Sudan University of science and Technology

College of Graduate Studies

In vitro Antimicrobial Activity of Methanolic Extract of *Cymbopogon schoenanthus* against Bacteria Isolated from Urinary Tract Infected Patients in Khartoum

دراسة فعالية مستخلص نبات المحريب كمضاد للجراثيم ضد البكتيريا المعزولة من عدوى المسالك البولية من مرضى في ولاية الخرطوم

A Dissertation Submitted In Partial Fulfillment For The Requirement of Master Degree In Medical Laboratory Science (Microbiology)

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الايـــــــه

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(قَالَ رَبِّ اشْرَحْ لِي صَدْرِي (25) وَيَسِّرْ لِي أَمْرِي (26) وَاحْلُلْ عُقْدَةً مِنْ لِسَانِي (27) يَفْقَهُوا قَوْلِي (28))

صدق الله العظيم

(سورة طه)

DEDICATION

To

My father

My mother

My brothers

My sisters

And to all my dear friends

AKNOWLEDGMENT

First of all grateful **ALLAH** great blessing. My sincere thanks and gratitude go to my Supervisor **Dr: Wafa Ibrahim Elhag** for her advices, interest and leadership throughout this study. Deep thanks to my colleagues **Mohammed Awad** and **Ahmed Hamid** and staff of Microbiology Department in Sudan university of Science and Technology for their help and patience.

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Abstract

Medicinal plants have been widely used in folk medicine. They are considered today as an interesting source of new drug due to their bioactive components. The genus, *Cymbopogon schenanthus (*Poaceae). is an aromatic plant seems important source of several bioactive compound use in traditional medicine .

This was descriptive and cross sectional study conducted during the period from June to October 2018, to determine the antimicrobial activity of *Cymbopogon schoenanthus* methanolic extract against both Gram positive and Gram negative bacteria isolated from urinary tract infected patients .

A total of 70 urine samples were collected from patients with Urinary tract infection symptoms . These specimens were inoculated onto Cystine Lactose Electrolyte Deficiency (CLED) media . The significant growth was observed and identified using conventional microbiological methods. The antibiotic susceptibility testing was performed using standard disk diffusion method. Then inhibitory effects of methanolic extracts of *Cymbopogon schoenanthus* were evaluated against fifty isolates of both Gram-positive and Gram-negative bacteria and two reference strains *(E.coli* ATCC 25922 *and S.aureus* ATCC 25923) using the agar well diffusion and dilution methods. The minimum inhibitory concentration (MIC) was assayed using the Broth microdilution test method.

Out 70 urine samples collected 68 (97%) revealed significant bacterial growth. The identified species were *E.coli* (20, 29.4%), *P.mirabilis* (14 , 20.5%) , *S.aureus* (10 , 14.6%) , , *Enterobacter spp* (9 , 13.2%) ,*K.pneumoniae* (8 , 11.7%), *and Ps. aeruoginosa (* 7 , 10%) .

The antibiotic susceptibility testing results showed that Cotrimoxazole had higher resistant rates (64.7%) followed by Ceftazidime (42.6%), nitroforuntoin (42.5%), Ciprofloxacin (23.5%), Gentamycin (22.1%) and Imipenem (5.3 %).

Regarding multidrug resistance the results showed 50(73.5%) of isolated bacteria were multidrug resistance strains, out of the (73.5%) (26%) were *E.coli* higher resistant rate followed by (22%) *P.mirabilis* .

Cymbopogon schoenanthus methanolic extract was effective against different isolates and reference strains , the most effective concentration is 100% followed 50% with equal activity against Gram positive and Gram negative bacteria . Minimum inhibitory concentration of *Cymbopogon schoenanthus* methanolic extract for tested bacteria was ranged from 12.5-1.56 (% w/v) .

The results of the present study suggest a scientifically traditional use of *Cymbopogon schoenanthus* as an antibacterial agent. Future studies are needed to investigate and explore its application in the environmental and medical fields. Also carry out more pharmacological and toxicological studies to assess their therapeutic efficiency and potential for commercial utilizations .

مستخلص الاطروحة

النباتات الطبية تستخدم على نطاق واسع في الطب الشعبي و تعتبر اليوم مصدرا مهما في صنع الادوية الجديدة نظرا لمكوناتها النشطة بيولوجيا. جنس الاذخرالمكي (النجيلية) من النباتات العطرية و يعتبرمصدرا مهما للمكونات البيولوجية المستخدمة في الطب التقليدي .

اجريت هٰذِه الدراسة المقطعية الوصفية و التجريبية في الفترة من يونيو حتى اكتوبر 2018 في ولاية الخرطوم لدراسة نشاط المستخلص الميثانولي لنبات المحريب على انواع مختلفه من البكتريا المعزولة من مرضى عدوى المسالك البولية .

تم جمع 70 عينة بول من مرضى مصابين بعدوى المسالك البولية . تم تزريع هذِه العينات في وسط ازراع بمزرعة سيستين اللاكتوز ناقصة لشحنات . لوحظ نموا واضحا للبكتريا و تم التعرف عليها بإستخدام الإختبارات البيوكيميائية التقليدية .

تم قياس حساسية البكتريا المعزولة لبعض المضادات الحيوية للبكتريا بواسطة طريقة الإنتشار الطبقي القياسي .كما تم إختبارفعالية مستخلص نبات المحريب على خمسين بكتريا معزولة موجبة الجرام وسالبة الجرام و البكتريتان المرجعيتان (الاشريكية القولونية ATCC 25922و المكورات العنقودية الذهبية ATCC25923) بواسطة طريقة انتشار الاغار الطبقي والتخفيف . و تم إختبارالتركيز المثبط الادنى بإستخدام طريقة تخفيف الاغار التسلسلي .

من 70 عينة بول 68 (97%) اعطت نموا واضحا في ا لأوساط الإستزراعية حيث كانت الانواع التي تم التعرف عليها الإشريكية القولونية(20 ,29.4%) , المتقلبة الرائعة (14, 20.5%) , المكورات العنقودية الذهبية (10, 14.6%) , انواع المعوية (9 , 13.2%) الكلبسيلة الرئوية (8 , 11.7%) و الزائفة الزنجارية (7 , 10%) .

وجدت الدراسة ان الكوترايموكزازول 64.7% لديه اعلى درجة مقاومة يليه السيفتازيديم 42.6% , نيتروفورونتين 42.5%,سيبروفلوكساسين 23.5%,جنتامايسين 22.1%والإمبنيم 5.3%.

فيما يتعلق بمقاومة الادوية المتعددة اظهرت النتائج ان 50(73.5%) من البكتريا المعزولة كانت من سلالات مقاومة الادوية المتعددة , من (73.5%) (26%) كانت للإشريكية القولونية اعلى درجة مقاومة , تليها المتقلبة الرائعة (22%) .

مستخلص نبات المحريب كان له فعالية على البكتريا الممرضة المقاومة للادوية المتعددة والبكتريا المرجعية. التركيز 100(w/v%) كان الأكثر فعالية يليه التركيز 50 (w/v%) . بفعالية متساوية على البكتريا سالبة الغرام والبكتريا موجبة الغرام . التركيز المثبط الادنى لمستخلص نبات المحريب للبكتريا المختبرة يتراوح بين 1.56 -12.5(w/v%).

نتائج هذه الدراسة تقترح علميا إستخدام المحريب كمضاد للبكتريا . هناك حاجة لعمل دراسات مستقبلية لتاكيد و إستكشاف استخداماته في المجالات الطبية و البيئية , بالاضافة لإجراء عدة دراسات دوائية و سمية لتقييم الفعالية العلاجية والاستخدامات التجارية الممكنة.

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1. INTRODUCTION

1.1. Introduction

Medicinal plants have been widely used in traditional medicine for several centuries for the treatment of many health-related ailments (Gasal *et al*., 2017). According to the World Health Organization (WHO), about 80% of people worldwide are currently depending on traditional medicine for their primary health care needs (khalil *et al*., 2017). Herbal drugs have found wide spread use in many countries because they are easily, available, cheaper and safer than synthetics drug (Retnam and De -Britto, 2007). *Cymbopogon schoenanthus* is one important, popular, odoriferous plant, well known in indigenous medicine in Sudan and Egypt. It is a perennial herb known locally as 'Mahareb' or 'Hamareb' in Egypt as 'Halfa Bar' and in Saudi Arabia known as 'Al-Ethker'. It is wildly distributed in northern and central regions of the Sudan and used as dried herb in the Herbalist Market. It is used locally as tea for treatment of digestive ailment and as flavoring compound. It is effective as renal antispasmodic and diuretic agent (Boulos, 1983). Also it is use as a protection against fever, anti-malarial, and anti-helminthic (especially against Guinea worms) (Ahmed *et al.*, 2018), and it was shown to possess sedative, digestive and anti-parasitic properties (Sousa *et al*., 2005). Norbert and Seth (2014) demonstrated that it is an antifungal and anti inflammatory agent used for the prevention and treatment of acute inflammatory skin conditions. In Saudi traditional medicine, it is mainly used as a diuretic to inhibit kidney stone formation and as an anti-infectious agent in urinary tract infections (Al-Ghamdi *et al.*, 2007).

Urinary tract infection (UTI) is the second most common infectious presenting in community practice. Worldwide, about 150 million people are

diagnosed with UTI each year (Gonzalez and Schaeffer, 1999). Almost 95 % of cases of UTIs are caused by bacteria (Bishop *et al*., 2007).

Urinary tract infection causing bacteria become more resistant to available antibiotics, there is urgent to explore new strategies for managing UTIs (Foxman, 2003) Antimicrobial resistance is a major and increasing global problem ,The first important factor in increasing microbial resistance is improper use of antibiotics (Frère and Rigali 2016). The other is incorrect and unreasonable antibiotics prescription (Soleymani, 2013). 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). 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) as UTIs causing bacteria become more resistant to available antibiotics , the need to explore new strategies for managing UTIs is clear ,this led to increase urgency for new intervention with availability , low cost , more effectiveness as antibacterial and aware about medicinal plants and their therapeutic potential against pathogenic bacteria (Foxman , 2010).

1.2. Rationale

The extensive uses of antimicrobial agents have invariably resulted in the development of antibiotic resistance, which has become a major problem worldwide (Kumar *et al.,* 2006). The most common UTI causing bacteria become more resistant to available antibiotics, this phenomenon led to explore new strategies to managing UTI and find novel alternatives (Foxman, 2010). Medicinal plants could be suitable alternative solution because they are effective, available, with affordable cost and minimal toxicity. The *Cymbopogon schoenanthus* Camel's hay (Mahareb) is an important, popular, odoriferous plant, well known in indigenous medicine in Sudan and Egypt. It is used locally as tea for treatment of digestive ailment and as flavouring compound. It is effective as renal antispasmodic and diuretic agent (Boulos, 1983). And the previous studies reported that the *Cymbopogon schoenanthus* has antibacterial effect.(Ahmed *et al.* , 2018).

To verify the claimed activity of this plant use to treat urinary tract infections, this study was designed to answer this question.

1.3. Objectives

1.3.1. General objective

To detect antibacterial activity of methanolic extract of *Cymbopogen schoenanthus* against bacterial strain isolated from urinary tract infected patient in Khartoum state during June to October (2018).

1.3.2. Specific objectives

1. To isolate and identify bacteria causing urinary tract infection

2. To assess the antimicrobial activity of commonly use antibiotics against UTI pathogens.

3. To determine the antimicrobial activity and Minimum Inhibition Concentration (MIC)of Methanolic extract of *Cymbopogons choenanthus* against bacterial isolates

4. To compare susceptibility of UTs clinical isolates with *Cymbopogon schoenanthus* methanolic extract with and commonly used antibiotics.

2. LITERATURE REVIEW

2.1. Medicinal plants

Plants have been utilized as a source of medicine for thousands of years and continue to play an important role globally in primary health care, mostly in developing countries (Balunas , 2005). The use of medicinal plants is increasing because people believe that they are safe for human consumption. There is also an increase in infectious diseases worldwide caused by both drug resistance and lack of sufficient affordable medicine for people living in poor communities. The discovery of drugs from medicinal plants may be one of the solutions in the fight against infectious diseases. The World Health Organization estimates that about 80% of the people rely almost exclusively on traditional medicine for their primary healthcare needs. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Maryam *et al*., 2011). According to the World Health Organization (WHO, 1978) "a medicinal plant" is any plant, which in one or more of its organ contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs. herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Mahesh., 2008).

Herbs have medicinal property due to presence of different active principles like alkaloids, volatile essential oils, glycosides, resins, oleoresins, steroids, tannins, terpenes and phenols (Magda *et al*., 2017). The medicinal properties of plants could be based on the antioxidant, antimicrobial and antipyretic effects of the phytochemicals in them (Adesokan et *al*., 2008). In Africa and other developing countries, these traditional medicines derived from plants have continued to form the basis of rural medical care. This is due to the fact that this medicine are easy to get and available in cheap prices (Mohamed, 2016). Sudanese medicinal plants have been reported to exert antimicrobial activity against viruses, bacteria and protozoa, encourage further more research , with more benefits for patient been safe and effective as antibacterial with consider of drug resistance strains (Khalid *et al.*, 2012) . The great diversity of Sudan's flora and unique geographical position has been suitable for survival and establishment of many valuable medicinal and aromatic plants. For instance, *Cymbopogon* species which belong to the family Poaceae (Graminae), it comprises about 180 species, sub species, varieties and sub varieties. It is native to warm temperate, tropical regions of the Old World and Oceania (Ivan *et al* ., 2017).

2.2. *Cymbopogon schoenanthus*

2.2.1. Scientific classification

Kingdom: Plantae .

Phylum: Magnoliophyta.

Class: Liliopsida.

Order: Poales.

Family: Poaceae.

Genus: Cymbopogon.

Species: Cymbopogon schoenanthus (L.) Spreng. (Blanco, (2009).

Synonym is *Cymbopogon proximu* , Camel hay .

2.3 Description

Cymbopogon schoenanthus is a herbal plant. Common name is camel's hay and is known locally as (Maharaib). It is a perennial herb, erect, tufted 9 cm long, culms slender, glabrous and 3 – 4 nodes. Leaf simple, alternate, linear 5-7 cm long, 1cm wide, sheathed apex spiny entire, and inflorescence spikelets highly branched 5 cm log (Eltahir and Abue reish, 2010).

2.4 Origin and distribution

The plant is widely distributed in Africa (northwest tropical, northeast tropical and east tropical), temperate Asia (western Asia and Arabia) and tropical Asia (Indian and Indo-China). In addition, *Cymbopogon schoenanthus* is found in the northern and Central Sudan (Clayton *et al*., 2005).

In the Sudan camel's hay is found in Red Sea State at sea coast, Wadi El Omari, Kassala State at Gallabat, Matamma, Khartoum State at Jebel Royan, Omdurman and Soba, Blue Nile, Kordofan State at Jebel Abu Sunun and Darfur State at East of El Fasher and Kutme (Broun and Massey , 1979). Banthorpe *et al*, (1976) reported that camel's hay plant was collected from different habitats such as Khartoum State at Merkhyat which has sand rock desert, Blue Nile State at Abu Gulfa which has arid clay plain and Jebel Abbel that has savannah, loam, Kordofan State at Jebel Kone which has sand dunes soil and Nuba Mountain with sandy loam soil.

2.5. Phytochemistry

The enormous information gathered from the ethno-pharmacological applications of *Cymbopogons* begged the investigation of its chemical constituents. These studies have led to the isolation of alkaloids, volatile and non-volatile terpenoids, flavonoids, carotenoids and tannins from every part of these plants (Opeyemi *et al* ., 2015)

2.6. The uses of *C.schoenanthus* in folk medicines

C.schoenanthus plant is used in traditional medicine prepared as tea, decoction, infusion or fumes. Decoction of the lower part of the plant is used for treating colic and fever. Infusion of the leaves is used to treat Stomach trouble and lower the blood pressure. Infusion of the flowers serves as a febrifuge. The infusion of the plant is used as diuretic, sudorific (induces sweating), emmengogue (aids menstruation), astringent, carminative (relieves flatulence), antirheumatic and cataplasm (compress) for wounds of Camels. The dried tufts of the plant, when burnt a fume inhaled, treats influenza and some neurotic diseases (Boulous, 1983).

El-Kamali and El-Amir (2010) mentioned that 'Mahareb' plant is used to treat constipation, intestinal complaints and as an appetizer. Ethnobotanic studies, carried out by Millago *et al.*, (1997) showed that this plant has been used in traditional pharmacoepia in Burkina Faso to treat the cough of infants and children. Also the plant showed sedative, digestive and perfumed properties with strong characteristic aroma (Sousa *et al.,* 2005). The herb is used as flavouring constituent in traditional Sudanese foods and drinks. (Khadri *et al.*, 2011) mentioned that the herb is consumed in salad and used to prepare traditional meat recipes in Tunisia. It is also used as a carminative, anthelmintic diaphoretic and to healing gout and prostate

diseases . An important use in Sudan is as an ingredient in the preparation of a traditional drink known as 'Hilomour' (Abdalla 2000).

In Saudi traditional medicine, it is mainly used as a diuretic to inhibit kidney stone formation and as an. anti-infectious agent in urinary tract infections (Al-Ghamdi *et al*., 2007)

2.7 Pharmacological values of *Cymbopogon schoenanthus*

Previous studies concerning biological activities showed that *Cymbopogon schoenanthus* oil exhibits insecticidal (Ketoh *et al*., 2006, Bassoule *et al*., 2003). antitrypanosomal (Khadri *et al.*, 2011), El-Kamali and El-Amir (2010) reported that ethanol extract of *Cymbopogon schoenanthus* showed relatively higher propensity to act on Gram-positive bacteria. EL- Fadul (2004) reported that aqueous extract of 'Mahareb' plant gave negative results of both Gram-positive bacteria and Gram-negative bacteria. Hashim and co-workers (Hashim *et al*., 2016) have demonstrated that *C. schoenanthus* essential oils represent an inhibitory effect against *S.aureus* methicillin sensitive (MSSA), *S. aureus* (MRSA), *Escherichia coli* and *Klebsiella pneumonia*, Khadri *et al.*, (2011) reported that the essential oil of *Cymbopogon schoenanthus* has antioxidant and acetylcholine esterase inhibitory activities, Radwan (1975) reported that the compound extracted from un saponifiable matter fraction of the petroleum ether from *Cymbopogon schoenanthus* is a bicyclic sesquiterpenediol called proximadiol or cryptmeridiol (C_{15} $H_{28}O_2$), which is responsible for the antispasmodic activity and used for the propulsion of renal and ureteric calculi. On the other hand, clinical, pathological, hematological and biochemical studies carried out by Ahmed (2000) investigated that the oral administration of the essential oil of *Cymbopogon proximus* in doses of 0.25, 0.5 and 0.1 ml/kg/day caused difficult breathing,

ruffled hair and nervous signs in the New Zealand rabbits. Lesions on both goats and rabbits were congestion of liver, heart, kidney and intestine. Also Al-Ghamdi *et al.*, (2007) demonstrated that camel's hay plant act as inhibitor of calcium oxalate nephrotoxicity. Moreover optimization studies showed that this plant has a high diuretic activity**.**

2.8. Urinary tract infections (UTI)

(UTI) is an infection that begins in the urinary system. It is the second most common disease after respiratory infection. The urinary tract consists of the kidneys, ureters, bladder and the urethra (Amit *et al.*, 2012) 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. Many other bacteria can also cause an infection for example: *Klebsiella* species, *Pseudomonas* species, *Enterobacter* species, *Proteus* species, *Mycoplasma* species, *Chlamydia* species, *Serratia marcescens* and *Neisseria* species.

Staphylococcus saprophyticus is related to sexual active women. *Proteus mirabilis* and *klebsiella* species are often multiply antibiotic-resistant. *Enterococcus faecalis, Pseudomon asaeruginosa* and *Staphylococcus aureus* are cause infection especially after catheterization or instrumentation. Acute uncomplicated UTI is usually due to one type of organism and chronic infection is often associated with more than one type of organism (Sleigh and Timbury, 1998).

2.9. Risk factors of Urinary tract infection (UTI)

There are a number of factors that increase the risk of developing urinary tract infection. Some of these are: sex, age, pregnancy, catheterization,

kidney stones, tumours, urethral strictures, neurological diseases, congenital / acquired anomalies of bladder, vesico-ureteric reflux, suppressed immune system, diabetes mellitus, enlarged prostate , ureteric stresses, etc.(Sklar *et al.,* 1987). Women are more susceptible than men, due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora. Additionally, the physiological increase in plasma volume during pregnancy decreases urine concentration and up to 70% pregnant women develop glucosuria ,encourages bacterial growth in the urine (Gulfareen *et al* ., 2010). Catheterization is major predisposing and infects the urinary tract factor for UTIs; during insertion of the catheter bacteria may be carried directly into the bladder. Most urinary tract pathogens originate in the fecal flora but only aerobic and facultative species such as Escherichia coli possess the attributes required to colonize in patients with various diseases, the incidence of urinary tract infection is 20% for diabetes mellitus,14% for hypertension, 80% for hydronephrosis and nephrolithiasis and greater than 50% for long term indwelling catheters. 25% of pregnant women with asymptomatic bacteriuria go onto develop acute pyelonephritis (Muhammad *et al* ,2004).

2.10. Common bacteria cause UTI

2.10.1. *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* causes 60-90% of urinary tract infection .It is one of the two important causes of neonatal meningitis and the agent most frequently associated with “traveller’s diarrhoea, a watery diarrhoea. (Cheesbrough, 2006).

2.10.2. *Klebsiella pneumonia* .

Gram-negative and non motile usually capsulated rods cause UTIs in Hospital patients. Antigenic analysis for capsular polysaccharide reveals that more than 80 serotype are recognized (Cheesbrough, 2009).
They grow well on ordinary media, with colonies which are often, but not always, large and mucoid (Sleigh and Timbury, 1998).

2.10.3. *Proteus species*

Gram negative pleomorphic rods, motile non capsulated they grow on selective enteric media (Cheesbrough, 2009). *Proteus mirabilis* is main *proteus* species of medical importance. It causes urinary infection commonly in the elderly and young male often following catheterization or cytoscopy. Also *Proteus* causes septicaemia and occasionally meningitis and chest infections (Cheesbrough, 2006).

2. 10.4. *Pseudomonas aeruginosa (P.aeruginosa)*

P. aeruginosa are Gram negative rods, 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, *P. aeruginosa* cause Skin infections, Septicemia, urinary tract infection, respiratory tract infection and eye infection (Cheesbrough, 2006).

2. 10.5. *Citrobacter* specie

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

2. 10.6. *Enterococcus faecalis* (*E. faecalis)*

E. feacalis are gram-positive cocci, 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 , and occasionally endocarditis or meningitis. It is a normal commensal of the vagina and intestinal tract (Cheesbrough, 2006).

2. 10. 7. *Staphylococcus aureus (S. aureus)*

S. aureus are Gram positive cocci grow well aerobically and in a carbon dioxide enriched atmosphere, but less well. Temperature range for growth is 10–42 ºC, with an optimum of 35–37 ºC. *S. aureus* causes boils, pustules, impetigo, infections of wounds, ulcers, burns, osteomyelitis, mastitis, septicaemia, meningitis, pneumonia and pleural empyema. Food-poisoning(rapid onset, no fever), toxic shock syndrome and toxic skin exfoliation(Cheesbrough, 2006).

2.10.8. *Staphylococcus saprophyticus*

Gram positive cocci of uniform size occurring in groups but also singly and pairs. They are non-capsulated. and non- motile , *S. saprophyticus* cause UTIs in sexually active women. The surface agglutinins of this pathogen determinant of the virulence promoting it colonizes urinary tract (Collee *et al*, 1996).

2.10.9. *Serratia marcescens:*

It has been reported to cause UTIs, and it is gram-negative rods, facultative anaerobe and it is resistant to cephalosporin (Cheesbrough, 2009).

2. 11. 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 led to emergence of resistance among the various strains of microorganisms. Multidrug resistance (MDR) is defined as insensitivity or resistance of microorganism to the administrated of antimicrobial medicine despite earlier sensitivity to it (Popeda and Pluciennik., 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 (WHO, 2014).

2.12. Classification of MDR Pathogens

2.12.1. Primary Resistance

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

2.12.2. 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). It may further be classified as follows:

2.12.2.1. 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).

2.12.2.2. 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 (Marks and Flood , 2014).

2.12.3 .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).

2.13. Back ground studies about antimicrobial activity of *Cymbopogon schoenanthus* against urinary tract causes bacteria

Previous study carried out by Mohamed *et al.,* (2016) in Egypt among 15 plant against UTI bacterial isolates , highest antibacterial activity was exhibit by cymbopogon shoenanthus ethanolic extract against all isolated bacteria *S.aureus , E.coli , K.pneumoniae , p .aeruoginosa* . inhibitory zones ranged from (24.6- 10mm).

In Sudia Arabia Khalil *et al.***,** (2017). Carried out study to evaluating the

antimicrobial activities of methanolic and aqueous of *Cymbopogon schoenanthus* on several pathogenic bacteria, fungi and virus. Antibacterial and antifungal activities were evaluated using the agar well diffusion

methods. The results showed that methanol extract of *C. schoenanthus* exhibit an antibacterial effect on several Gram-positive Gram bacteria (*E. faecalis, S. saprophyticus* ATCC 49907, *S. saprophyticus* isolate, *S. aureus* ATCC 25923, *S. aureus* and *S. pyogenes* isolate) and negative bacteria (*E. coli , Ps. aeruginosa , K.pneumonia , P. mirabilis and S. Paratyphi* B). All extracts tested (aqueous extract, and methanol extract) were found to have an antiviral effect on HSV1; whereas, no antifungal effect was detected on both *Candida albicans* and *Aspergillus nige r.*

The largest inhibition zones were exhibited by Gram negative *Salmonella paratyphi B* isolate (22±1 mm) followed by *P. mirabilis* isolate (20.67±0.58mm) and Gram positive *S. aureus* isolate (19.3±0.58 mm). In Saudi Arabia Gasal *et al.*,(2016). reported results about inhibitory effects of water extracts of C. *schoenanthus* against ten isolates of both Gram positive and Gram-negative bacteria using the agar well diffusion and dilution methods. Results: The *C. schoenanthus* extract was effective against *Escherichia coli, Staphylococcus aureus, methicillin-sensitive* (MSSA*) S. aureus* (MRSA) *and Klebsiella pneumonae* . But not effective against *Staphylococcus saprophyticus*.

In Sudan test recorded by Ahmed *et al.*, (2018) against six *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923. high activity reported against both Gram-positive, Gram-negative bacteria and standard microorganism , results showed effect of *C .schoenanthus* extract against *Aspergillus nige r* ATCC 1015 , *Candida albicans* ATCC 7596, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 53657, the two tested fungus however no activity observed against *Pseudomonas aeruginosa.*

The largest inhibition zones were exhibited by *E coli* ATCC 25922, (78mm)

3. MATERIALS AND METHODS

3.1. Study design

This was descriptive and cross sectional study.

3.2. Study area

This study was conducted in El faysial Hospital in Khartoum state .

3.3. Study duration

This study was conducted in period from June to October 2018.

3.4. Study population

Patients with Urinary Tract Infections.

3.5. Inclusion criteria

Patients admitted to hospital with urinary tract infections signs and symptoms.

3.6. Exclusion criteria

Patients with UTIs symptoms but starting antibiotics.

3.7. Sample size

Seventy patients randomly enrolled in this study.

3.8. Data were collection tool

Data was collected from hospital records.

3.9. 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.

3. 10. Experimental work:

3.10.1. 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. The specimens were inoculated under aseptic condition using standard loop on cysteine lysine electrolyte deficient (CLED) media (HI Media laboratories _ india) . The inoculated media were incubated aerobically at 37℃ for 18-24 hrs. then examined for growth.

3.10.2. Macro and microscopic examination

Macroscopic examination was done to detect the colour 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. urine samples were then centrifuged and the deposit was tested for the presence of pus cell, red blood cell, and yeast cell, (Cheesbrough, 2006).

3.10.3. Identification of the clinical isolates:

The growth was observed, significant growth more than 10^5 CFU/ml was identified by standard microbiological procedures including the following steps:

3.10.3.1. Colonial morphology

Colonial morphology used as first identification steps focusing on colony

Size, colour , edge and fermentation of lactose in CLED agar .

3.10 .3.2. 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 examined microscopically by oil immersion lens (x100)

3. 10.3. 3. Biochemical tests

According to Gram results, suitable biochemical tests were selected for identification of pathogens.

3.11. Identification of Gram positive cocci:

3.11.1. Catalase test

The test was carried out as describe by Barrow and Feltham (1993). 0.5ml of 3% H_2O_2 was placed on clean tubes, and one colony of the tested culture from Nutrient Agar (HI Media - india) was picked with a wooden stick and added to the tubes. A positive reaction was indicated by production of air bubbles.

3.11.2. Coagulase test

The test was used to identify *S.aureus* which was coagulase positive from other Staphylococci species which were coagulase negative. Coagulase causes plasma to clot by converting fibrinogen to fibrin. On clean slide place drop of distilled water and emulsify colony of tested organism then add loop full of plasma on the suspensions and mixed gently the results was clumping of organisms within 10 seconds (Cheesbrough, 2006).

3.11.3. DNAase test

The test was used to differentiate *S.aureus* (positive) from other Staphylococci species (negative). The tested organism was culture on a medium which contain DNA (HI Media – India), after overnight incubation the colonies were tested by flooding the plate with a weak hydrochloric acid (HCl). The acid precipitates un hydrolysed DNA. DNase produced colonies were surrounded by clear area indicating DNA hydrolysis (Cheesbrough, 2006).

3.11.4. Mannitol fermentation test

This medium was used to differentiate S.aureus from other Staphylococci species. A portion of colony was inoculated on mannitol salt agar (HI Media – India) containing 75 g\l sodium chloride and incubated aerobically at 37ºC for 18-24 hrs. Fermenting mannitol: medium turns yellow (Cheesbrough, 2006).

3.12. identification of Gram negative rod:

3.12.1. Oxidase test

The technique was described by Barrow and Feltham (1993). Strips of filter paper was soaked in 1% solution of tetra methyl-p-phenylenediamine dihydrochloride and dried in hot air oven and then placed on clean glass slide bacterial colony by sterile glass rod and rubbed on filter paper strip. If purple colour developed with 5-10 seconds, the reaction was considered positive.

3.12.2. Indole production test

the tested organism was cultured on to peptone water broth media (HI Media – India). indole production is detected by kovac‘s reagent which contain 4-dimethylaminobenzaldehyde. If there is pink ring the result was indicated positive. If there is no pink ring in surface the result indicated negative (Cheesbrough, 2006).

3.10.3. Citrate utilization test

In this test the organism has ability to use citrate as only source of carbon. By straight wire the tested organism was cultured in Kosser‟s citrate medium (HI Media – India) contain sodium citrate, ammonia salt and indicator bromo-thymol blue showing turbidity ,incubated for 24 hours in 37℃. The change in colour of the indicator from green to blue was considered as positive no change indicate Negative (Cheesbrough, 2006).

3.10.4. Urease test

In the test organism produce urease enzyme break down urea and produce ammonia, which make pH media alkaline, in the presence of phenol red as indicter. The tested organism was inoculated in the christensen's urea agar (HI Media – India) and incubated for 24 hours in 37$_o$C. Positive: pink color. Negative: no change (Cheesbrough, 2006).

3.12.5. Motility test

Motility is used for the detection of motility of gram-negative enteric bacilli. Semisolid agar (HI Media – India) use for the detection of bacterial motility. Bacterial motility can be observed directly from examination of the tubes following incubation. Growth spreads out from the line of inoculation if the organism is motile. Highly motile organisms provide

growth throughout the tube. Growth of non motile organisms only occurs along the stab line. (Murray, *et al*., 2007) .

3.12.6. Kligler Iron Agar test (KIA)

The test based on the ability of bacteria to fragment sugar (lactose and glucose), production of gas and H_2S. Under aseptic condition KIA (HI media – India) was incubated with organism under test by using straight wire loop. Then incubated at 37°C for overnight. Then change in color crack and production of H_2S was observed (Cheesbrough, 2006).

3.13. Antimicrobial susceptibility testing

3.13.1. 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 standards (Cheesbrough, 2006). Inoculated on to Muller Hinton agar (Hi Media – india), The following antibiotic disks were used: Ceftazidime (30 mcg), Imipenem (10mcg), Gentamycin (10mcg), Cotrimoxazole (25mcg) Nitrofurantoin (30 mcg) and Ciprofloxacin (5mcg). Plates were left at room temperature to dry and incubated at 37℃ for 18-24 hrs. Zones of inhibition were measured in mm and the result was interpreted according to standardized chart.

3. 14. Quality control

3. 14. 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. 14. 2. Control susceptibility testing method

The quality control strain *S. aureus* ATCC 25923 and *E. coli* ATCC25922 were 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. 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 test.

3.15 Reading and interpretation of antimicrobial susceptibility testing

The diameter of each zone of inhibition (including the diameter of the disk) was measured to nearest millimeter using ruler. Zones interpreted according to CLSI interpretation chart . The susceptibility of isolates was reported according to manufacture standard zone size interpretative manual. Sensitive organisms were when the zone of inhibition was equal or greater than the standard (Jan ., 2016).

3.16. Preparation of *C.schoenanthus* methanolic extract:

Extraction was carried out according to method described by Sukhdev *et. al.* (2008): 150 gm of plant sample was coarsely powdered using mortar and pestle. Coarsely sample was extracted by soaking 80 % methanol for about five days with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extract of each parts combined together. The yield percentages were calculated as followed:

Weight of extract / weight of sample * 100

3. 17. Cup plate method

The agar well diffusion method was done on Muller Hinton Agar (MHA) medium for the assay of the antimicrobial activity of *C.schoenanthus* methanolic extracts against fifty (50) multidrug resistant isolated bacteria, 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 (8mm diameter) on MHA medium. 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)). Under aseptic conditions 100 µl of three concentrations of *C.schoenanthus* extracts (100 (%w/v), 50 (%w/v), and 25(%w/v),) were introduced into the wells. The plates were allowed to stand for 1hour in the refrigerator 4 ℃ for diffusion of the extract to take place and incubated at 37 ℃ for 24 hrs. Methanol was used as negative control. Zone of inhibitions were measured (in mm) and the mean were calculated (Aneja and Joshi, 2009).

Antimicrobial activity of extract was determine depending on the study of Mukhtar and Ghori, (2012) whom reported that the diameters of inhibition zones were measured in millimeters >9mm zones was considered as inactive; 9-12mm as partially active and >13 mm as active .

3.18. Minimum inhibitory concentration (MIC) test

The MIC test was performed according to the CLSI guidelines (Gasal *et al.,* 2017) with some modifications. The tested extracts were serially diluted in nutrient broth medium. Duplicate tubes of each dilution (12.5, 6.25, 3.125, 1.65, mg/ml) were inoculated with 200 µl (5 x 10^5 CFU/ml) of appropriate bacterial suspension. Then, cultures were incubated at 37°C for 24 h. MICs were considered as the least concentration of each extract with no visible bacterial growth in terms of turbidity (Demarsh *et al.,* 2001) .

3. 19. Statistical Analysis

Data were computed and analyzed by using Statistical Package for Social Sciences (SPSS) computer software version 16.5 to check frequency, mean , and standard deviation.

4. RESULTS

Seventy patients suffering of UTI symptoms and signs were enrolled in this study. Out of the total 68 (97%) showed significant bacterial growth while the remaining 2 (3%) were negative (Figure 1).

Out of 68 isolates bacteria , *E.coli* was common isolated bacteria (20 . 29.4%) , followed by *P. mirabilis* (14 , 20.5%) , *Enterobacter* (9 , 13.2%), *S.aureus* (10 ,14.7%) , *K. pneumonia* (8 , 11.7%) and *Ps.aerogenosa* (7, 10.2%) . (Table 1).

The antibacterial susceptibility test of clinical isolates showed that most of isolated bacteria were sensitive to Imepenem (64 , 94%), followed by Gentamycin (53, 77.9%), and while most them were resistant to Co-trimoxazole (44 ,64.7%), followed by Ceftazidime (29 , 42.6%) and nitrofurantoin (29 , 42.6%). (Table 2).

Regarding multidrug resistance , 50 (73.5%) of isolates were multidrug resistance including *E. coli*(13, 26%), *Proteus mirablis* (11, 22%) *S. aureus* (8, 16%) , *Ps. aeruginosa* (7, 14%) , *K. pneumonia* (6, 12%) and *Enterobacter spp* (5, 10%) , (Table 3).

Antimicrobial activity of *Cymbopogon schoenanthus* methanolic extract was firstly screened against reference strains (*S.aureus* ATCC 25923 and *E. coli* ATCC 25922). Then tested against multidrug resistance strains with different concentrations that has been applied were 100 , 50 and 25%(w/v) The result revealed that the highest inhibition zone of methanolic extract at concentration 100 % was 20.8±2.4 mm in *E.coli* and the least inhibition zone measured 17 mm in *Ps. aeruoginosa* (Table 4).

Minimum inhibitory concentration of *C. schoenanthus* methanolic extract carried out on the isolated bacteria that showed positive results using the

agar well diffusion test., maximum growth of bacteria was achieved at 24h for the broth micro-dilution test, MIC ranged from (12.5mg\ml – 1.56 mg\ml) . (Table 5).

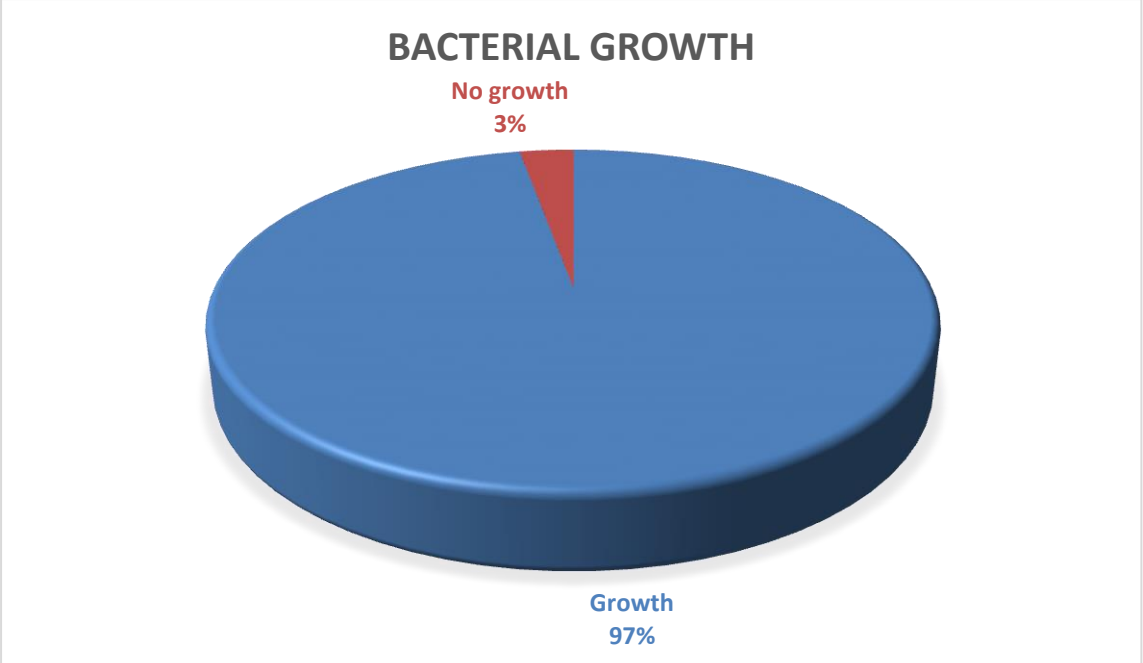

Figure 1: percentage of UTIs among study population (n=70).

Table 1. Frequency and percentage of isolated bacteria from urinary tract infected patients

Isolated bacteria	Frequency	Percentage
E.coli	20	29.4%
P. mirabilis	14	20.6%
S.aureus	10	14.7%
Enterobacter	9	13.2%
K. pneumonia	8	11.7%
Ps.aerogenosa	7	10.2%
Total	68	100%

Table(2) :Antimicrobial Susceptibility testing results of UTIs Isolates

Antibiotics	Imi		Gent		Cipr		CAZ		COT		Nitro	
Tested organisms	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
Ps. Aeruoginosa N= 7	85.7 6	14.3 1	71.4% 5	28.6% 2	71.4% 5	28.6% 2	28.6% 2	71.4% 5	28.6% 2	71.4% 5	57.1% 4	42.9% 3
K .pneumonea N=8	87.5% 7	12.5% 1	62.5% 5	37.5% 3	62.5% 5	37.5% 3	50% 4	50% 4	50% 4	50% 4	75% 6	25% 2
P .mirabilis N=14	92.8% 13	7.1% 1	78.6% 11	21.4% 3	78.6% 11	21.4% 3	78.6% 11	21.4% 3	42.9% 6	57.1% 8	35.7% 5	46.3% 9
E.coli N=20	100% 20	_ 0	90% 18	10% 2	90% 18	10% 2	70% 14	30% 6	50% 10	50% 10	75% 15	25% 5
S.aureus N=10	100% 10	_ 0	50% 5	50% 5	40% 4	60% 6	20% 2	80% 8	10% 1	90% 9	40% 4	60% 6
Enterobacter N=9	88.9% 8	11.1% 1	77.8% 7	22.2% 2	100% 9	_ 0	55.6% 5	44.4% 4	22.2% 2	77.8% 7	55.6% 5	44.4% 4
Total and%	94.1% 64	5.9% 4	77.9% 53	22.1% 15	76.5% 52	23.5% 16	57.4% 39	42.6% 29	35.3% 24	64.7% 44	57.4% 39	42.6% 29

Key : Imi : Imipenem , Gent: Gentamycin , Cip: Ciprofloxacin , CAZ : Ceftazidime , COT: Cotrimoxazole , Nitro: Nitroforuntoin

Table 3. Frequency and percentage of multi drug resistance bacteria isolated from urinary tract infections .

Isolated bacteria	Frequency	Percentage
E.coli	13	26%
P. mirabilis	11	22%
S.aureus	8	16%
Enterobacter	5	10%
K. pneumonia	6	12%
Ps.aerogenosa	7	14%
Total	50	100%

Table 4. Antibacterial activity of *C. schoenanthus* methanolic extract against different isolates

Isolated bacteria	Mean of inhibitory zone mm		
	Conc of 100 mg/ml	Conc of 50mg/ml	Conc of 25mg/ml
Escherichia coli	20.8±2.4	15.9±2.3	12.3±1.9
K.pneumonia	19.6±1.1	14.6±1.3	12.0±1.4
S.aureus	19.5±1.4	14.6±1.8	12.0±2
Ps .aeruogenosa	19.2±0.8	14.8±1.9	11.2±1.6
P.mirabilis	18.8±2	14.5±3.3	11.6±2.9
Enterobacterspp	18.8±1.8	14.8±3.1	12.0±2
E.coli ATCC 25922	30±0.0	26±04	23±0.7
S.aureus ATCC25923	20±0.0	18±0.8	14±2

Values represent means ± standard deviations of zones of inhibition. Analyzed by (SPSS version 16.0).

>13 mm : active , 9-12 mm: partial active , <9 mm : not active .

Table (5): minimum inhibitory concentration MIC) of *C . schoenanthus* methanolic extract against different isolates

Bacterial species	**MIC mg\ml**
E.coli	1.56
K.peumonia	12.5
S.aureus	3.125
P.aeruogenosa	12.5
P.mirabilis	1.56
Enterobacterspp	3.125
E.coli ATCC	1.56
S.aureusATCC	3.125

5. DISCUSSION

5.1 Discussion

Due to prescription of antibiotics without sensitivity testing microorganisms develop resistance to many antibiotics , in addition to this many of them are known to side effects , therefore there is need to screen local medicinal plants with possible antibacterial properties to find novel alternatives (Ahmed *et al.,* 2000) .

This study was carried out to evaluate antimicrobial activity of *C.schoenanthus* against selected bacteria isolated from patients with urinary tract infections. Seventy urine samples were collected (n=70) only 68 samples (97%) showed bacterial growth . the common isolated bacteria were *E. coli* (20, 29.4%), *p. mirabilis* (14 , 20.5%) and *S.aureus* (10 ,14.7%) , this results were in agreement with Agalu *et al*., (2014) whom reported the common isolated bacteria were *E. coli , Proteus mirabilis* and *S.aureus* .

The results of antibiotic showed that Co-trimoxazole had high resistant rates 64.7%, followed by Ceftazidime 42.6% and nitrofurantoin 42.6% *pseudomonas aeruogenosa* reported high rate of resistance to most antibiotics such as Co-trimexazole (71.4%), and ceftazidime (71.4%). This study results was in agreement with Bitsori *et al.,* (2011) reported that *Ps.aeruogenosa* from children UTI were resistance to ceftazidime (13.9%). Imipenem showed high activity rate against most isolated bacteria may be due to it was not commonly used yet , this study results agreed with Marchiam e*t al.,* (2008) who reported imipenem is considered the drug of choice against *E.coli, Ps .aeruginosa, Acinetobacter spp, Klebsiella spp, and Enterobacter spp.*

C. schoenanthus methanolic extract used in this study had shown antimicrobial effect on Gram positive bacteria (*S.aureus*) and Gram negative bacteria (*E.coli, K .pneumonia , P.mirabilis , Enterobacter spp, and Ps.aeruginosa* , this results agreed with Hashim *et al*., (2016) who demonstrated that *C. schoenanthus* extract represent an inhibitory effect against *S.aureus* methicillin sensitive (MSSA), *S. aureus* (MRSA), *Escherichia coli* and *Klebsiella pneumonia*, and slightly agreed with results reported by Mohamed *et al.,* (2018) whom reported Inhibitory effect against *S.aureus, E. coli and Klebsiella* but not against *Pseudomonas aeruginosa.*

For all concentration (100% (w/v), 50% (w/v) and 25% (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 by Esimone *et al.* (1988), who reported that extract of *C .schoenanthus* inhibit the growth of various microorganisms at different concentrations. where the increase in the concentration of extracts corresponded to the increase of diameter of inhibition zones.

In this study no difference was observed in sensitivity rate between Gram positive and Gram negative bacteria to *C.schoenanthus* methanolic extract, this result agreed with study done by Deans and Ritchia (1987), and disagreement of study was carried out by EL-Kamali and EL-Amir (2010) to exanimate the antibacterial activity of ethanol extracts obtained from eight Sudanese medicinal plants. In their study, scientists have demonstrated that *C .schoenanthus* showed relatively higher propensity to act on Gram- positive bacteria. Gasal *et al.,* (2017) reported that the variation might be due to many factors including the method of extraction, climatic, seasonal and geographical conditions and harvest time. The largest

inhibition zones were exhibited by Gram negative isolated *E. coli* to different concentrations of *C.schoenanthus* (100, 50 and 25 % (w/v)) zone of inhibition was (20.8±2.4mm) . the result was agreement with Gustafson *et al*., (1998) who reported that *E.coli* susceptible to *C. schoenanthus* extract more than *S.aureus*. The antimicrobial of *C. schoenanthus* methanolic extract has been evaluated in vitro against isolates and standard organisms (*E.coli* ATCC 25922 and *S. aureus* ATCC 25923). Our Study revealed that methanolic extract of *C.schoenanthus* inhibit bacterial growth with MIC ranged from 25-1.56 % (w/v).

5.2. Conclusions

Cymbopogon shoenanthus possesses high antibacterial activities against pathogenic bacteria *(E. coli, P.aeruginosa, K.pneumoniae, S.aureus,. Proteus mirabilis and Enterobacter spp),* that cause UTI in human, *and* standard organisms *(E.coli ATCC 25922 and S.aureus ATCC 25923*). The more effective concentration was 100 % (w/v), zone of inhibition was increased with the increase of concentration of extracts. methanolic extract of *C.shoenanthus* had the same activity against Gram positive and negative bacteria . MIC of methanolic extract of isolates and standard organisms range from (1.56 % -12.5 % (w/v).

5.3. Recommendations

- Examine methanolic extract of *C. Schoenanthus* on different isolates and use different methods and different solvents for extraction process.
- Pharmacological, toxicological studies should be carried out to assess their therapeutic efficiency and potential for commercial utilizations.
- More research is required to validated these results.

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Appendices

Appendix (1): biochemical tests for isolated bacteria

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

Isolated bacteria	Biochemical tests				KIA			
	Indole	Urease	*Citrate*	Motility	Slop	Butt	Gas	H_2s
E.coli	+ve	-ve	-ve	M	Y	Y	+ve	-ve
P.aeruginosa	-ve	-ve	+ve	M	R	R	-ve	-ve
P. mirabilis	-ve	+ve	+v	M	R	Y	+ve	+ve
Enterobacter	-ve	-ve	+ve	M	Y	Y	+ve	-ve
K.pneumoniae	-ve	+ve	+ve	N	Y	Y	+ve	-ve

Key: R: red, Y: yellow, +ve: positive, -ve: negative , M : motile, N: non motile

biochemical properties of Gram positive bacteria isolated from patients with urinary tract infections

Isolated bacteria	**Catalase**	**DNase**	**Manitole ferment**
S.aureus	+ve	+ve	+ve

Key: +ve: positive, -ve: negative

Appendix(2): Media and reagents

1-DNase Test Agar

Formula in grams per liter

Casein Peptone 15,00

Soy Peptone.....5,00

Sodium Chloride..... 5,00

Deoxyribonucleic Acid.....2,00

Bacteriological Agar..... 15,00

Final pH 7,3 ± 0,2 at 25ºC

Preparation

Suspend 42 grams of the medium in one litre of distilled water. Mix well to obtain a homogeneous suspension. Heat with frequent agitation and boil for one minute. Sterilize in an autoclave at 118-121°C (15 lbs. sp.) for 15 minutes. Cool to 45-50°C and pour into sterile Petri dishes. If desired, add 5% blood to the medium without mannitol to prepare a blood agar medium.

2-**Mannitol Salt Agar**

Formula in grams per liter

Sodium Chloride.....75,00

Peptone Mixture..... 10,00

D-Mannitol 10,00

Beef Extract..... 1,00

Phenol Red.....0,025

Bacteriological Agar..... 15,00

Final pH 7,4 ± 0,2 at 25ºC

Preparation

Suspend 111 grams of the medium in one litre of distilled water. Mix well and heat with frequent agitation until

complete dissolution. Boil for one minute. Sterilize in autoclave at 121°C (15 lbs. of steam pressure)
for 15 minutes. Pour into Petri dishes.

3- Mueller Hinton Agar

Formula in grams per liter

Beef Infusion..... 2,00

Casein Peptone H 17,50

Starch..... 1,50

Bacteriological Agar 17,00

Preparation

Suspend 38 grams of medium in one liter of distilled water. Mix well. Heat agitating frequently and boil for about one minute. Dispense and sterilize in autoclave at 116 - 121°C (15 lbs.sp) for 15 minutes. Cool to 45° or 50° C and add defibrinated blood if desired. The blood mixture should be chocolated by heating to 80° C for 10 minutes if Neisseria development is desired. Do not overheat. to remelt the cold medium, heat as
briefly as possible.

4- Simmons Citrate Agar

Formula in grams per liter

Ammonium Dihydrogen Phosphate .....1,00 Dipotassium Phosphate..... 1,00

Sodium Chloride.....5,00

Sodium Citrate 2,00

Magnesium Sulfate 0,20

Bacteriological Agar..... 15,00

Bromthymol Blue.....0,08

Final pH 6,9 ± 0,2 at 25ºC

Preparation

Suspend 24,3 grams of the medium in one liter of distilled water. Mix well and heat with frequent agitation until
completely dissolved. Dispense in tubes and sterilize in the autoclave at 121ºC (15 lbs sp.) for 15 minutes. Cool
the tubes in a slanted position so that the base is short (1-1,5 cm. deep). Alternatively, the media can be poured
into petri plates.

5- Peptone Water

Formula in grams per liter

Bacteriological peptone.....10,00

Sodium Chloride 5,00

Final pH 7,2 ± 0,2 at 25ºC

Preparation

Suspend 15 grams of the medium in one liter of distilled water. Dissolve the medium completely. Distribute into appropriate containers and sterilize in autoclave at 121ºC (15 lbs sp) for 15 minutes

6- Kligler Iron Agar

Formula in grams per liter

Peptone mixture 20,00

Lactose 10,00

Sodium Chloride..... 5,00

Dextrose 1,00

Ferric Ammonium Citrate 0,50

Sodium Thiosulfate.....0,50

Phenol Red 0,025

Bacteriological Agar 15,00

Final pH 7,4 ± 0,2 at 25ºC

Preparation

Suspend 52 grams of the medium in one liter of distilled water. Mix well and heat with frequent agitation. Boil for

one minute. Dispense into tubes and sterilize at 121º C (15lbs. pressure) for 15 minutes. Allow to cool in a slanted position so as to obtain butts of 1’5-2 cm. Depth. For greater accuracy, Kligler Iron Agar should be used on the day of preparation or melted and solidified before use.

8- **Urea Agar (Christensen)**

Formula in grams per liter

Gelatin Peptone..... 1,00

Dextrose 1,00

Sodium Chloride..... 5,00

Monopotassium Phosphate 2,00

Urea 20,00

Phenol Red.....0,012

Final pH 6,8 ± 0,2 at 25ºC

Preparation

Dissolve 29 grams of the medium in 100 ml. of distilled water. Sterilize by filtration. Separately dissolve 15 grams of agar in 900 ml. of distilled water by boiling. Sterilize in autoclave at 121°C (15 lbs.sp) for 15 minutes. Cool to 50°C and add to the 100 ml. of the sterile Urea Agar Base. Mix well and dispense aseptically in sterile tubes. Leave the medium to set in a slanted position so as to obtain deep butts. At a pH of 6.8 to 7.0 the solidified medium should have a light pinkish yellow colour. Do not remelt the slanted agar.

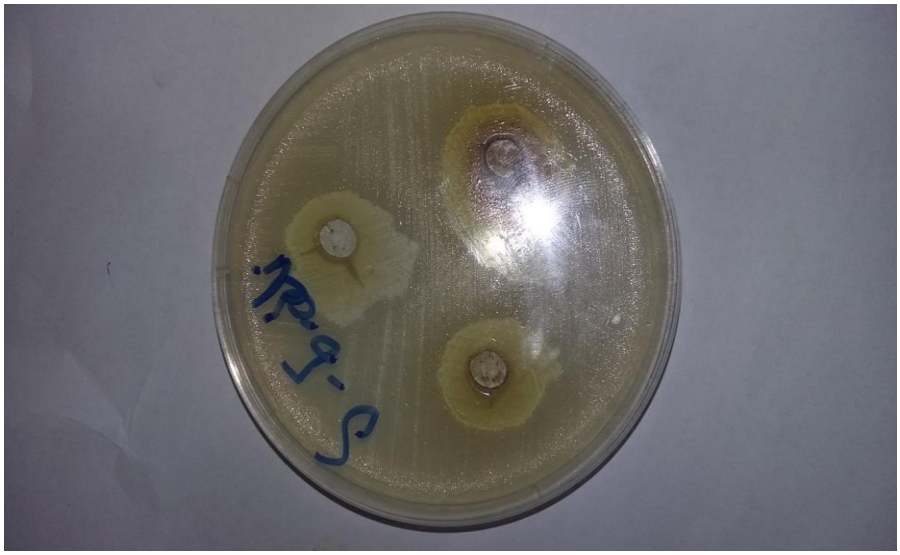
Appendix (3): interpretation chart for antimicrobial susceptibility testing

Antibiotic disk	Sensitive	Resistance
Ceftazidime (25 mcg)	≥21 mm	≤ 17mm
Nitroforuntoin(30 mcg)	≥ 17 mm	14≤ mm
Ciprofloxacin. (5 mcg)	≥21 mm	≤ 15 mm
Imipenem. (30 mcg)	≥23 mm	≤ 19 mm
Gentamicin (30 mcg)	≥17 mm	≤ 14 mm
Co-Trimoxazole(25 mcg)	≥16 mm	≤10 mm

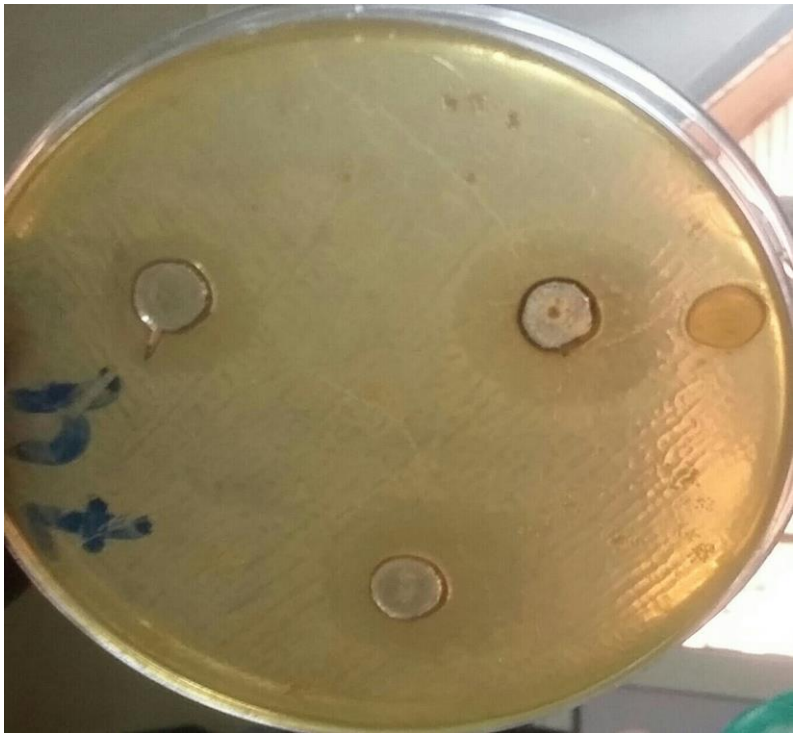
Apendex (4) : cultiure media and tool

Figeure 1 : *Cymbopogon Schoenanthus* plant

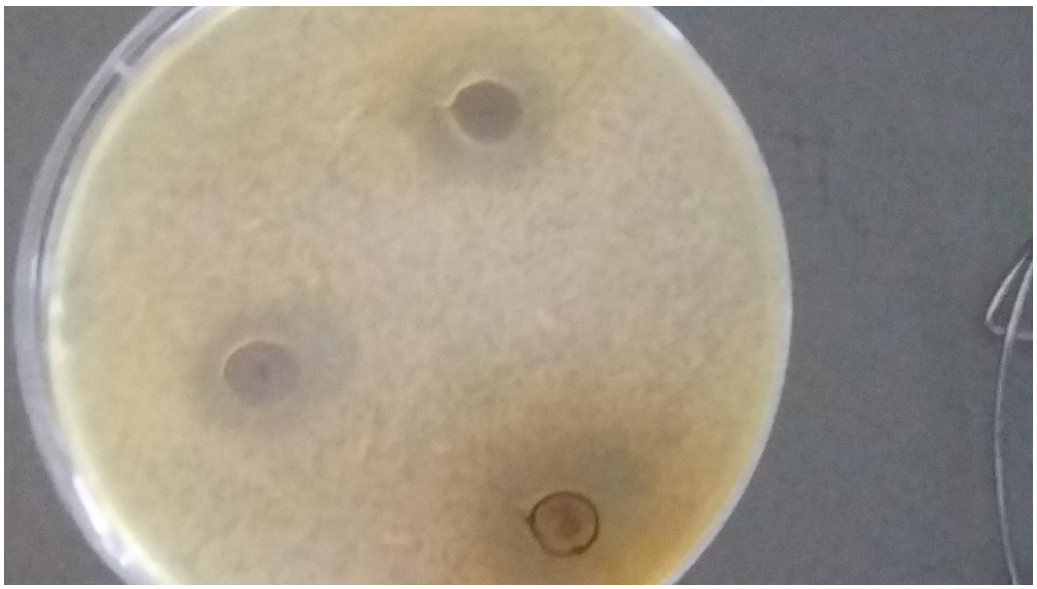
Figure (2) : Antimicrobial susceptibility of *E.coli*to Gentamycin, Cotrimoxazole, Imipenem, Cefatzidime ,Ciprofloxacin and Nitrofurontoin



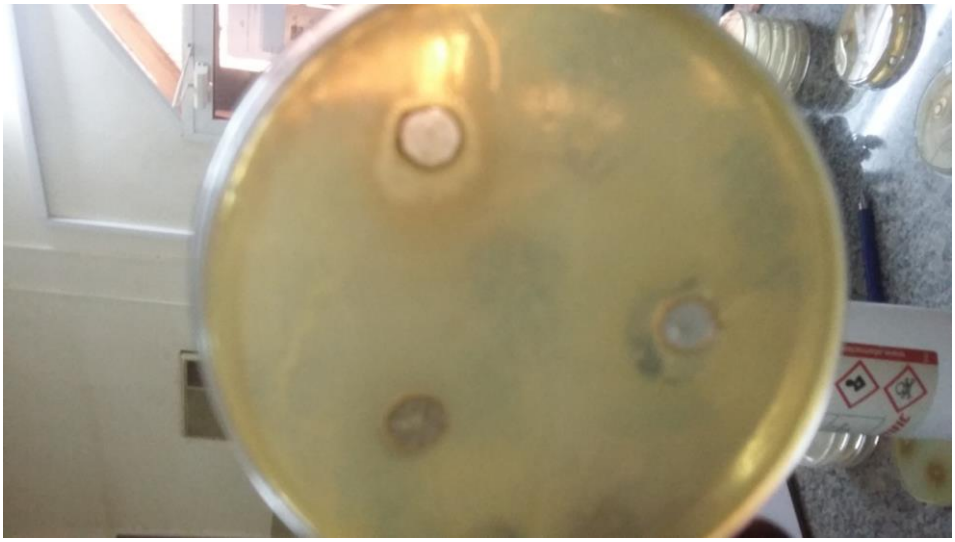
Figure(3) : The inhibition zone of Cymbopogon schoenanthus extract against *Escherichia coli* ATCC25922



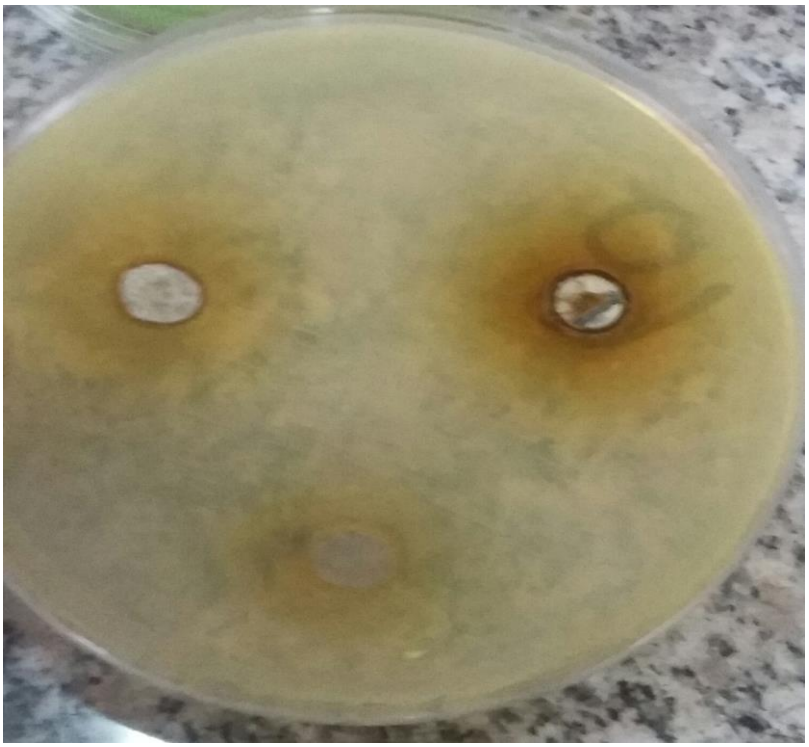
Figure(4) : The inhibition zone of Cymbopogon schoenanthus extract against *E.coli*



Figure(5): The inhibition zone of Cymbopogon schoenanthus extract against *S. aurous* .



Figure(6)The inhibition zone of Cymbopogon schoenanthus extract against *Klebsiella species*



Figure(7):The inhibition zone of *Cymbopogon schoenanthus* extract against *Enterobacter species*