81.4%	26 37.1%	44 62.9%	20 28.6%	50 71.4%	17 24.3%	53 75.7%	63 90%	7 10%	70	17.5 ±1 A	15.5 ±1 A	13.5 ±0.5 A	12 ±0.8 P.A	10 ±0.5 P.A	N.A
K. pneumonae	7 77.8%	2 22.2%	7 77.8 %	2 22.2%	4 44.4%	5 55.6%	3 33.3%	6 66.7%	7 77.8%	2 22.2%	9	19 ±1.6 V.A	17 ±2 A	15.4 ±2 A	13.5 ±1.8 A	11 ±1.9 P.A	N.A
Citrobacter spp	1 100%	-	1 100 %	-	-	1 100%	-	1 100%	1 100%	-	1	22 ±1 V.A	20 ±1.5 V.A	18 ±0.5 A	16 ±1.1 A	11 ±0.8 P.A	N.A
E. feacalis	5 62.5%	3 37.5%	5 62.5%	3 37.5%	6 75%	2 25%	4 50%	4 50%	5 62.5%	3 37.5%	8	16.5 ±1.8 A	14.5 ±2.2 A	13 ±2.1 A	9 ±1.6 N.A	8 ±1.9 N.A	N.A
P. aeruginosa	3 60%	2 40%	3 60%	2 40%	3 60%	2 40%	1 20%	4 80%	5 100%	-	5	21 ±1 V.A	19 ±0.8 V.A	17 ±1.3 A	16 ±1.5 A	13 ±1.8 A	N.A
Proteus spp	4 80%	1 20%	4 805	1 20%	2 40%	3 60%	2 40%	3 60%	3 60%	2 40%	5	17.5 ±1.8 A	15 ±1.6 A	11 ±1.2 P.A	9 ±0.9 N.A	8 ±0.9 N.A	N.A
S. epidermidis	-	1 100%	1 100%	-	1 100%	-	1 100%	-	1 100%	-	1	15 ±1.1 A	14 ±1.5 A	13 ±1.2 A	12 ±0.5 P.A	8 ±0.5 N.A	N.A
S. aureus	-	1 100%	-	1 100%	1 100%	-	1 100%	-	1 100%	-	1	13 ±0.5 A	11 ±0.8 P.A	10 ±0.7 P.A	9 ±0.4 N.A	8 ±0.5 N.A	N.A
Total &%	33 35.3%	67 64.7%	47 45.1%	53 54.9%	37 37.3%	63 62.7%	29 29.4%	71 70.6%	86 84.3%	14 15.7%	10 0	-	-	-	-	-	-

Table 4: Comparison the activity of *A. indica* leaves methanolic extract with activity of commonly used antimicrobial against control strain

Bacterial isolates	Ciprofloxacin 5mg		Gentamycin 10mg		Cotrimoxazole 25mg		Ceftazidime 30mg		Imipenem 10mg		T	Methanolic extract concentrations (% w/v)					
	S	R	S	R	S	R	S	R	S	R	%	50	25	12.5	6.25	3.125	1.5
E.coli ATCC	1 100%	-	1 100%	-	1 100%	-	1 100%	-	1 100%	-	1	24 ±0.4 V.A	22 ±1 V.A	20 ±1 V.A	-	-	NA
S. aureus ATCC	1 100%	-	1 100%	-	1 100%	-	1 100%	-	1 100%	-	1	22 ±0.8 V.A	20 ±1.7 V.A	18 ±2 A	17 ±2 A	13 ±0.5 A	NA

Key: S: Sensitive, R: Resistant, T: Total, A: Active, P.A: Partial active, V.A: Very active.

Zones of inhibition in millimeters

Ciprofloxacin: Resistant < or = 15 mm Sensitive > 21

Gentamycin: Resistant < or = 14 mm Sensitive > 17

Cotrimoxazole: Resistant < or = 10 mm Sensitive > 16

Ceftazidime: Resistant < or = 17 mm Sensitive > 21

Imipenem: Resistant < or = 19 mm Sensitive > 23

Not active: <9 mm, Partial active: 9-12 mm, Active: 1-18 mm, Active: 1-18 mm

Very active > 18mm.

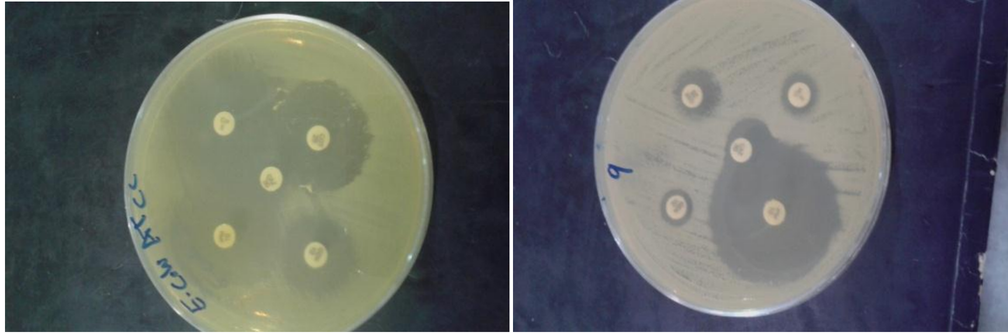

Fig. 5: Antimicrobial susceptibility of *E.coli* ATCC 25922on left and at right *E. coli* MDR to Gentamycin, Cotrimoxazole, Cefatzidime, Imipenem and Ciprofloxacin.

4. 7. Antimicrobial activity of *A. indica*

In this study methanolic extract of *A.indica* showed antimicrobial activity against strains of *S. aureus*, *P. aeruginosa*, MDR *E. coli*, *Proteus* spp, *S. epidermidis, Citrobacter* spp, *K. pneumonia, E. feacalis, S.aureus* ATCC 25923 and *E. coli* ATCC 25922. Results were expressed as mean ±SD. The statistical significance was established at $P < 0.05$.

4. 8. Interpretation of results

After 24 hours incubation antibacterial activity result were expressed in diameters of inhibition zones were measured in millimeters <9mm zones was considered as inactive; 9-12mm as partially active while 13-18 mm as active and >18 mm as very active (Mukhtar and Ghori, 2012).

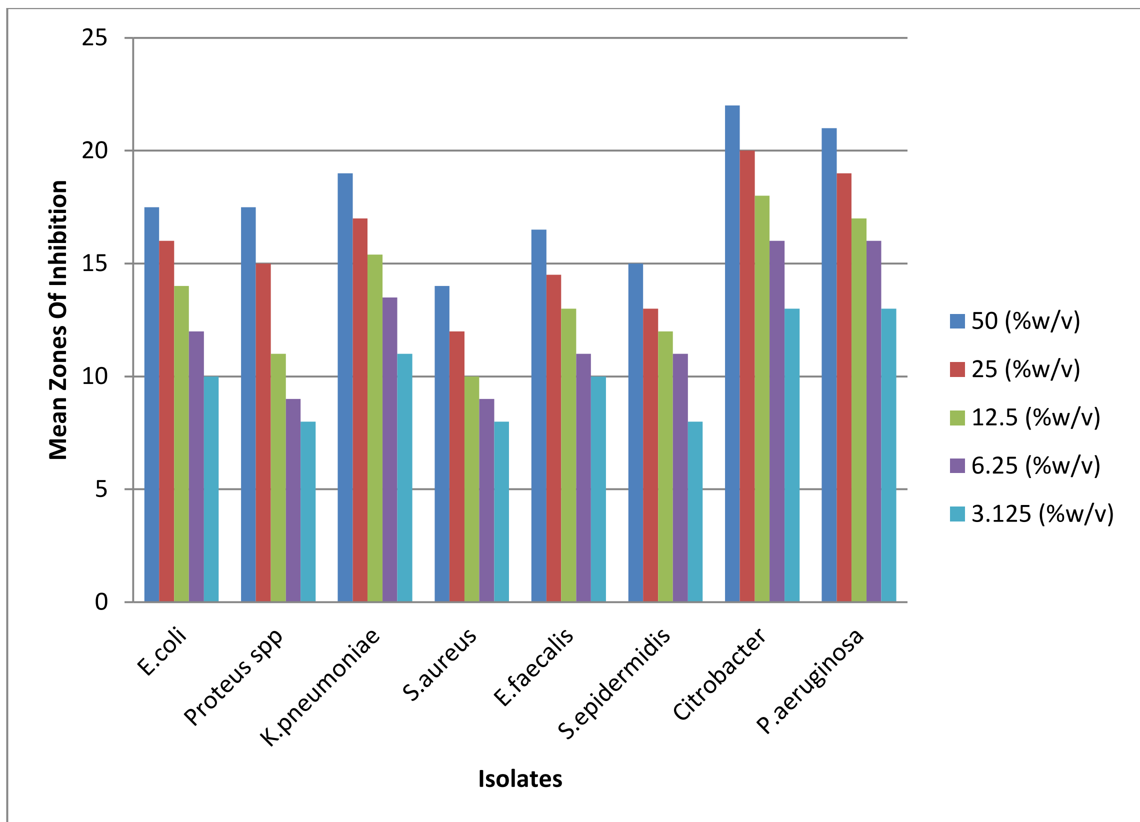

Fig. 6: Mean of inhibition zones after in vitro exposure of isolates to *A.indica* methanol extract in different concentrations (diameter in millimeter).

4. 9. Minimum Inhibitory Concentration (MIC) of *A.indica* methanolic extract obtained by agar diffusion method

Table 5: Showed (MIC) of *A. indica* leaves methanolic extract

Bacteria species	Methanolic extract
E. coli	3.125mg/ml
P. aeruginosa	3.125 mg/ml
Proteus spp.	3.125 mg/ml
K. pneumoniae	3.125 mg/ml
E. faecalis	3.125 mg/ml
S. aureus	3.125 mg/ml
S. epidermidis	3.125 mg/ml
Citrobacter spp.	3.125 mg/ml
E. coli ATCC	12.5 mg/ml
S. aureus ATCC	3.125 mg/ml

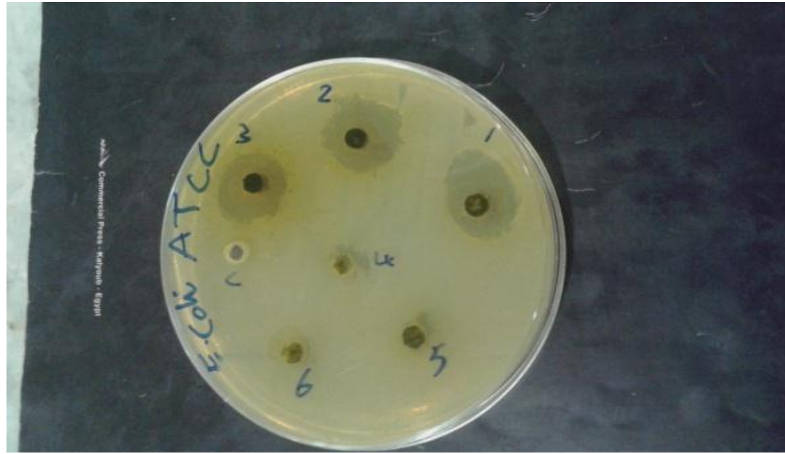

Fig. 7: Activity of *A. indica* leaves methanolic extract on *E. coli* ATCC 25922 with concentrations 1= 50 (%w/v), 2= 25 (%w/v), 3= 12.5 (%w/v), 4= 6.25 (%w/v)

5= 3.125 (%w/v) and C= control methanol

Fig. 8: Activity of *A.indica* leaves methanolic extract on left MDR *E. coli* and on right *P. aeruginosa* with concentrations 50 (%w/v), 25 (%w/v), 12.5(%w/v), 6.25(%w/v) and 3.125(%w/v) from 1 to 5 respectively and control methanol at C whole.

4.10. Gas chromatography results

GC-MS Chromatography of methanol leaves extract of *A. indica* clearly showed 45 peaks indicating the presence of 45 phytochemical compounds, the information about 45 compounds are mention in Appendix 1 and 29 of them showed antibacterial activity.

Table 6: Active antibacterial compounds in methanolic extract of Neem

NO	Active ingredient compounds	%
1	Arabino-Hex-1-enitol,1, 5-anhydro-2-deoxy	15.55
2	1, 3-Propanediol, 2-(hydroxymethyl)-2-nitro-	10.34
3	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	8.55
4	n-Hexadecanoic acid	7.75
5	.beta,-D-Glucopyranose ,1, 6-anhydro-	6.17
6	Phytol	4.06
7	4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy	3.89
8	Dihydroxyacetone	3.38
9	Benzoofuran, 2, 3-dihydro-	3.06
10	Eicosyne	2.38
11	Hydroxymethylfurfural	2.03
12	Dodecanol	1.88
13	9, 12, 15, Octadecanoic acid,(z, z, z)-	1.76
14	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	1.58
15	1, 2, 3-Propanetriol,1-acetate	1.41
16	Furan, 2, 5-dimethyl-	1.33
17	2-Methoxy-4-vinylphenol	1.29
18	Dodeccanoic acid	1.23
19	5-Pyrimidinol, 2-methyl-4-(methylthio)-	1.22
20	4-Methyl-2, 5-dimethoxybenzaldehyde	1.18
21	4-(H)-Pyridine, N-acetyl-	1.09
22	Phenol, 2, 6-dimethoxy-	1.07
23	Tetradecanoic acid	0.96
24	Octadecanoic acid	0.82
25	2, 5-Dimethyl-4-hydroxy-3(2H)-furanone	0.66
26	Pentanedioic acid, 3, 3-dimethyl-, monomethylester	0.65
27	Pentanoic acid, 4-oxo-	0.65
28	Octadecanal	0.64
29	Hexadecanoic acid, methyl ester	0.48

CHAPTER FIVE

5.1 DISCUSSION

Among this study urinary tract infection was more in females (60%) than males (40%). Women are more prone to UTIs than men because in females, the urethra is much shorter and closer to the anus than males (Jawetz and Melnick *et al.,* 1995). The female genital tract is closely related to the bladder and this relationship makes the spread of disease possible from one tract to the other (Epstein, 2010). The most frequently isolated bacteria was *E. coli* 70%, this may be due to that *E. coli* found as normal flora in intestinal tract, skin and vagina, However under predisposing condition they can multiply rapidly and are capable of adhering to uroepithelial cell, producing infection (Kunin, 1987 and Clarridge *et al.,* 1987). This study in agreement with studies of Irving *et al*. (2006) and Kebira *et al*. (2009), whom reported that urinary tract infection was more in females and most of infections are caused by *E. coli*. *E. coli* showed high rate of resistance to antibiotic used in this study, this in agreement with report of WHO (2015), that reported resistance to urinary antibiotic most common in *E. coli*, also in agreement with Niranjan and Malini (2013) whom reported that 76.51% of *E.coli* isolated from patients with UTI is resistant. The result showed that Ceftazidime had high resistant rates 70.6% followed by Ciprofloxacin 64.7%, Cotrimoxazole 62.7%, Gentamycine 54.9% and Imipenem 15.7%, increasing rate of resistant of this antibiotics that due to overuse and misuse of these medication, as well as a lack of new drug developed by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements (Gould and Bal, 2013; Centers for Disease Control and Prevention, 2013; Viswanathan, 2014 and Michael *et al*., 2014). On other

hand Imipenem showed high sensitivity rate 84.3%, may be due to that there was not commonly used.

A. indica leaves methanolic extract used in this study had shown antimicrobial effect as presented in different tables and graphs used in results interpretation, the effect was shown on all tested bacteria. For all extracts (50 (% w/v), 25 (% w/v),12.5 (% w/v), 6.25 (% w/v), and 3.125 (% w/v)) used during this study, the bactericidal activity increased with the increase of the extract concentration, this means the inhibition zones was higher on plates that contain extract with low dilution factor, this is also observed in the report done by Esimone *et al*. (1988), whom reported that extract of plants inhibit the growth of various microorganisms at different concentrations. This is similar to the results recorded in this study where the increase in the concentration of extracts corresponded to the increase of diameter of inhibition zones exception 100 (% w/v), did not showed zone of inhibition this may be due to that it was more concentrated and contain small amount of water that help in diffusion and uptake of effective compounds and some compounds were its more concentrated didn't produced any effect. The study in agreement with Okemo *et al.* (2001) whom reported that crude of Neem plant was very effective against *S. aureus* and *E. coli*. They found that an extract of concentration 50 (% w/v), has significant reduce growth of *S. aureus* after 24 hrs incubation, while extract increasing concentration completely wiped out viable bacteria in lesser time. The results are similar to a study that carried out by Gajendrasinh *et al*. (2012), whom showed that *E. coli* was the most susceptible bacterium to Neem leaf extract and not similar to a study carried out by Uwimbabazi *et al*. (2015) that showed *E. coli* resistant to all extracts of *A. indica* used, but effective against *S. aureus* and disagree with Yehia *et al*. (2011) whom reported that Chloroform and methanol extracts were high effective against *P. vulgaris* and *Micrococcus luteus* and a lesser

extent on *E. coli*, *K. pneumoniae*, *E. faecalis* and *Streptococcus fecalis*. *A.indica* leaves methanolic extract is more effective against gram negative bacteria than gram positive bacteria may be because gram negative bacteria cell wall lacks Peptidoglycan and contain more lipopolysaccharideand lipoprotein (Levinson, 2012). The antimicrobial of *A. indica* leaves extract has been evaluated *in vitro* against isolates and standard organisms (*E. coli* ATCC 25922 and *S. aureus* ATCC 25923). The Study revealed that methanolic extract of Neem leaves performance inhibition of bacterial growth with MIC 3.125 (% w/v). Moreover the results showed that the *E. coli* multidrug resistant was susceptible to methanolic extract with inhibition zone 17.5±1mm and MIC 3.125 (% w/v). These principles are actually the defensive mechanism plant against different pathogens (Hafiza, 2000).

Furthermore phytochemical compounds of *A.indica* (Neem) methanolic leaves extract was determined by gas chromatography showed 45 compounds, 29 of them contain antibacterial constituents (phyto-constituents alkaloids, glycosides flavonoid, phenolic compounds, steroids, triterpenoids, cartenoides and tetra-triterpenoids azadirachtin) (Koona and Budida, 2011). There is study on Neem phytochemical compounds conducted by Akpuaka *et al.* (2013) in Pakistan that showed 45 compounds with minimum different with the study compounds may be the content varies considerably due to environmental, genetic factors and climates.

5.2. Conclusions

A.indica possesses high antibacterial activities against pathogenic bacteria (*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis*, *S. aureus*, *S. epidermidis*, *Proteus* spp and *Citrobacter* spp, that cause UTI in human), and standard organisms (*E. coli* ATCC 25922 and *S. aureus* ATCC 25923). The more effective concentration was 50 (% w/v), zone of inhibition was increased with the increase of concentration of extracts ($P<0.05$). Gram negative bacteria were more susceptible to *A. indica* leaves methanolic extract than Gram positive bacteria. MIC of methanolic extract of isolates and standard organisms range from (3.125 (%w/v)-12.5 (%w/v)). Gas chromatography analysis of *A. indica* methanol leaves extract showed 45 compounds, 29 of them have antibacterial activity.

5.3. Recommendations

1-Further work in *A. indica* from different locations and different parts of plant.

2-Study of antibacterial activity and determination of MIC of *A. indica* by using different solvents should be done.

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

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APPENDICES

Appendix 1: Compound information of *A. indica leaf methanol* extract analysis by Gas chromatography

1/Arabino-Hex-1-enitol, 1, 5-anhydro-2-deoxy

Formula: $C_6H_{10}O_4$ CAS: 26566-29-0 Mol Weight: 146 RetIndex: 1371

Compound name: Arabino-Hex-1-enitol, 1, 5-anhydro-2-deoxy-$$ Glucal $$1, 5-Anhydro-2-deoxyhex-1-enitol # $$.

2/ Benzene, Propyl-

Formula: C_9H_{12} CAS: 103-65-1 Mol Weight: 120 RetIndex: 992

Compound name: Benzene, Propyl- $$n-Propylbenzene $$ Isocumene $$ Propylbenzene $$1-Phenylpropane $$1-Phenylpropane $$UN2364.

3/Benzoofuran, 2, 3-dihydro-

Formula: C_8H_8O CAS: 496-16-2 Mol Weight: 120 RetIndex: 1036

Compound name: Benzoofuran, 2, 3-dihydro-$$ Coumaran $$ Dihydrobenzofuran $$ Kumaran $$2, 3-Dihydro.

4/.beta.-d Mannofuranoside, O-geranyl

Formula: $C_{17}H_{32}O_{26}$ CAS: 0-00-0 Mol Weight: 232 RetIndex: 2651

Compound name: .beta.-d Mannofuranoside, 1-O-(10-undecenyl)-$$10-Undecenyl hexofuranoside # $$.

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

Formula: C6H10O CAS: 498-07-7 Mol Weight: 162 RetIndex: 1404

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

6/cis-Vaccenic acid

Formula: C18H34O2 CAS: 506-17-2 Mol Weight: 282 RetIndex: 2175

Compound name: cis-Vaccenic acid$$11-Octadecenoic acid, (Z)-$$(Z)-11-Octadecenoic acid$$.

7/Cyclopentane, 1-acetyl-1, 2-epoxy-

Formula: C7H10O2 CAS: 15121-02-5 Mol Weight: 126 RetIndex: 920

Compound name: Cyclopentane, 1-acetyl-1, 2-epoxy-$$1-(6-Oxabicyclo[3. 1. 0]hex-1-yl) ethanone# $$.

8/Cyclopropyl carbinol

Formula: C4H8O CAS: 2516-33-8 Mol Weight: 72 RetIndex: 664

Compound name: Cyclopropyl carbinol $$ Hydroxymethylcyclopropane $$ Cyclopropanemeththanol $$ Cyclopropylmethanol $$ Cyclopropanemethyl $$.

9/3-Deoxy-d-mannonic acid

Formula: C6H12O6 CAS: 0-00-0 Mol Weight: 180 RetIndex: 1704 Compound name: Deoxy-d-mannonic acid $$ 3-Deoxyhexonic acid# $$.

10/1.2-diacetylhydrazine

Formula: C4H8N2O2 CAS: 3148-73-0 Mol Weight: 116 RetIndex: 1219

Compound name: 1.2-diacetylhydrazine $$ N, N-'Diacetylhydrazine $$1, 2-Diacetylhydrazine $$ Acetic acid, acetylhydrazide $$ Hydrazime, 1, 2, diacetyl-$$ NS.

11/Dihydroxyacetone

Formula: C3H6O3 CAS: 96-26-4 Mol Weight: 90 RetIndex: 941

Compound name: Dihydroxyacetone $$ 2-Propanone, 1, 3-dihydroxy-$$ Chromelin $$ Dihyxal $$ Otan $$ Oxantin $$ Oxatone $$ Soleal $$ Triulose $$ Viticolo.

12/2, 5-Dimethyl-4-hydroxy-3(2H)-furanone

Formula: C6H8O3 CAS: 3658-77-3 Mol Weight: 128 RetIndex: 1022

Compound name: 2, 5-Dimethyl-4-hydroxy-3 (2H)-furanone $$ 3(2H)-Furanone, 4-2, 5-dimethyl- $$ 3(2H)-Furanone, 2, 5-dimethyl-4-hydroxy-$$Alleton.

13/1-Dodecanol

Formula: C12H256O5 CAS: 112-53-8 Mol Weight: 186 RetIndex: 1457

Compound name: 1-Dodecanol $$ Dodecyl alcohol $$ n-Dodecan-1-ol $$

n-Dodecanol $$ n-Dodecyl alcohol $$ AlcoholC-12 $$ Dodecanol $$.

14/Dodeccanoic acid

Formula: $C12H24O2$ CAS: 143-07-7 MolWeight: 200 RetIndex: 1570

Compound name: Dodeccanoic acid $$ n-Dodecanoic acid $$ Neo-fat12 $$ Aliphat no.4 $$ ABL$$ Dodecyclic acid $$ Lauric acid $$ Laurostearic acid $$ Neo fat.

15/Eicosyne

Formula: C20H38 CAS: 71899 Mol Weight: 278 RetIndex: 2027

Compound name: Eicosyne $$ 9-Icosyne# $$.

16/ 4-((1E)-3-Hydroxy-1-Propenyl)-2-methoxy

Formula: C10H12O3 CAS: 0-00-0 Mol Weight: 180 RetIndex: 1653

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

17/Furan, 2,5-dimethyl-

Formula: C6H8O CAS: 625-86-5 Mol Weight: 96 RetIndex: 732

Compound name: Furan, 2, 5-dimethyl- $$ 2, 5-Dimethyl furan $$2, 5-Dimethylfurane $$.

18/2-Furanmethanol

Formula: C5H6O2 CAS: 98-00-0 Mol Weight: 98 RetIndex: 885

Compound name: 2-Furanmethanol $$ Furfyl alcohol $$ alpha-Furfury l alcohol $$ alpha-Furylcarbinol $$ Furfuralcohol $$ Furylcarbinol $$.

19/.gamma.-Elemene

Formula: C15H24 CAS: 29873-99-2 Mol Weight: 204 RetIndex: 1431

Compound name: .gamma.-Elemene $$1-Methyi-2-(1-methylethenyl)-1-vinylcyclohexane), (1R-trans) - $$ (-)-gamma-Elemene $$ o-Mer.

20/Hexanoic acid

Formula: C6H12O2 CAS: 142-62-1 Mol Weight: 116 ReIndex: 97

Compound name: Hexanoic acid $$ Caproic acid $$ n-Caproic acid $$ n-Hexanoic acid $$ n-Hexoic acid $$ n-Hexylic acid $$ Butylacetic acid $$ Capronic acid.

21/ 2-Hexadecene, 3, 7, 11, 15-tetramethyl-[R[R*, R*-(E)]]-

Formula: C20H40 CAS: 14237-73-1 Mol Weight: 280 RetIndex: 1802

Compound name: 2-Hexadecene, 3, 7, 11, 15-tetramethyl-[R[R*, R*-(E) -[]$$ (2E)-3, 7, 11, 15-Tetramethyl-2-hexadecene # $$.

22/Hexadecanoic acid, methyl ester

Formula: C17H34O2 CAS: 112-39-0 Mol Weight: 270 RetIndex: 1878

Compound name: Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester $$ n-Hexadecanoic acid methyl ester $$ Metholene 2216 $$ Methyl hexadecanoic $$.

23/Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester

Formula: C19H38O4 CAS: 23470 Mol Weight: 330 RetIndex: 2498 Compound name: Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester $$ Palmitin, 2-mono-$$ Palmitic acid. beta.-monoglyceride $$ 2-Hexadecanoyl $$.

24/4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy

Formula: C6H8O4 CAS: 28564-83-2 Mol Weight: 144 RetIndex: 1269

Compound name: 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-$$ 3, 5-Dihydroxy -6-methyl1-2, 3-dihydro-4H-pyran-4-one $$ 2, 3-dihydro-3, 5-dihydroxy-.

25/ 5-Hydroxymethylfurfural

Formula: C6H6O3 CAS: 67-47-0 Mol Weight: 126 RetIndex: 1163

Compound name: 5-Hydroxymethylfurfural$$2-Furancarboxaldehyde, 5(hydroxymethyl)- $$ 2-Furaldhyde, 5-(hydroxymethyl)-$$ HMF $$ 5-(Hydroxymethyl).

26/4-(H)-Pyridine, N-acetyl-

Formula: C7H9NO CAS: 67402-83-9 Mol Weight: 123 ReIndex: 1032

Compound name: (H)-Pyridine, N-acetyl-$$ 1-Acetyl-1, 4-dihydropyridine #$$.

27/Maleic anhydride

Formula: C4H2O3 CAS: 108-31-6 Mol Weight: 98 RetIndex: 979

Compound name: Maleic anhydride $$2, 5-furandione $$ cis-Butenedioic anhydride $$ Dihydro-2, 5dioxofuran $$ Maleic acid anhydride $$Toxilic anhydride $.

28/2-Methoxy-4-vinylphenol

Formula: C9H10O2 CAS: 7786-61-0 Mol Weight: 150 RetIndex: 1293

Compound name: 2-Methoxy-4-vinylphenol $$ Phenol, 4-ethyenyl-2methoxy -$$ Phenol, 2-methoxy-4vinyl- $$ 4-Hydroxy-3-methoxystyrene $$ P-Vinylguaiacol

29/4-(4-Methyl-piperazin-1-yl)-1, 5, -dihydro- imidiazol-2-one

Formula: C8H14N4O CAS:0-00-0 Mol Weight: 182 RetIndex: 1598 Compound name: 4-(4-Methyl-piperazin-1-yl)-1, 5, -dihydro-imidiazol-2-one $$ 4-(4-Methyl-1-piperazinyl)-1, 5-dihydro-2H-imidazol-2-one# $$.

30/4-Methyl-2, 5-dimethoxybenzaldehyde

Formula: C10H12O3 CAS: 4925-88-6 Mol Weight: 180 RetIndex: 1473

Compound name: 4-Methyl-2, 5-dimethoxybenzaldehyde$$2, 5-Dimethoxy-4-methylbenzaldhehyde # $$.

31/ n-Hexadecanoic acid

Formula: C16H32O2 CAS: 57-10-3 Mol Weight: 256 RetIndex: 1968

Compound name: n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid $$ Pentadecanecarboxylic acid $$ 1-Pentadecanecarboxylic.

32/ 1, 2, 3, 4, 5, 6, 7, 8-Octahydro-2-naphthol, 4-methylene-2, 5, 5-trimethyl-$$

Formula: C6H8N2OS CAS: 35231-61-9 Mol Weight: 156 RetIndex: 1366

Compound name: 1, 2, 3, 4, 5, 6, 7, 8-Octahydro-2-naphthol, 4- methylene-2, 5, 5-trimethyl- $$ 2, 5, 5-Trimethyl-4-methylene-1, 2, 3, 4, 5, 6, 7, 8-octahydro-2naphthalenol $$.

33/Octadecanoic acid

Formula: C18H36O2 CAS: 57-11-4 Mol Weight: 284 RetIndex: 2167

Compound name: Octadecanoic acid $$ Stearic acid $$ n-Octadeconic acid $$ Humko Industrene R $$ HystreneS-97 $$ Hystrene T-70 $$.

34/Octadecanal

Formula: C18H36O CAS: 638-66-4 Mol Weight: 268 RetIndex: 1999

Compound name: Octadecanal $$ Stearaldehyde $$ Octaddecyl aldehyde $$ Stearyl aldehyde $$ n-Octadecanal $$.

35/ 9, 12, 15, Octadecanoic acid, (z, z, z)-

Formula: C18H30O2 CAS: 463-40-1 Mol Weight: 278 RetIndex: 2191
Compound name: 9, 12, 15, Octadecanoic acid, (z, z, z) -$$ Linolenic acid $$ All-cis-9, 12, 15-Octadecatrieonic acid $$ cis, cis, cis-9, 12 $$.

36/Pentanoic acid,4-oxo-

Formula: C5H8O3 CAS: 123-76-2 Mol Weight: 116

Compound name: Pentanoic acid, 4-oxo-$$ Levulinic $$.beta-Acetylpropionic acid $$.gamma-Ketovaleric acid $$ Levnlic acid $$.

37/ Pentanedioic acid, 3, 3-dimethyl-, monomethylester

Formula: $C_8H_{14}O_4$ CAS: 27151-66-2 Mol Weight: 174 RetIndex: 1256

Compound name: Pentanedioic acid, 3, 3-dimethyl-, monomethylester $$ 5-Methoxy-3, 3-dimethyl-5-oxopentanoic acid# $$.

38/Phenol, 2, 6-dimethoxy-

Formula: $C_8H_{10}O_3$ CAS: 91-10-1 Mol Weight: 154 RetIndex: 1279 Compound name: Phenol, 2, 6-dimethoxy-$$ 2, 6-dimethoxy-$$ Pyrogallol 1, 3-dimethyl ether $$ Syringol $$ 1, 3Dimethoxy-2-hydroxybenzene $$ 2-Hydroxy-1, 3-dimethoxybenzer

39/Phytol, acetate

Formula: $C_{22}H_{42}O_2$ CAS: 0-00-0 Mol Weight: 338 RetIndex: 2168

Compound name: Phytol, acetate

40/Phytol

Formula: $C_{20}H_{40}O$ CAS: 150-86-7 Mol Weight: 296 RetIndex: 2045

Compound name: Phytol $$ 2-Hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-,[R[R*, R*-(E)]] - $$ trans-phytol $$ 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol-, (2E, 7R, F).

41/1, 2, 3-Propanetriol, 1-acetate

Formula: $C_5H_{10}O_4$ CAS: 106-61-6 Mol Weight: 134 RetIndex: 1091

Compound name: 1, 2, 3-Propanetriol, 1-acetate $$ Acetin, 1-mono-$$ .alpha-Monoacetin $$ Glycerol. Alpha-monoacetate $$1-Monoacetin $$ 2, 3-Dihydroxy.

42/1, 3-Propanediol, 2-(hydroxymethyl)-2-nitro-

Formula: $C_4H_9NO_5$ CAS: 126-11-4 Mol Weight: 151 RetIndex: 1444

Compound name: 1, 3-Propanediol, 2-(hydroxymethyl)-2-nitro- $$ Isobutylglycerol, nitro- $$ Methane, trimethylnitro- $$ Nitroisobutylglycerol $$ Nitrotris.

43/ 5-Pyrimidinol, 2-methyl-4-(methylthio)-

Formula: $C_6H_8N_2OS$ CAS: 35231-61-9 Mol Weight: 156 RetIndex: 1366

Compound name: 5-Pyrimidinol, 2-methyl-4-(methylthio)-

$$ 2-Methyl-4-(methylsulfanyl)-5-pyrimidinol# $$.

44/Tetradecanoic acid

Formula: $C_{14}H_{28}O_2$ CAS: 544-63-8 Mol Weight: 288 RetIndex: 1769 Compound name: Tetradecanoic acid $$ Myristic acid $$ n-Tetradecanoic acid $$ n-Tetradecoicacid $$ Neo-fat14 $$ UnivolU316 $$ 1-Triecanecarboxylic.

45/3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol

Formula: $C_{20}H_{40}O$ CAS: 102608-53-7 Mol Weight: 296 RetIndex: 2045

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

Appendix 2:

Fig. 9: Collection of *A. indica* and drying in shadow and ground by using mortar and pestle.

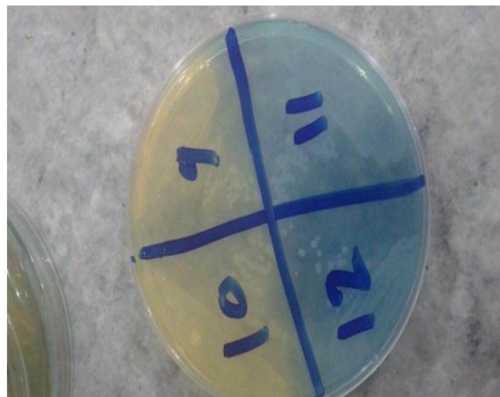

Fig. 10: Growth of bacteria on CLED; yellow color indicate lactose ferment while blue color indicate non- lactose ferment.

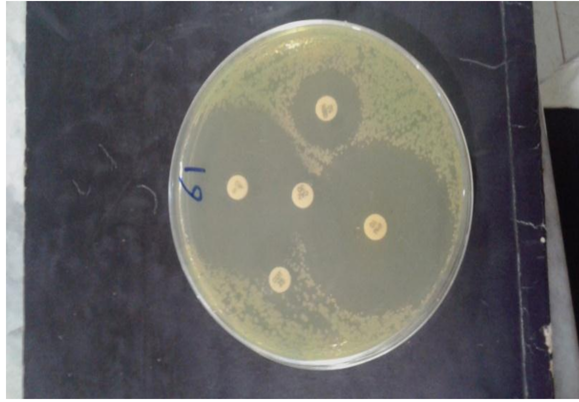

Fig. 11: Antimicrobial susceptibility of *P. aeruginosa* to Gentamycin, Cotrimoxazole, Imipenem, Cefatzidime and Ciprofloxacin.

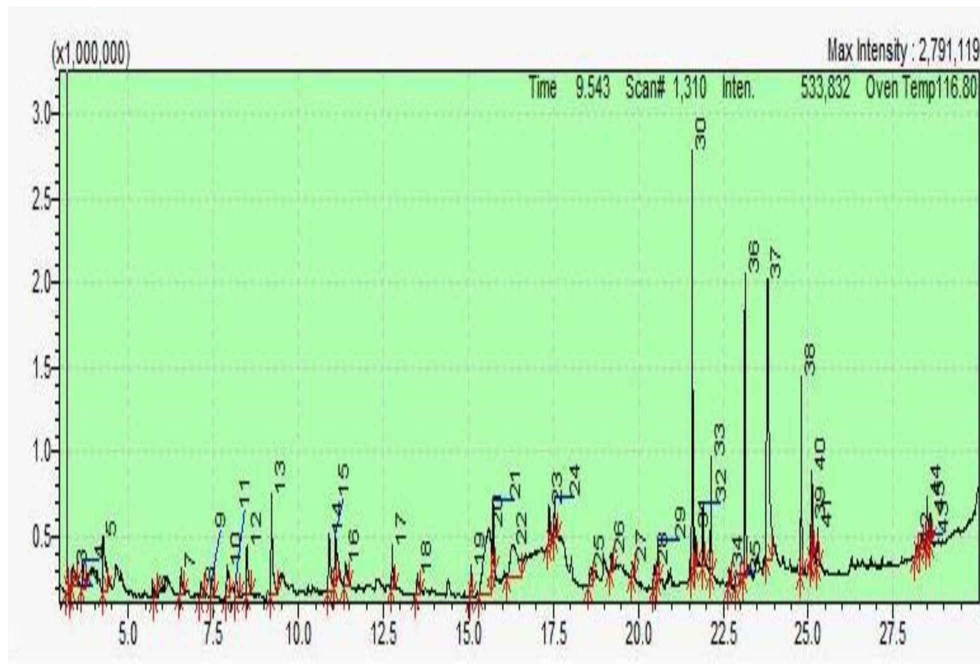

Fig. 12: G C-MS Chromatogram of methanol extract of A.indica leaves clearly

showed 45 peaks.

Appendix 3

Table 7: The weight and yield of *A. indica* extract by methanol extraction method

Weight of sample	Weight of extract	Yield%
50 g	9.396 g	18.792%

Table 8: Gas chromatography analysis of *A. indica* leaves methanolic extract

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	3.187	61715	0.12	1.2-diacetylhydrazine
2	3.291	91928	0.18	Maleic anhydride
3	3.360	697490	1.33	Furan, 2,5-dimethyl-
4	3.642	520048	0.99	2-Furanmethanol
5	4.250	1771223	3.38	Dihydroxyacetone
6	5.777	87602	0.17	Hexanoic acid
7	6.546	568075	1.09	4(H)-Pyridine, N-acetyl-
8	7.145	337651	0.65	Pentanoic acid, 4-oxo-
9	7.438	345518	0.66	2,5-Dimethyl-4-hydroxy-3(2H)-furanone
10	7.898	921764	1.76	Cyclopentane, 1-acetyl-1,2-epoxy-
11	8.160	400511	0.77	Cyclopropyl carbinol
12	8.482	989759	1.89	4-(4-Methyl-piperazin-1-yl)-1,5,-dihydro-in
13	9.205	2024228	3.87	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydrox
14	10.898	1602819	3.06	Benzofuran, 2,3-dihydro-
15	11.083	1063793	2.03	5-Hydroxymethylfurfural
16	11.383	736824	1.41	1,2,3-Propanetriol, 1-acetate
17	12.758	672886	1.29	2-Methoxy-4-vinylphenol
18	13.504	558988	1.07	Phenol, 2,6-dimethoxy-
19	15.077	390898	0.75	.gamma.-Elemene
20	15.595	5413061	10.34	1,3-Propanediol, 2-(hydroxymethyl)-2-nitr
21	15.729	984166	1.88	1-Dodecanol
22	16.305	3231797	6.17	.beta.-D-Glucopyranose, 1,6-anhydro-
23	17.374	643550	1.23	Dodecanoic acid
24	17.543	615544	1.18	4-Methyl-2,5-dimethoxybenzaldehyde
25	18.583	639901	1.22	5-Pyrimidinol, 2-methyl-4-(methylthio)-
26	19.208	229739	0.44	1,2,3,4,5,6,7,8-Octahydro-2-naphthol, 4-me
27	19.833	240291	0.46	3-Deoxy-d-mannonic acid
28	20.468	601874	1.15	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxy
29	20.571	502505	0.96	Tetradecanoic acid
30	21.587	4477277	8.55	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
31	21.671	482805	0.92	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[
32	21.908	711383	1.36	Phytol, acetate
33	22.139	1245731	2.38	9-Eicosyne
34	22.681	252396	0.48	Hexadecanoic acid, methyl ester
35	22.890	472612	0.90	.beta.-d-Mannofuranoside, O-geranyl
36	23.139	4054321	7.75	n-Hexadecanoic acid
37	23.806	8138363	15.55	Arabino-Hex-1-enitol, 1,5-anhydro-2-deoxy
38	24.796	2126530	4.06	Phytol
39	25.085	359002	0.69	cis-Vaccenic acid
40	25.110	922046	1.76	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
41	25.279	427690	0.82	Octadecanoic acid
42	28.188	224616	0.43	Benzene, propyl-
43	28.318	338933	0.65	Pentanedioic acid, 3,3-dimethyl-, monomet
44	28.499	826555	1.58	Hexadecanoic acid, 2-hydroxy-1-(hydroxym
45	28.604	332762	0.64	Octadecanal
		52339170	100.00	

Questionnaire

Sudan University of Sciences and Technology

College of Graduate Studies

The Antimicrobial Activity of *Azadirachta Indica* (Neem) Against Bacteria Isolated from Urinary Tract Infected Patients in Khartoum State

Questionnaire for requirement of MSc degree

Date ……………………………………………………………….…………

Place of sample collection………………………………………………...

ID number…………………………………………………………………

Ward……………………………………………………………………….

Sex………………………………………………………………………..

Age……………………………………………………………………….

Type of Specimen…………………………………………………………

Isolated Bacteria …………………………………………………………

Comment on antimicrobial susceptibility testing

Sensitive to……………………………………………………………….

Intermediate to…………………………………………………………...

Resistant to………………………………………………………………