Sudan University of Science and Technology

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Antimicrobial Activity of *Solenstemma argel* Extract against Selected Uropathogenic Isolates In Khartoum State

فعاليه النشاط المضاد لمستخلص الحرجل علي بكتريا مختاره ومعزولة من التهاب المسالك البوليه بولاية الخرطوم

A dissertation Submitted in Partial fulfillment for the Requirements of M.Sc in Medical Laboratory Sciences (Microbiology)

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الآيـة

بسم الله الرحمن الرحيم

قال تعالى:

قَالُوا سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَاۖ إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

سورة البقرة الآية ٣٢

Dedication

To my father, mother ,brother

and sister with great love

Acknowledgement

First of all praise to Allah, at the start, the end and forever for his blessings throughout the long path of this research. My thanks and gratitudes to my supervisor Dr. Ahmed Ibrahim Hashim for his guidance throughout the period of this study. Special thanks are extended to my friend Nijood for her assistance in the collection of the bacteria and my family for their continuous support and encouragement.

Abstract

An alarming increase in bacterial strains resistant to existing antibacterial agents demands a renewed effort to seek agents effective against pathogenic bacteria resistant to antibacterial.

The aim of this study was to study the antibacterial activity of different concentration of methanol extracts of medicinal plant *Solenstemma argel* using cup-plate agar diffusion method on selected uropathogenic isolates bacteria.

The study was conducted in Khartoum State, Sudan between February and April 2017.

One hundred selected uropathogenic bacterial clinical isolates were used in this study. The most frequent of the isolates were *E.coli* 60(60%), followed by*K.Pneumonia7*(7%),*P.mirabilis*10(10%),*Ps.aeruginosa* 10(10%),*S.aureus* 8(8%), and *E.faecalis* 5(5%).

The methanol extract of *Solenstemma argel* was screened for it is antimicrobial activity against uropathogenic clinical isolates. Which showed antimicrobial activity against both Gram positive and Gram negative bacteria.

It was clear that the most powerful effect was observed in case of *E.faecalis*; moderate action against *E.coli; K.pneumoniae; P.mirabilis*;*S.aureus*; and low effect against *Ps.aeruginosa*. The clinical isolates of E.*faecalis*, *E. coli* , *S. aureus* and *Ps. aeruginosa* was sensitive to all concentrations of *Solenstemma argel* extract. The clinical isolates of K. *pneumoniae* and *P.mirabilis* was sensitive to 100% (w/v) and 50%(w/v) of *Solenstemma argel* extract but resistant to 25%(w/v) concentration.

المستخلص

الزياده المقلقة في سلالات البكتريا المقاومه للمضادات البكتيريه الحاليه تتطلب جهدا متجددا للحصول علي عناصر فعالة ضد البكتيريا الممرضة والمقاومة للمضادات البكتيرية.

هدف هذه الدراسه اختبار فعالية مستخلص الميثانول للنبات الطبي الحرجل ,بتراكيز مختلفه باستخدام طريقة الانتشار عن طريق الحفر علي لوحات الاكار ,علي البكترية المعزولة من عينات طبيه من التهاب المسالك البولية.

اجريت هذه الدراسه في ولاية الخرطوم في الفترة من فبراير الي ابريل 2017.

تم جمع مائة عينة من عينات بكتيريه معزولة من البول وكانت كالاتي الاشريكيه القولونيه 60(60%) و الكلبسيلا الرئوية 7(7%) و المتقلبه الاعتيادية10(10%) و الزائفة الزنجاريه10(10%) و العنقودية الذهبية 8(8%)و المكورات السبحية البرازية5 (5%).

وقد اظهر مستخلص ميثانول للحرجل فعالية ضد كل من البكتريا موجبه الغرام والبكتريا سالبه الغرام حيث كانت الفعاليه العاليه ضد بكتريا المكورات السبحية البرازيةو الفعالية المتوسطة ضد كل من الاشريكية القولونية والكلبسيلا الرئويه والمتقلبة الاعتياديه والعنقوديه الذهبية وكانت اقل فاعلية ضد الزائفة الزنجارية.

وكانت كل من الاشريكيه القولونيهو الزائفة الزنجاريه والعنقودية الذهبية والمكورات السبحية البرازية حساسه لكل تراكيز الحرجل التي اختبرت في هذه الدراسه. أما الكلبسيلا الرئويةو المتقلبه الاعتيادية كانت حساسه لكل من تركيز 100% وتركيز 50% فقط ومقاومه لمستخلص الحرجل في تركيز 25% .

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

Microbial infection is major public health problem in the developing countries. Antibiotics are used to treat these infections. Due to the mis use of antibiotics, the incidence of multiple antibiotic resistance among human pathogens is increasing; this has forced the scientists to search for new antimicrobial substances from natural sources (Deshpande, 2013).

Plants possess active ingredients for defense against plant pathogens many of these antimicrobial substances were found to produce the same affects against human pathogens. Hence; researchers started screening plenty of plant extracts and essential oils against various human pathogens. Screening medicinal plants for antimicrobial activity has led to encouraging results

However, it is essential to investigate the toxicity of the plant (Kavitha and Salish, 2013). Antibiotics have undesirable side effects while the emergence of previously uncommon infections is also a serious medical problem. Over75% of the antimicrobial in clinical use are of natural origin and most of them are obtained from fungal source (Kavitha and Salish, 2013).

The plant argel is a member of the family *Asclepiadaceae* that comprises numerous medicinal plants, like *Calotropisprocera*, *Marsdeniaobyssinicna* and *Huerniamecrocarpa*, known for their cardiac activity. argel grows naturally in the northern parts of the Sudan and extends from Berber to Abu-Hamad, especially the Rubatab area. It is also widely distributed throughout North Africa (Egypt, Libya and Algeria) and Saudi Arabia (Ahmed, 2004).

Plant has been used in treating human diseases for thousands of years. In certain African countries, up to 90% of the population still relies exclusively on plant as source of medicienes. Environmental degradation provides threats to biological diversity but the sub Saharan region still boasts wide variety of indigenous species. There reconsider able economic benefits in development of indigenous medicines and use medicinal plants for the treatment of various diseases (Linda JB and David ,2006) .

1.2Rationale

The continuous emergence of multidrug resistance bacteria is a major threat to the live of human around the globe. Antibiotics and synthetic chemotherapeutic agents were thought of as miracle tablets that cure all infectious diseases. This fact was shortly found to be incorrect as Methicillin resistance strains emerged since the 1960s (Pakleung, 2011).

Plants produces antimicrobial substances that protect the plants against plants pathogens. The research has proved that antimicrobial substances produced by plants could produce the same effects on human pathogens. *Solenstemma argel* is one of the medicinal plants that are used in Sudan and other countries in order to treat bacterial infections like urinary tract infection(Pakleung, 2011).

1.3.1General Objective

To evaluate the antibacterial activity of *Solenstemma argel* extract against selected bacterial clinical isolates.

1.3.2Specific Objective-

1-To determine the antimicrobial potential of methanolic extract of *Solenstemma argel* extract against selected urinary tract pathogens

2-To isolate, identify and to determine the antimicrobial sensitivity of

Solenstemma argel extract against uropathogenic clinical isolates against selected antibiotics

3- To determine the antimicrobial potential of different concentrations of solvent Alcohol

CHAPTER TWO

LITERATURE REVIEW

2.1. Urinary Tract Infections (UTIs)

UTI is the most common bacterial infection encountered in the care setting in the United States (Rechtsteiner, 2011*).*

Recurrent UTI is defined as uncomplicated UTIs in 6 months or more traditionally, as ≥3 positive cultures within the preceding 12 months Most recurrences occur within the first months after the primary infection and there can often be clustering of infections. When the initial infection is caused by *Escherichia coli*(*E. coli*), there is a higher risk of re-infection within the first 6 months. When there is recurrent infection with the same organism despite adequate therapy, it is considered a relapse (Annette *et al.*, 2010*).*

2.1.1.Pathogenesis of aerobic bacteria cause urinary tract infection

Symptomatic UTI infection in a healthy human is a complex event. It is initiated when potential urinary pathogens from the bowel or in some cases from the vagina (as a result of direct inoculation during sexual activity).

Virulence and fitness factors include flagella, toxin and polysaccharide coating and the other properties that assist the bacteria in avoiding the human response*(*Johnson, 2003*).*

2.1.2 Symptoms of UTI

burning feeling during urination, dysuria, frequency, urgency, urinary in continence, suprapubic discomfort, sensation of in complete bladder emptying post void, fever, back pain, abdominal palpation, vaginal

discharge, hematuria, nocturia,offensive smelling without purulent (Andrew, 2010*).*

2.1.3. Sterile pyuria

Is defined as the presence of 10 or more white cells per cubic millimeter in a urine sample, 3 or more white cells pre high-power field of urine, positive result on Gram's stain of urine specimen.

Sterile pyuria is the present finding of white cells in the urine in the absence of bacteria as determined by wet preparation techniques (Horan *et al.*, 2008).

2.1.4. Causes of Sterile pyuria

causes related to infection: Current use of antibiotic, recently treated UTI within past 2 weeks, gynecologic infection, urethritis due to prostatitis, balanitis, appendicitis, viral infection of the lower

genitourinary tract, genitourinary tuberculosis, fungal infection and parasitic disease such as trichomonas's (Horan *et al.*, 2008).

causes not related to infection: Presence used of urinary catheter, recent cystoscopy, urinary tract stone, foraged body such us surgical partials in the urethra, pelvic irradiation, urinary fistula, rejection of a renal transplant and inflammatory disease such as systematic lupus erythematous (Horan *etal*.,2008).

2.1.5 Routes of infection

In healthy patients most uropathogens originate from rectal flora and enter the urinary tract via the urethra into the bladder. This is known as the ascending route and uropathogens initially adhere to and colonise urothelium of the distal urethra. Enhancement of this route is exacerbated in patients with soiling around the perineum, in patients with urinary catheters and in females that use spermicidal agents. In patients with established cyctitis up to 50% of infections may ascend into the upper urinary tracts and most episodes of pyelonephritis are caused by ascension of bacteria from the bladder through the ureter and into the renal pelvis. Bacterial ascent is aided by conditions such as pregnancy and ureteral obstruction as these condtions inhibit ureteral peristalsis. Bacteria that reach thr renal pelvis can penetrate the renal parenchyma through the collecting ducts and disrupt the renal tubules (Kebira *et al.*,2009).

In healthy individual's infection of the kidney through the haematogenous routes is uncommon. Occasionally, the renal parenchyma may be breeched in patients with Staphylococcus aureus bacteramia or Candida fungaemia that via the lymphatics. Conditions associated with the lymphatic route are retroperitoneal abscesses and severe bowel infections (Kebira *et al.*, 2009) .

2.1.6 Complicated and uncomplicated UTI

Complicated UTI can occur in either the upper or lower urinary tract but is accompanied by an underlying UTIs are classified as complicated or uncomplicated (Khan and Ahmed ,2001)**.**

2.1.6.1Complicated UTI

Underlying host factor such as age,catheterisation,diabetes mellitus and spinal cord injury predispose to complicated UTIs. In complicated UTIs less virulent uropathogens (that rarely cause disease in a normal urinary tract)

can cause significant damage to anabnormal urinary tract. Studies have demonstrated associations between Group B streptococcal bacteremia, Candida and Enterococci with complicated UTIs in the elderly population (Khan and Ahmed 2001).

2.1.6.2UncomplicatedUTI

UTI can be classified as either complicated or uncomplicated depending on underlying host factors and on underlying uropathogens as illustrated The etiology of uncomplicated UTIs has remained constant over the last 2 to 3 decades with E.coli accounting for the vast majority of cases. Previously, female patients with uncomplicated UTI generally remained sensitive to a trimethoprim-sulfamethoxazole combination and the traditional approach to therapy had been an empirical short-course treatment with this antibiotic regimen. Unfortunately, number of more recent studies have demonstrated increasing antimicrobial resistance among uropathogens causing uncomplicated cystitis and traditional antibiotic regimens have been questioned(Ronald,2003).

2.2Hargel (*Solenostemma argel*)

Hargel (*Solenostemma argel*) is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances (Shayoub, 2003). Herbs had been used all cultures throughout history. The primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine (Shayoub, 2003). Certainly herbs used in some infections, cough, cold, stomach upset, indigestion, catarrh, constipation and so on (WHO, 2002*;* Mcntyres2003). Herbal medicine has provided the world's population with safe, effective and low cost natural substances (medicine) for centuries (Shayoub, 2003).

Hargel leaves are used in indigenous medicine for the treatment of some diseases such as the disease of liver and kidney. It is an effective remedy for bronchitis and is used to treat neuralgia. It is used as incense in the treatment of measles and sometimes crushed and used as remedy for healing wounds. The leaves are infused to treat gastro-intestinal cramps and stomach colic (Sulieman *et al*., 2009) reported that hargal aqueous extracts have antimicrobial activity against two fungi (Aspergillus niger and Pennicilium italicum) and two Gram negative bacteria (*Escerichia coli* and *Salmonella typhi*).

Morphological description

A perennial, 60 cm high, with several vigorous stems. The leaves are, oval, leathery and covered with fine hairs. The numerous flowers have white petals, and a strong smell. Their inflorescences are giving the plant attractive look. The fruits are thick, 5 cm. long and 1.5-2 cm wide, green with violet lines; they contain pubescent seeds. The plant has a long flowering period from March to June (El-kamali,2001).

Regional

Algeria, Libya and Egypt. No particular threat is reported for Algeria, but in Egypt the plant is vulnerable because of its intensive overuse. The largest population of *Solenostemma argel* grows in the upper part of the WadiAllaqi conservation area and from 1989 on has been protected by Egyptian law. The plant is cultivated on a farm in the downstream part of WadiAllaqi to increase its population and promote the cultivation of this economically important plant. Hargel is Tropical plant that spreads across the central Sahara to the Sinai and the southeastern (Arabian) desert . Ecology The plant grows in extremely dry conditions with a yearly rainfall of around 50-100 mm. It grows on the gravelly soils and on the stony and pebbly soils (El-

kamali, 2001).Part used the leaves and stems which are collected in the spring and prepared as an infusion, a decoction or a powder. This can be taken by mouth or used externally.

Constituents

Acylated phenolic glycosides, namely argelin and argelosid, choline, flavonoids, monoterpene and preganeglucoside, sitosteroland a triterpenoidsaponin (El-kamali, 2001).

Pharmacological action

Anti-inflammatory activity and antimicrobial activity. It is used for colds, diabetes, respiratory troubles, rheumatism, stomach pain, urinary infection. The bitter sap from the stem is used for cold (El-kamali, 2001).

Chemical composition

Leaf of hargel is characterized by high carbohydrates (64.8%) and low crude fiber (6.5%). In addition the leaf contained 15 % protein, 1.6% crude oil, 7.7% ash, and 4.4% moisture content. The results recorded by Marwa revealed that the leaf contained high potassium (0.54%), calcium (0.06%), magnesium (0.03%) and sodium (0.01%), but it is characterized by low cupper (0.0001%), ferrous (0.002%), manganese(0.002%) and lead (0.001%). The protein fractionation of leaf is characterized by high albumins (16.7%), non- nitrogenous protein (15.3%),Prolamine (11.7) and low globulins (8.7%) and glutulin (6.2%).Leaf continedphytic acid (3.2g/100g and tannin content (0.4%)(El-kamali, 2001).

CHAPTER THREE

MATERIALS & METH ODS

3.1 Study design

This study was a descriptive cross sectional laboratory based study.

3.2 Study duration

This study was conducted between February and April 2017

3.5 Sample size

One hundred bacteria (n=100) selected from clinical bacterial isolated.

3.6 Collection and Identification of bacteria

Bacteria used was obtained from Nutrient glycerol broth at -20ºC. Then the samples were inoculated under aseptic condition on Cysteine Lysine Electrolyte Deficient

(CLED) for the isolation and identification of the pathogens. The inoculated culture media were incubated aerobically at 37ºC for 18-24 hours and examined for significant growth.

3.6.1 Identification of the clinical isolates

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

3.6.2 Colonial morphology

Colonial morphology used as first identification depending on size, color, edges and fermentation of lactose in CLED.

3.6.3 Preservation and storage of isolated organism

Isolated organisms were kept in nutrient agar slope at 4ºCfor further identification and susceptibility tests. Nutrient glycerol broth used for long stage preservation for isolates at -20ºC.

3.6.4 Microscopic examination

Fixed and dried smears were prepared from growth. The smear was` stain with Gram stain firstly crystal violet stain was applied for 30- 60 sec, washed with water followed by 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 (×100) to detected the Gram reaction and formed of bacteria.

3.6.5 Biochemical test

Biochemical tests including Kliglar Iron Agar test(KIA),(Himedia India)indole, urease, citrate,motilitytest,esculin hydrolysis and catalase test as well as inoculation on differential selected media such as Mannitol Salt Agar (MSA) was used to distinguish between the clinical isolates.

3.6.5.1 Indole test

The test organism is cultured in a medium which contain tryptophan, indole production was detected by Kovac's reagent which contain 4-d.This react with indole to give red color compound .

3.6.5.2 Urease test

The test organism was cultured in medium contain urea and indicator phenol red. The enzyme breaks down the urea to give ammonia and carbon dioxide which release the ammonium showing change in the color from yellow to pink color.

3.6.5.3 Citrate test

The test organism was cultured in medium contain sodium citrate, ammonia salt and indicator bromo-thymol blue showing turbidity and change in color of the indicator from green to blue due to the alkaline reaction,

3.6.5.4The motility test

Depend on the properties of bacteria structure which contain flagella or no.

3.6.5.5 Esculin test

The test organism was cultured in esculin agar yellow in slope, the H_2S produced when the sulphur-containing amino acids are decomposed to give black color.

3.6.5.6 Catalase test

Depend on the enzyme act as breakdown of hydrogen peroxide to give oxygen and water indicated produced by air bubble.

3.6.5.7-Kliglar iron agar

Test was used for detect in of H_2S and gases production sugar fermentation(lactose), help identification of enterobacteriasae.

3.6.5.8 Mannitol salt agar

Used to help differeinated the *Staphylococcus* species and *Streptococcus*, to detect the mannitol fermentation to change color from yellow to pink color or no mannitol ferment.

3.6.5.9 DNA test

The test organism cultured in medium contain DNA, after inoculation and incubation at overnight used HCL acid ,which precipitates the hydrolyzed DNA to give clear zone around the colonies.

3.7 Modified Kirby–Bauer Method

Isolated pathogens were tested against antibiotics for susccebility test by using Kirby-Bauer disk diffusion method in which 3-5colonies were touched by sterile standard wire loop then emulsified into sterile normal saline and adjusted to 0.5 McFarland standard (Cheesbrough, 2006).

Antibiotic discs (Hi Media laboratories PV+Ltd, India) were applied on inoculated plates: Ciprofloxacin (30 mcg), Gentamicin (10mcg), and

Imipenem (10 mcg) Ceftazidime (30 mcg), Vancomycin (30 mcg) and Ami Kacin (20 mcg), Aztreonam, Tetracycline, Piperacillin, Cotrimoxazand Nitrofurantoin.

Plates were left at room temperature and incubated at 37C ^{o} for 18-24 hrs.

Zone of inhibition were measured in mm and results interpreted according to standardized chart(Srinivasan *et al.*, 2001).

3.8Collection and identification of plant material

Solenstemma argel was collected from farmer in Northern state in nuri area. The dried *(Solenstemma argel*) in shadow and cleaned from dust and grass,samples were identified and extracted in Industrial Research Institute in Khartoum Bahri.

3.9 Preparation of extract

Extraction was carried out according to the method described by (Sukhdev*et al.,* 2008). 50grams of *Solenstemma argel* was grinded using mortar and pestle and extracted with 80 % methanol using sox hlet extractor apparatus.

Extraction carried out for about eight hours till the solvent returned green colour at the last siphoning time. Solvent was evaporated under reduced pressure using rotary evaporator apparatus. Finally the extract was allowed to dry in Petri dish and the yield percentage was calculated as followed:Weight of extract obtained / weight of plant sample * 100

3.10Agar diffusion method

The agar well diffusion using Mueller Hinton Agar medium was done to screen the antimicrobial activity of *Solenstemma argel* extracts against selected pathogens. The bacteria were activated by inoculating the strains in nutrient agar plate and incubated at 37^{o}C for 18-24 hrs. Each strain was suspended in sterile normal saline. Then 0.1 ml of inoculums (adjusted according to McFarland standard) was inoculated onto the molten Mueller

Hinton agar media and after proper homogenization it was poured into sterile petri dishes. Wells were made into the seeded plates by using crock-borer size (10mm).Various concentrations of *Solenstemma argel*(100%, 50%, 25%)(w/v) were introduced into the wells subsequently and all the plates were incubated at 37ºC for 24 hrs (Srinivasan *et al.,* 2001).

Positive control was Chloramphenicol concentration and negative control was methanol, each step was under strict aseptic conditions. Bacterial growth was determined by measuring the diameter of the zone of inhibition (in mm) and the mean were calculated.The inhibition zone with diameter less than 12mm were considered as having no antibacterial activity (Srinivasan *et al.,* 2001).

3.11 Statistical Analysis

All collected data were analyzed using Microsoft Office Excel 2007.

CHAPTER FOUR

RESULTS

4. Results

Antimicrobial activity of methanolic extract of *Solenstemma argel* in various concentrations 100%(w/v),50%(w/v) and 25%(w/v) was determined against selected Gram positive and Gram negative organisms including *Escherichia coli*(60%),*Klebsiella pneumoniae*(7%), *Proteus mirabilis* (*10%*), *Enterococcus faecalis*(5%), *Pseudomonas aeruginosa*(10%) and *Staphylococcus aureus(8%)(table4.1).*The Screening of antimicrobial activity of extracts was done by agar well diffusion method using cork borer No.5. Each strain was tested in triplicate and the means were presented in tables as the diameter zone of inhibition exhibited around the wells containing the antimicrobial extracts as well as controls measured in mm.

Table (4.1)Frequency of clinical isolated bacteria

Species	**Frequency**	**Percent**
E.coli	60	60%
K.pneumoniae	7	7%
P.mirabilis	10	10%
E.faecalis	5	5%
Ps. aeruginosa	10	10%
S.aureus	8	8%
Total	100	100%

The biochemical tests results for Gram negative and Gram positive isolates are show in table 4.2 and 4.3

Table (4.2) Biochemical results of Gram negative bacteria isolated from patients with urinary tract infection

	Biochemical tests							Motility Test
	Indole	Urease	Citrate	KIA				
				Slope	Butt	Gas	H_2S	
E. coli	+ve	- ve	- ve	Y	Y	+ve	- ve	+ve
K. pneumoniae	- ve	+ve	+ve	Y	Y	- ve	- ve	-ve
P. mirabilis	+ve	+ve	+ve	R	Y	+ve	+ve	+ve
Ps.aeruginosa	-ve	-ve	+ve	R	R	-ve	-ve	+ve

Table (4.3) Biochemical results of Gram positive bacteria isolated from patients with urinary tract infection

Isolates	**Catalase**	**Mannitol Fermentation**	**DNase Test**	**Esculin hydrolysis**
E. faecalis	-ve	-ve	-ve	+ve
S.aureus	+ve	+ve	+ve	-ve

The result of antimicrobial activity of antibiotics are presented in tables 4.4,4.5 and 4.6 The bacteria have shown variable reactions to the antibiotics used in the tests(table 4.4),(table 4.5) and (table 4.6)

Tables (4.4) Results of the sensitivity of the isolated uropathogenic strains against selected antibiotics

		E.coli	*K.pneumoniae*	*E. faecalis*	*Ps.aeruginosa*	*P.mirabilis*	*S.aureus*
Cotrimoxazole	S	41 (40%)	20(20%)	0	31(50%)	7(7%)	12(12%)
	R	0	0	12(14%)	0	7(7%)	0
Nitrofurantion	S	41 (56%)	20(27)	12(14%)	31(50%)	7(7%)	12(15%)
Vancomycin	S	41 (56%)	20(27)	12(14%)	31(50%)	7(7%)	12(15%)
	R	0	0	0	0	7(7%)	0

*Note :S=sensitive, R=resistance

Table(4.5) Results of antimicrobial sensitivity test of Gram positive bacteria

	Microorganism		
Antibiotics		E. faecalis	S.aureus
Gentamycin	S	12(37.5%)	12(37.5%)
Augmentin	S	12(37.5%)	12(37.5%)
Erythromycin	S	12(37.5%)	12(37.5%)
Ciprofloxacin	S	12(37.5%)	12(37.5%)
Fuidicacid	S	12(37.5%)	12(60%)
Chloramphenicol	S	12(37.5%)	12(57.90%)

Table (4. 6)Results of antimicrobial sensitivity tests of Gram negative bacteria

Antibiotics		Microorganism			
		E .coli	*K.pneumoniae*	*P.mirabilis*	*Ps.aeurginosa*
Amikacin	S	41(60%)	20(29%)	7(10%)	31(50%)
ceftazidime	S	41(60%)	20(29%)	7(10%)	31(50%)
Aztreonam	S	41(60%)	20(29%)	7(10%)	31(50%)
Tetracycline	S	41(60%)	20(29%)	7(10%)	31(50%)
Piperacillin	S	41(60%)	20(29%)	7(10%)	31(50%)
Imipenem	S	41(60%)	20(29%)	7(10%)	31(50%)

The results of antimicrobial activity of argel are presented in tables 4.7,4.8 and 4.9

The effect of argel on the bacterial isolates was high (4.7)

Table (4.7) Mean diameter inhibition zone (mm) of 100% of *Solenstemma argel* against bacterial isolates

Isolated bacteria	Mean diameter of inhibitionzones
E .coli	19
P .mirabilis	19.4
Ps. aeruginosa	17.4
K .pneumonia	19.1
E .faecalis	20.2
S.aureus	19.3

*Note that any diameter less than 12mm means that there was no antimicrobial activity since the size of the crock borer was 10 mm.

The concentration of argel at 50% and 25% gave the higher effect on all the tested organisms(Table 4.8 and 4.9 respectively)

Table (4.8) Mean diameter inhibition zone (mm) of 50% of *Solenstemma argel* against bacterial isolates

Isolated bacteria	Mean diameter inhibition Zones
E .coli	16.2
P .mirabilis	16
Ps.aeruginosa	15.4
*K. pneumonia*e	14.5
E.faecalis	16.4
S.aureus	15.3

*Note that any diameter less than 12mm means that there was no antimicrobial activity since the size of the crock borer was 10 mm.

Table (4.9) Mean diameter inhibition zone (mm)of 25% of *Solenstemma argel* against bacterial isolates

Isolated bacteria	Mean diameter of inhibition zones
E .coli	12.6
P .mirabilis	11.4
Ps.aeurginosa	12.3
K .pneumonia	10.5
E .faecalis	13.0
S.aureus	12.3

*Note that any diameter less than 12mm means that there was no antimicrobial activity since the size of the crock borer was 10 mm.

CHAPTER FIVE

Discussion

5.1. Discussion

The misuse of antimicrobial drugs has led to emergence of resistance to many antibiotics. Therefore, there is a need to screen local medicinal plants for possible antibacterial properties (Khan *etal* ., 2009).

According to the results, the effect of *Solenstemma argel on* isolated bacteria *E.coli, K.pneumoniae*, *S.aureus, E.faecalis, P.aeruginosa* and *P.mirabili*s,showed higher effect on *E.faecalis* and this result is similar to those reported by Abdalhady, which showed the high effect of *Solenstemmaargel* on *E.faecalis* ,and moderate actions against *E. coli,S. aureus, K.pneumoniae* and*P.mirabilis,and* low effect against *Ps.aeruginosa* (Abdalhady*etal*., 1994).

The means diameter of growth inhibition zone of clinical bacterial isolates has increased proportionally when the concentration was raised..

The best inhibition zone obtained by methanol extract of *Solenstemma argel* was 20.2mm in diameter against *E.faecalis* and 19.4mm against clinical isolates of *P.mirabilis* also 19.3mm against clinical isolates of *S.aureus* at concentration of 100%(w/v). Followed by 19.1mm against *K.pneumonia*e and 19mm against *E.coli* and 17.4mm against clinical isolates of *Ps.aeruginosa* at concentration of 100%(w/v).

The clinical isolates of *E.coli* were sensitive to all the selected antibiotic as well as the extract of *Solenstemma argel* and this result is similar to those reported by Abdalmonim ,who reported the higher concentration gave maximum effect which decreased with dilution, clear zone of inhibition was shown by the extract against *E. coli*(Abdalmonim, 2009).

The clinical isolates of *P.mirabilis* were resistant to some selected antibiotics but was sensitive to100%(w/v) and 50%(w/v) of *Solenstemma argel* extract but resist to 25%(w/v) concentration.

E. faecalis tested was resistant to cotrimoxazole and sensitive to all concentrations of *Solenstemma argel* extract, similar results were obtained by Faten , who reported the most powerful effect was observed against *E. faecalis* ,which showed antimicrobial activity against both Gram positive and Gram negative bacteria (Faten *et al*., 1994).

The clinical isolates of *S. aureus* was sensitive to all concentrations of *Solenstemma argel* extract.

The clinical isolates of *Ps.aeruginosa* was sensitive to all concentrations of *Solenstemma argel* extract and this result is disagreed to those reported by Faten,in this study reported there was no effect of *Solenstemma argel* against *Ps. aeruginosa* (Faten *et al*., 1994).

The clinical isolates of *K. pneumoniae* was sensitive to 100%(w/v) and 50%(w/v) of *Solenstemma argel* extract but resist to 25%(w/v) concentration.

The different between studies may be attributed to the use of different solvents, sample size,inoculums,climate and location of collection of samples, and preparation of patients,different extracted methods and finally isolated bacteria(Khan *et al.*,2009).

5.2 Conclusion

In conclusion the results of this study showed that the methanolic extract of *Solenostemma argel* has is it high effects antimicrobial potential against urinary pathogens.

5.3 Recommendation

-Study the toxicity of the active ingredients.

-More research is required to verify these results.

-Further investigations with reference strains are essential before it is possible to draw any final conclusion since, high concentrations were required for the inhibition of urinary pathogens.

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Appendix 1

Appendix (1) Mueller Hinton II Agar

Ingredients g/L

beef infusion 300.0

Casein acid Hydrolysate 17.50

Starch 1.50

Agar 17.00

preparation:

Suspend 38g of the powder in 1 litre of Distilled water Mix thoroughly.

Heat, to completely dissolve the powder.Autoclave at 1210C for 15 minutes.

Appendix (2) : CLED Agar (Cystine-Lactose-Electrolyte-Deficient Agar)

Ingredients g/L

Enzymatic Digest of Gelatin 4 g

Enzymatic Digest of Casein 4 g

Beef Extract 3 g

Lactose 10 g

L-Cystine 0.128 g

Bromthymol Blue 0.02 g

Agar 15 g

Final pH: 7.3 ± 0.2 at 25°C

preparation:

Suspend 36g of the powder in 1 litre of Distilled water, Mix thoroughly.

Heat with frequent agitation and boil for 1 minute to completely dissolve the

powder. Autoclave at 1210C for 15 minutes.

Appendix (3): Nutrient agar

Ingredients g/L

Peptic digest of animal tissue 5.00

Beef extract/yeast extract 3.00

Agar 15.00

NaCl 5.00

pH is adjusted to neutral (7.4) ± at 25 °C.

preparation

Suspend 23 g of the powder in 1 litre of Distilledwater . Heat with frequentagitation and boil for 1 minute to completely dissolve the powder. Autoclaveat 121˚C for 15 minutes.

Appendix (4) :Christensen's Urea Agar

Ingredients g/L

Sodium Chloride 5.00

Monopotassium Phosphate 0.8

Dipotassium Phosphate 1.2

Peptone 1.00

Dextrose 1.00

Phenol Red 0.012

Agar 15.0

Final pH 7.4 ± 0.2 at 25 ° C.

Preparation

Suspend 24 grams in 950 ml of distilled water heat boil to dissolve completelyAutoclave at 121 ° C for 15 minutes.then cool to 50° C addAseptically 40% urea solution and mix well , dispense in strile tube andallow to set at slant position

Appendix (5) Simmon's Citrate Agar

Ingredients g/L

Sodium Chloride 5.0

Sodium Citrate 2.0

Ammonium Dihydrogen Phosphate 1.0

Dipotassium Phosphate 1.0

Magnesium Sulfate 0.2

Bromothymol Blue 0.08

Agar 15.0

Final pH 7.4 ± 0.2 at 25 ° C

Preparation

Suspend 24.28 grams in 100 ml of distilled water heat boil to dissolvecompletelyAutoclave at 15 Ibs pressure (121° C) for 15 minutes. Then cool to50° C aseptically dispense in sterile tube and allow to set.

Appendix (6) Peptone Water

Ingredients g/L

Sodium Chloride 5.0

Peptic digest of animal tissue 10.0

Final pH 7.4 ± 0.2 at 25 ° C

Preparation

Suspend 15 grams in 100 ml of distilled water heat boil to dissolve completelyAutoclave at 15 Ibs pressure (121° C)for 15 minutes. Then coolaseptically, dispense in sterile tube and allow to set

Appendix (7) :Kligler Iron Agar (KIA)

Ingredients g/L

Peptic digest of animal tissue 15

Lactose 10.0

Proteose Peptone 10.0

Sodium Chloride 5.0

Beef Extract 3.0

Yeast Extract 3.0

Dextrose 1.0

Sodium Trisulphate 0.3

Ferrous Sulfate 0.2

Phenol Red 0.024

Agar 15.0

Preparation

Suspend 57.52 grams in 1000 ml of distilled water heat boil to dissolvecompletelyAutoclave at 15 Ibs pressure (121° C)for 15 minutes. Then coolaseptically, dispense in sterile tube and allow to set slant position

Appendix (8) :Mannitol Salt Agar (MSA)

Ingredients g/L

Proteose Peptone 10.0

Sodium Chloride 10.0

Beef Extract 1.0

D-mannitol 10.0

Phenol Red 0.025

Agar 15.0

Preparation

Suspend 111.02 grams in 1000 ml of distilled water heat boil to dissolvecompletelyAutoclave at 15 Ibs pressure (121° C) for 15 minutes. Then coolaseptically, dispense in sterile tube and allow to set slant position

Appendix (9):DNase Test Agar

Enzymatic Digest of Casein 15 g

Enzymatic Digest of Animal Tissue 5 g

Sodium Chloride 5 g

Deoxyribonucleic Acid 2 g

Agar 15 g

Final pH: 7.3 ± 0.2 at 25°C

Preparation

Suspend 24 g of the medium in 100ml of Distilled water .Heat with frequentagitation and boil for one minute to completely dissolve the medium.Autoclave at 121°C for 15 minutes

Appendix (10) :Antibiotic Disc

CIP Ciprofloxacin 30 mcg/disc

Gen Gentamicin 10 mcg/disc

CXR Ceftriaxone 30mcg/disc

CefoCefoxitin 30 mcg /disc

VA Vancomycin 30 mcg/disc

CAZ Ceftazidime 30 mcg /disc

Imp Imipenem 10 mcg/disc

AMC Amoxicillin 20mcg/disc

Appendix(11)Table effect of different concentrations *Solenstemma argel* against *E.coli*

Solenstemmaargel	Mean inhibition zones (mm)
100 w/v (%)	19
50 w/v (%)	16.2
25 w/v (%)	12.6

*Note that any diameter less than 12mm means that there was no antimicrobial activity since the size of the crock borer was 10 mm.

Appendix(12)Table effect of different concentrations of*Solenstemmaargel* against *P.mirabilis*

Solenstemmaargel	Mean Inhibition zones (mm)
100 w/v (%)	19.4
50 w/v (%)	16
25 w/v (%)	11.4

*Not that any diameter less than 12mm means that there was no antimicrobial activity since the size of the crock borer was 10 mm.

Appendix(13)Table effect of different concentrations of*Solenstemmaargel* against *Ps.aeruginosa*

Solenstemmaargel	Mean Inhibition zone(mm)
100 w/v (%)	17.4
50 w/v (%)	15.4
25 w/v (%)	12.3

*Note that any diameter less than 12mm means that there was no antimicrobial activity since the size of the crock borer was 10 mm.

Appendix(14)Table effect of different concentrations of*Solenstemmaargel* against *K.pneumonia*e

Solenstemmaargel	Mean Inhibition zones(mm)
100 w/v (%)	19.1
50 w/v (%)	14.5
25 w/v (%)	10.5

*Note that any diameter less 12mm means that there was no antimicrobial activity since the size of the crock borer was 10 mm.

Appendix(15)Table effect of different concentrations of*Solenstemmaargel* against *E.faecalis*

Solenstemmaargel	Mean inhibition zones (mm)
100 w/v (%)	20.2
50 w/v (%)	16.4
25 w/v (%)	13.0

*Note that any diameter less than 12mm means that there was no antimicrobial activity since the size of the crock borer was 10 mm.

Appendix(16)Table effect of different concentrations *of solenstomma argel* against *S. aureus*

Solenstemmaargel	Mean inhibition zones (mm)
100 w/v(%)	19
50 w/v(%)	15
25 w/v(%)	12

*Note that any diameter less than 12mm means that there was no antimicrobial activity since the size of the crock borer was 10 mm.