

CHAPTER ONE

1.INTRODUCTION

In recent years, herbal medicines have become an integral part of the primary health care system of many nations (Fajimi and Taiwo, 2000). The investigation of certain indigenous plants for their antimicrobial properties may yield useful results. Plants have variety of compounds with potentially significant therapeutic application against human pathogens including bacteria, fungi or virus (El astal *et al.*, 2005). The current research on natural molecule and products primarily focuses on plants. Since they can be sourced more easily and be selected based on their ethno-medical uses (Arora and Kaur, 2007). Recently, some higher plant products have attracted the attention of microbiologists to search some phytochemical components for their exploitation as antimicrobials such plant products would be biodegradable and safe to human health (Kumar *et al.*, 2008; Wang *et al.*, 2010). Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced as Ayurveda, Unani, and Siddha. Several plant species are used by many ethnic groups for the treatment of various ailments (Dhar *et al.*, 1968). However, very little information is available on such activity of medicinal plants and out of 4, 00,000 plant species on earth only a small number has been systematically investigated for their antimicrobial activities. There is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. The belief that “Green Medicine” is safe and more dependable than the costly synthetic drugs many of which have adverse side effects (Nair and Chanda, 2007). The clinical

microbiologists have great interest in screening of medicinal plants for antimicrobial activities and phytochemical as potential new therapeutics. The need to screen plants for pharmaceuticals is particularly urgent in the light of rapid deforestation and the concurrent loss of biodiversity throughout the world (Dalziel, 1956).

1.1. Rationale

Plants commonly used in traditional medicine are assumed to be safe. This safety is based on their long usage in the treatment of diseases, This study idea arose during the working in university of Africa –Collage of Sciences collaboration with Research Center for Aromatic plants, and to verify activity of *senna* as antimicrobial agents. This plant-based, traditional medical system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al*, 2007).

1.2. Objectives

1.2.1. General objective

To evaluate the antibacterial activities of *Senna alexandrina* mill extracts on some pathogenic bacteria isolated from urinary tract infection.

1.2.2. Specific objectives

1. To isolate and identify the pathogenic bacteria from urinary tract Infections .

2. To study the antibacterial activity of *Senna alexandrina* mill extracts on some pathogenic bacteria isolated .

3. To compare the antibacterial activities of crude extracts of selected plants against the isolated bacteria with most commonly used antibiotics .

4. To identify the major ingredients in the extracts of the tested plants.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Medicinal plants

According to the World Health Organization (WHO), the use of traditional herbal medicine has spread not only in the developing countries, but also in the industrialised regions, as a complementary way to treat and to prevent diseases. The pharmacological properties of the medicinal plants have been attributed to the presence of active constituents which are responsible for important physiological functions in living organisms (O'zcan *et al.*, 2004, Yamashita *et al.*, 2005). Consequently, medical practitioners are also prescribing herbal medicine teas and herbal extracts as a supplementary type of treatment in everyday problems caused by our modern civilization (Gomez *et al.*, 2007). Sudan has an immense diversity and variation in vegetation and is one of the richest countries with regard to phytopharmaca. Although herbal remedies are often perceived as being natural and, therefore, safe, they are not principally free from adverse effects. While many investigations of the quality values of medicinal plants are being reported in the current literature, less emphasis has been made on the metal content of herbal products (Gomez *et al.*, 2007). such as renal failure, symptoms of chronic toxicity and liver damage (Gomez *et al.*, 2007). One very important feature when considering the health effects of trace elements is their slow accumulation in tissues, even at low doses. Hence, acute effects are reported very rarely, whereas chronic exposure can lead to the build-up of higher concentrations and onset of disease. Trace element toxicity can

manifest with non-specific symptoms and, often, epidemiology is the only possible approach to ascertain their role (Prasad 2008). WHO estimates that 80% of the developing world's people rely on herbs for their primary health care needs (IUPAC, 2007). This is also the case in Sudan (personal communications). Sudanese medicinal plants in dried form have been exported to different African, Asian, European, North and South American countries since 1952. Sudan imports a variety of plant species for use in traditional medicine in their crude form or as herbal teas. Plant materials are mainly imported from Egypt, Syria, India, China, Niger, Guatemala, Saudi Arabia and Tanzania, as well as other nearby African countries. This cost the country about 900,000 USD annually (Khalid *et al.*, 2007).

For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Bonjar and Farrokhi *et al.*, 2004). *Cassia* species have been of medical interest due to their good therapeutic value in folk medicine (Abo *et al.*, 2000). Working with different medicinal plants extract showed that they can lyses thrombus as streptokinase according to Gennaro and Remington., (2000) and Sweta *et al.*, (2006). The plant sap can act against microorganisms by preventing the growth of microbial colony (Hammer *et. al.*, 1999). Some of the plants extracts also increase lethality of the cell due to their known cytotoxic effect. Brine shrimp lethality bioassay is performed for evaluating the level of toxicity according to the method of (Persoone, 1980) and Goldstein *et al.*, 1974). Keeping this fact in the consideration, the attempts were made to establish physiochemical standards of the plant *Cassia senna* (Common names- senna, Indian senna, English- sanay, hindi- sana ka pat) belonging to family Fabaceae.

2.2. Utilization of Medicinal plants as A Source of Antimicrobial Agents:

According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. (Gislene *et al.*, (2000).

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. (Gislene *et al.*, 2000).

There is currently a large and ever expanding global population base that prefers the use of natural products in treating and preventing medical problems. This has influenced many pharmaceutical companies to produce new antimicrobial formulations extracted from plants or herbs. Virtually all cultures around the globe have relied historically, and continue to rely on medicinal plants for primary health care. There is currently a worldwide upsurge in the use of herbal preparations and the active ingredients isolated from medicinal plants in health care. Up to 40% of modern drugs are derived from natural sources, using either the natural substance or a synthesized version (Jasim& Najii., 2003).

Some antibiotics have become almost obsolete because of drug resistance and consequently new drugs must be sought for. Herbal treatment is one possible way to treat diseases caused by multidrug resistant bacteria. The use of plant extracts and phytochemicals, with known antibacterial properties, may be of immense importance in

therapeutic treatments. In the past few years, a number of studies have been conducted in different countries to prove such efficiency (Indranil *et al.*, 2006).

2.3. Botanical ethnopharmacological and phytochemical profiles of the tested plants:

2.3.1. *Senna alexanderina* :

Synonyms:

Cassia acutifolia Delile ,*Cassia angustifolia* Vahl, *Cassia lanceolata* Forssk,*Cassia senna* L. and *Senna angustifolia* Vahl. Although recognized as two distinct species in many pharmacopoeias, *Cassia acutifolia* Delile and *C. angustifolia* Vahl. are considered botanically to be synonyms of the single species. *Cassia senna* L.(WHO., 1988).

2.3.2. Selected vernacular names:

Sanna mekka , Sana sana, Alexandria senna, Alexandrian senna, cassia, eshrid, falajin, fan xie ye ,filaskon maka, hindisana, illesko, Indian senna, ma khaam khaek, makhaam khaek, mecca senna, msahala, nelaponna, nelatangedu, nilavaka, nilavirai, nubia senna, rinji, sanai, sand hijazi, sanjerehi, sen de alejandria, sen de la india, senna makki, senna, senamikki, sennae folium, sonamukhi, Tinnevelly senna, true senna (Farnsworth *etal.*, 1995).

Common names:

Alexandrian senna ,Indian senna ,senna ,Tinnevelly senna ,true senna and senna akki .

2.3.3. Scientific classification:

Kingdom : Plantae.

Order : Fabales.

Family : Fabaceae.

Sub-family : Caesalpinioideae.

Genus : *Senna*.

Species: *alexanderina* .

2.3.4. Description:

Low shrubs, up to 1.5 m high, with compound paripinnate leaves, having 3–7 pairs of leaflets, narrow or rounded, pale green to yellowish green. Flowers, tetracyclic, pentamerous, and zygomorphic, have quincuncial calyx, a corolla of yellow petals with brown veins, imbricate ascendentprefl oration, and a partially staminodial androeceum. The fruit is a broadly elliptical, somewhat reniform, flattened, parchment-like, dehiscent pod, 4–7 cm long by 2 cm wide, with 6 to 10 seeds (Farnsworth *etal.*, 1995).

2.3.5. General appearance:

Macroscopically, the leaflets are lanceolate or lanceolate- ovate, unequal at the base, with entire margin, acute-mucronate apex and short, stout peti-
* Adopted from the volume 1 of WHO monographs on selected medicinal plants. 1 C. *Italic* Mill. Is listed in the

Malian pharmacopoeia . 364 WHO monographs on medicinal plants commonly used in the Newly Independent States (NIS) oles; sometimes broken; 1.5–5 cm in length and 0.5–1.5 cm in width , bearing fine pubescence of appressed hairs, more numerous on the lower surface (WHO ,1988).

2.3.6. Organoleptic properties:

The color is brownish yellow. (WHO ,1988). The odor is characteristic, and the taste is mucilage- like and then slightly bitter (WHO ,1988).

2.3.7. Geographical distribution:

The plant is indigenous to tropical Africa. It grows wild near the Nile river from Aswan to Kordofan, and in the Arabian peninsula, India and Somalia .(Youngken, 1950) . It is cultivated in India, Pakistan, and the Sudan. (Youngken, 1950).

2.3.8. General identity tests:

Macroscopic, microscopic examinations, and microchemical analysis and thin-layer chromatographic analysis for the presence of characteristic sennosides (sennosides A–D) (3–5). (European pharmacopoeia 1995).

2.3.9. Antioxidant Activity

The methanolic extract of *Cassia* species seeds shows stronger antioxidant activity. It was found that it exhibits stronger antioxidant activity as compared to Alphotocopherol . The phenolic active

Component, alaternin and nor-rubrofusarin glucoside isolated from extract of *Cassia species* also showed a potent free radical scavenging activity. (Chakrabarty and Chawla., 1983).

2.3.10. Antimutagenic Activity

Antimutagenic activity of a methanol extract of *Cassia species* seeds were demonstrated against aflatoxin B1 with the *Salmonella typhimurium* assay. The numbers of revertants per plate decreased significantly when this extract was added to the assay system using *Salmonella typhimurium* TA100 and/or TA98. The methanol extract was then sequentially partitioned with CH₂Cl₂, nbutanol and H₂O. The CH₂Cl₂ and n-butanol fractions possessed antimutagenic activity but

the H₂O fraction was inactive. Column chromatography using silica gel yielded pure chrysophanol, chrysoobtusin and aurantio obtusin from CH₂Cl₂ fraction *Cassiaside* and rubro-fusarin gentiobioside from the n-BuOH fraction. Each of these compounds demonstrated significant antimutagenic activity.

2.3.11. Antibacterial Activity:

De-alcoholized extract of *Cassia species* seeds inhibited the growth of *Micrococcus pyogenes* var. albus, *Micrococcus citreus*, *Cornebacterium diphtheria*, *Bacillus megatherium*, *Salmonella typhosa*, *Salmonella paratyphi*, *Salmonella schottmuelleri* and *Escherichia coli*103. (Singh 1990).

2.4. General Methods of Extraction of Medicinal plants

Many methods were used to extract the most bioactive compounds from medicinal plants these were :

2.4.1 Maceration:

During this process, the powdered crude plant is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the damp solid material is pressed, and the combined liquids are clarified by filtration or decantation after standing (Handa *et al.* , 2008).

2.4.2 Infusion:

Fresh infusions are prepared by macerating the crude plant for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude plants (Handa *et al.* , 2008).

2.4.3. Digestion:

This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable (Handa *et al.* , 2008).

2.4.4. Decoction:

In this process, the crude medicinal plant is boiled in a specified volume of water for a defined time: it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat-stable constituents (Handa *et al.* , 2008).

2.4.5. Percolatin:

With this procedure, the plant materials is moistened with solvent and all allowed to swell before being placed in one of series of percolation chamber. The material is repeatedly rinsed active with with solvent until all ingredients have been removed. Solvent is reused until it is saturated. New solvent is used on plant material that is almost completely exhausted and then re-used on subsequently less batches. This methods is more active in obtained active ingredients than the maceration technique (Raaman, 2006).

2.5. Hot Continuous Extraction (Soxhlet) :

In this methods, the finely ground crude plant materials is placed in a porous bag or "thimble" made of strong filter paper , which is placed in the chamber of the soxhlet apparatus .The extracting solvent in flask is heated , and its vapors condense in condenser. The condensed extractant drips into the thimble containing the crude drug . When the level of liquid in chamber is siphoned into flask. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method , compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent (Handa *et al .*, 2008).

2.6. Aqueous Alcoholic Extraction by fermentation ;

The extraction procedure involves soaking the crude plant material , in the form of either a powder or a decoction , for a specified period of a time , during which It undergoes fermentation and generates alcohol in situ: this facilitates the extraction of the active constituents contained in

the plant material . The alcohol thus generated also serves as a preservative . If the fermentation is to be carried out in an earthen vessel, it should not be new ; water should first be boiled in the vessel . In large –scale manufacture, wooden vates , porcelain jars or metal vessels are used in place of earthen vessels (Handa *et al .* , 2008).

2.7. Counter-current Extraction:

This is a highly effective process whereby solvent flows in the opposite direction to plant material . Unlike maceration and percolation , which are batch processes, this method is continuous . Screw extractors and carousel extractors are two types of equipment used for counter current extraction

2.8. Steps Involved in the Extraction of Medicinal plants:

According to the method adopted by (Handa *et al .* , 2008) the steps of extraction includes:

2.8.1. Size Reduction:

The dried plant material is disintegrated by feeding it into a hammer mill or a disc pulverizer which has built – in sieves . The particle size is controlled by varying the speed of the rotor clearance between the hammers and the lining of the grinder and also by varying the opening of the discharge of the mill. Usually , the plant material is reduced to a size between 30-40 meshes, but this can be changed if the need arises. (Handa *et al .* , 2008)

2.8.2. Extraction :

Extraction of the plant material is carried out in three ways:

- 1.Cold aqueous percolation
- 2.Hot aqueous extraction(decoction)
- 3.Solvent extraction (cold or hot) (Handa *et al .*, 2008)

2.8.3. Filtration:

The extraction so obtained is separated out from the macr (exhausted plant material) by allowing it to trickle into a holding tank through the built- in false bottom of the extractor, which is covered with a filter cloth. The macr is retained at the false bottom , and the extract is received in the holding tank. From the holding tank, the extract is pumped into a sparkler filter to removed fine or colloidal particles from the extract. (Handa *et al .*, 2008)

2.8.4. Spray Drying:

The filtered extract is subjected to spray drying with a high pressure pump at a controlled feed rate and temperature, to get dry powder. The desired particle size of the product is obtained by controlling the inside temperature of the champer and by varying pressure of the pump. The dry powder is mixed with suitable diluents or excipients and blended in a double cone mixer to obtain a homogeneous powder that can straight away used, for example, for filling in capsules or making tablets.

2.8.5. Concentration:

The enriched extract from percolators or extractors, known as miscella, is fed into a wiped film evaporator where it is concentrated under vacuum to produce a thick concentrated extract. The concentrated extract is further fed into a vacuum chamber dryer to produce a solid mass free from solvent. The solvent recovered from the wiped film evaporator and vacuum chamber dryer is recycled back to the percolator extractor for the next batch of plant material. The solid mass thus obtained is pulverized and used directly for the desired pharmaceutical formulations or further processed for isolation of its phytoconstituents. (Handa *et al.*, 2008)

2.9. Urinary tract infection (UTI):

The urinary system is composed of kidney, ureters, bladder and urethra. The main function of it is filtration and excretion of waste products to outside body and play a major role in water and salt and balance in the body fluids, urine which is formed by the kidneys passes to bladder through thin tubes called ureters, and via urethra to outside the body (Shao HF *et al.*, 2003)

The urinary tract is common site of infection. While 1% of male and 3% of female infants and children develop a urinary tract infection (UTI), 20% of females will have at least one UTI during life time. Bacteria periodically enter the female urinary bladder from the urethra in small numbers. They are able to bind, multiply, colonize, and invade the urinary tract in sequential order. Whether, infection ensues depends on the virulence and inoculum size of the microorganism and the adequacy of host defense mechanisms (Ferhat and Cibali., 2004).

Bacterial colonization of urine within this tract (bacteriuria) is common, result in microbial invasion of tissues responsible for the manufacture, transport and storage of urine, infection of the upper urinary tract consisting of kidney and its pelvis, is known as (pyelonephritis), infection of the lower urinary tract may involve the bladder (cystitis), urethra (urethritis), because all portion of the urinary tract are joined by a fluid medium, infection at any site may spread to involve other areas of the system (Rayan and Ray; 2004).

2.9.1. Etiology of UTI:

Over 95% of urinary tract infections are caused by a single bacterial spp, and 90% of these are *Escherichia coli*, other Enterobacteriaceae, *Pseudomonas* and Gram positive bacteria become increasingly frequent with chronic, complicated, and hospitalized patient. The Gram positive bacteria *Enterococci* are the most important, *Staphylococcus saprophyticus*, coagulase negative Staphylococci, is now recognized as the etiology in a significant majority of symptomatic infection in young sexually active women. Yeast particularly species of *Candida*, may be isolated from catheterized patients receiving antibacterial therapy, and from diabetic individuals (Dromigny *et al.*, 2002).

2.9.2. Treatment:

Urinary tract infection (UTI) can be treated for just 1-3 days with oral trimethoprim – sulfamethoxazole (TMP-SPH) or an oral penicillin e.g. ampicillin. However, *E. coli* sepsis requires treatment with parenteral antibiotics e.g. a third generation cephalosporin such as cefotaxime with or without an aminoglycoside such as gentamicin. Antibiotic therapy is usually not indicated in *E. coli* diarrhoeal disease (Miller *et al.*, 2004).

In vitro surveillance data from across the United States indicate that 10%–20% of urinary *Escherichia coli* isolates from female outpatients are resistant to trimethoprim - sulfamethoxazole (TMP-SMX) (Miller *et al.*, 2004).

Alternative therapies for uncomplicated urinary tract infections in women include fluoroquinolones and nitrofurantoin, but the activities of these agents against TMP- SMX –resistant isolates are rarely reported (TMP-SMX) (Miller *et al.*, 2004).

CHAPTER THREE

3. Materials & Methods

3.1. Type of the study

This is a descriptive cross sectional laboratory – based study .

3.2. Study Area

This study was conducted at Khartoum State, Sudan during the period from March to May 2014. Samples were taken from patients admitted at Khartoum Teaching Hospital and Omderman Teaching Hospital.

3.3. Study population

Patients who visited Khartoum Teaching Hospital and Omderman Teaching Hospital suffering from UTI.

3.4. Inclusion criteria

Patients admitted to these hospitals with urinary tract infection were recruited in this study .

3.5. Exclusion criteria

Patient under medical treatment or those already on antimicrobial treatment were excluded .

3.6. Ethical consideration

This study was approved by College of Graduate Studies of Sudan University of Science and Technology and Khartoum Teaching Hospital and Omderman Teaching Hospital and Verbal consents were taken from the patients .

3.7. Sample Size

Hundred individuals were recruited ,for taking urine randomly irrespective of sex and age.

3.8. Data collection

Data were collected from the patients using structured questionnaire involving ; age , sex , symptoms and medications.

3.9. Data analysis

Data were analyzed using Microsoft Office Excel 2007 and SPSS version 11.5.

3.10. Methods

3.10.1. Collection of specimens:

3.10.2. Urine:

The patient was asked to pass the first drops of urine and collect the mid stream urine in sterile universal container and the container was closed immediately. After collection, boric acid was added as preservative. Each specimen was labeled by code number before examination.

3.10.3. Culture:

Urine specimens were cultivated on (Cystine lactose Electrolyte Deficient) CLED medium and incubated aerobically at 37°C for overnight.

3.10.4. Identification of Isolated bacterial:

The colonial morphology and fermentation of lactose were examined macroscopically on CLED , this was observed by yellow color.

3.10.5. Gram's Stain:

Smear was prepared and fixed gently by flaming over with crystal violet for 1 minute, washed with D.W and covered with lugol's Iodine for 1 minutes, washed and decolorized with alcohol for 15 seconds, washed immediately with D.W and covered with saffranin for 2 minutes washed with D.W ,dried by air and examined microscopically with X100 (Cheesbrough, 2000).

3.11. Biochemical tests:

3.11.1. Indole test:

The organisms were inoculated by using wire loops in about 3 ml of sterile peptone water, incubated aerobically at 37°C for overnight. Drops of Kovac's reagent was added. Red ring indicates formation of indole (+ve) and yellow or brown color indicates negative result (Cheesbrough, 2000).

3.11.2. Citrate Utilization test:

The test based on the ability of an organism to utilize citrate as the sole source of carbon and ammonium salt as the sole source of nitrogen. Kossler's citrate broth was inoculated using wire loop full of organism and incubated aerobically at 37°C for overnight. Presence of bright blue color with turbidity indicates positive result and no change indicates negative result (Cheesbrough, 2000).

3.11.3. Urease test:

The organism was cultivated using straight wire in agar medium containing urea and phenol red, (when bacteria is urease producer it will break down the urea & produce ammonia). Change in pH of the medium to alkaline leads to change in color into pink color (+ve result) and no change indicates negative result (Cheesbrough, 2000).

3.12. Sugar fermentation, amino acid utilization and H₂S production:

The organism was inoculated in KIA by using straight wire by stabbing the butt and streak the slope, incubated aerobically at 37°C for over night to observe the yellow butt (glucose fermentation), yellow slope (lactose fermentation), air bubblers or cracks (formation of gas) and blacking (formation of H₂S). (Cheesbrough, 2000).

3.13. Sensitivity Testing:

Sensitivity test was performed by modified Kirby-bauer disc diffusion method. Well Isolated colonies of similar appearance were touched by using sterile loop and emulsified in 2ml of sterile normal saline; the turbidity of the suspension was matched to the turbidity of McFarland standard (0.5%). Then sterile swab was immersed in the suspension excess was removed by pressing and rotating swab against the side of the test tube above the level of the suspension. The plate of Muller – Hinton agar was inoculated by swab and allowed to dry for 2 minutes, using sterile forceps the antibiotic disc was placed on the inoculated plate and within 30 seconds of application the plate was incubated plate and within 30 second was incubated aerobically at 37°C for 24 hours. Each zone of inhibition was measured in (mm) and interpreted by interpretive chart, and then organism reported as sensitive and as resistant. (Cheese brough, 2000).

3.14. Test microorganisms

3.14.1. Standard microorganisms:

<i>Escherichia coli</i>	ATCC 25922(Gram -ve bacteria)
<i>Kelebsiella pnemoneae</i>	ATCC 53657(Gram -ve bacteria)
<i>Pseudomonas aeruginosa</i>	ATCC 27853 (Gram -ve bacteria)
<i>Staphylococcus aurous</i>	ATCC 25923(Gram +ve Bacteria)

National Collection of Type Culture (NCTC), Colindale, England.

American Type Culture Collection (ATCC) Rockville, Maryland, USA.

These standard bacteria isolates were provided by Medicinal and Aromatic plants Center , khartoum- Sudan .

3.15. Determination of minimum inhibitory concentration (MIC) by agar plate dilution method:

Plates were prepared in the series of increasing concentrations of the plant extraction in the following order 100, 50, 25, 12.5. The bottom of each plate was marked off into 4 wells. The viable organisms tested were grown in broth over night to contain 10^8 CFU / ml.

Loop-full of diluted culture was spotted with a standard loop that delivers 200 microliter(-Concentration 10 mg | ml (100%) at 0.1 ml |cup.) on the surface of each segment and then incubated at 37 °C for 24 hours.

The principle of the agar plate dilution is the inhibition of the growth on the surface of the agar by the plant extracts incorporated into the medium.

The end point (MIC) is the least concentration of antimicrobial agent that completely inhibits the growth. Results were reported as the MIC in mg/ml.

3.16. Extraction of the Medical plant

3.16.1. Collection and preparation of plant Samples

The selected plants for this work was *Alexandria senna* , they were obtained from AL- Sooq AL- Arabi in Khartoum . The dried plant samples were cleaned from dust and grass then they were separately crushed to a powder by using sterilized mortar and pestle.

3.16.2. Extraction by Soxhlet Extractor

Fifty grams from Sudanese *Alexandria senna* were extracted sequentially into 500 ml chloroform and methanol. Resulting extraction in two solvents were evaporated and concentrated using the rotary evaporator at 50 C. At the end of this work two types of extraction were obtained (Abeysinghe,2010).

3.16.3. Preparing the plant Extraction for Testing the Antibacterial Activity

Each type of extraction was dissolved in methanol and four different concentrations were prepared 100%, 50% , 25%, and 12.5% for testing their antibacterial activity by (one gram with ten from solvent , that represented (100%) and one ml from 100% with one ml of solvent (50%) and so on . (Toudert *et al* , 2009)

CHAPTER Four

4. Results

4.1. Bacteriological result :

Sixty six patients (66%) were examined from Khartoum Hospital and 34 (34%) from Omdurman Hospital . (Table 1)

Table 2 shows males and females distribution which were 33(33%) males and 67(67%) females.

Table 3 show the growth of bacteria in urine samples , 63 (63%) showed growth whereas 37 (37%) with no growth ,Gram reaction revealed that 30 (48%) isolates were Gram positive and 33 (52%) were Gram negative , (table 4). , The isolated were identified phenotypically as follows : *Staphylococcus aureus* 21 (33.3%) , *Klebsiella pneumoniae* 6 (9.5%) ,*Protus mirabilis* 7(11.1%), *Enterococcus faecalis* 4(6.3%) , *Escherichea coli* 12 (19%), *Pseudomonas aeuroginosa* 5 (7.9%), *C.albicans* 5 (7.9%) and *Citrobacter* 3 (4.7%) (table 5) (figure 1).

Table (1) Distribution of Specimens according to hospital

Hospital	frequency	Percentage
Khartoum	66	66%
omdurman	34	34%
Total	100	100%

Table (2) Distribution of Males and Females samples

Sex	frequency	Percentage
Males	33	33%
Females	67	67%
Total	100	100%

Table (3) Frequency of bacterial growth

Bacteria	frequency	Percentage
Growth	63	63%
Non Growth	37	37%
Total	100	100%

Table (4) Gram reaction for isolated bacteria

Gram reaction	frequency	Percentage
Gram positive	30	48%
Gram negative	33	52%
Total	63	100%

Table (5) Type of bacteria isolated and percentage

Bacteria	frequency	Percentage
<i>S.aureus</i>	21	33.3%
<i>Entero.fecales</i>	4	6.3%
<i>E.coli</i>	12	19%
<i>K.pneumoniae</i>	6	9.5%
<i>Protus mirabilis</i>	7	11.1%
<i>Pseudo.aeruginosa</i>	5	7.9%
<i>Citrobacter spp</i>	3	4.7%
<i>C.albicans</i>	5	7.9%
Total	63	100%

The minimum inhibitory concentration (MIC) of *Senna alexandrina* extracts using chloroform, methanol and drugs on ATCC organisms are shown in table 6,7 and 8.

Table (6) The MIC of *Senna alexandrina* leaves chloroform extract to standard organisms (ATCC)

Extract	Solvent	Conc.	<i>St.aureus</i>	<i>E.coli</i>	<i>K.pneum.</i>	<i>Ps.aerog.</i>
<i>Senna alexandrina</i> leaves	CH ₂ CL ₃	100	20	17	14	12
		50	19	15	11	9
		25	15	9	-	-
		12.5	11	-	-	-

Note : CH₂CL₃ (Chloroform).

-Concentration 10 mg | ml (100%) at 0.1 ml |cup.

Interpretation of Results :

-Mean diameter of growth inhibition zone(MDIZ) in (mm).

IF MDIZ :-

-More than 18 sensitive

-14-18 moderate

-Less than 14 Resistant

(-) No activity

P-value = insignificant

Minimum inhibitory concentration (MIC):

-*St.aureus* =19 mm (50) more effective .

-*E.coli* =17 mm (100)

-*K.pneumoniae* = 14mm (100)

-*P.aeruginosa* = 12mm(100)

Table (7) The MIC of *Senna alexandrina* leaves methanolic extract to standard organisms (ATCC)

Extract	Solvent	Conc.	<i>St.aureus</i>	<i>E.coli</i>	<i>K.pneum.</i>	<i>P.aerog.</i>
<i>Senna alexandrina</i> leaves	MeoH	100	15	17	14	18
		50	14	15	11	15
		25	12	11	-	10
		12.5	9	7	-	-

Note :

-MeoH (methanol)

-Concentration used 10mg| ml (100%) at 0.1 ml |cup.

Interpretation of Results :

Mean diameter of growth inhibition zone(MDIZ) in (mm).

IF MDIZ :

More than 18 sensitive

14-18 modrate

Less than 14 resistant

(-) No activity

Minimum inhibitory concentration (MIC):

-*S.aureus* = 15 mm (100).

-*E.coli* = 15 mm (50).

-*K.pneumoniae* = 14mm (100).

-*P.aeruginosa* = 15 mm (50).

Table (8) Antibacterial activity of reference drugs against Standard organisms (ATCC)

Drug	Conc. Used mg/ml	Standard organism used MDIZ mm			
		<i>St.aureus</i>	<i>E.coli</i>	<i>K.pneum.</i>	<i>P.aerog.</i>
Ciprofloxacin	5	33	34	26	24
Gentamicin	10	31	29	22	10
Amoxicillin	20	7	19	-	-
Co-trimoxazole	23	35	30	16	-

The MIC of the *Senna alexandrina* of isolated organisms is shown in table 9 using different concentration of the methanol extracts, 100 mg/ml concentration was more effective on all the isolates whereas the concentration of 50 mg/ml had an effect on five groups of isolates, the concentration 12.5 mg/ml had no activity on all isolates.

Table (9) Number of isolated bacteria and their minimum inhibitory Conc. (MIC) of methanol extract of *Senna alexandrina* leaves at different concentration

Bacteria	Concentration of extract (mg/ml) and No. of organism(%)			
	100	50	25	12.5
No.isolated				
<i>S.aureus</i> 21(100%)	15(71.4%)	4(19%)	2(9.5%)	-
<i>E.fecales</i> 4(100%)	4(100%)	-	-	-
<i>E.coli</i> 12(100%)	3(25%)	5(41.6%)	4(33.3%)	-
<i>K.pneumoniae</i> 6(100%)	6(100%)	-	-	-
<i>Prot. mirabilis</i> 7(100%)	4(57.1%)	3(42.8%)	-	-
<i>Ps.aeruginosa</i> 5(100%)	2(40%)	3(60%)	-	-
<i>Citrobacter</i> 3(100%)	2(66.6%)	1(33.3%)	-	-

Note : (-) No activity .

The antibacterial activity of the tested antibacterial drugs against isolated bacteria is shown in table 10. All isolates were sensitive to gentamicin and ciprofloxacin ,whereas the other two drugs showed variable effects on bacterial growth .

The MIC of *senna alexandrina* extracts using chloroform at different concentrations on the groups of the isolates is shown in table 11, All the isolates were sensitive to the extract at 100 mg/ml concentration but no activity of the extract at 12.5 mg/ml .

Table.(10) Number of isolated bacteria and their minimum inhibitory Conc. (MIC) of Chloroform extract of *Senna alexandrina* leaves at different concentration

Bacteria	Concentration of extract (mg ml) and No. of organism(%)			
	100	50	25	12.5
<i>St.aureus</i> 21(100%)	13(61.9%)	6(28.5%)	2(9.5%)	-
<i>E.fecales</i> 4(100%)	4(100%)	-	-	-
<i>E.coli</i> 12(100%)	7(58.3%)	3(25%)	2(16.6%)	-
<i>K.pneumoniae</i> 6(100%)	6(100%)	-	-	-
<i>Prot. Mirabilis</i> 7(100%)	4(57.1%)	2(28.5%)	1(14.2%)	-
<i>Ps.aeruginosa</i> 5(100%)	3(60%)	2(40%)	-	-
<i>Citrobacter</i> 3(100%)	3(100%)	-	-	-

Note: (-) = No activity.

Table (11) Antibacterial activity of reference drugs against isolated bacteria

Bacteria	Ciprofloxacin	Gentamicin	Amoxacillin	Co-trimoxa.
<i>St.aureus</i>	S	S	S	S
<i>E.fecales</i>	S	S	S R	S
<i>E.coli</i>	S	S	R	S
<i>K.pneumoniae</i>	S	S	R	S R
<i>Prot. mirabilis</i>	S	S	S R	S
<i>Ps.aeruginosa</i>	S	S	R	R
<i>Citrobacter</i>	S	S	R	R

Note:

(S) Sensitive , (R) Resistant .

Gas chromatography results as show in table 12 and figure 1 revealed that *senna alexandrina* contain 12 phytochemical components The active ingredients was not evident by this study .

Table (12) Gas chromatography analysis of *Senna alexandrina* leaves

peak	Retention time	Area	Area%	Name
1	41.225	3151055	7.11	5-o-acetyl-thio-octyl-beta-l-rhamnofuranoside
2	43.187	4722094	10.66	Acetic acid 2(2-tert-butyl-4methyl-6-oxo phenyl
3	43.987	3151342	7.11	Beta-l-rhamnofuranoside
4	48.098	2649324	5.98	Stigmasterol
5	52.315	7989693	18.03	Gamma-sitosterol
6	53.480	4809991	10.85	Stigmata-5,24(28)-dien-3-ol (3.beta.)
7	54.992	4425562	9.99	Stigmata-5,24(28)-dien-3-ol (3.beta.22E) –(CAS) Stigmasterol
8	57.181	8740372	19.72	(3.beta)-(CAS)24.beta-Ethyl-5delta-cholesten-3beta
9	57.827	2645532	5.97	Fucosterol
10	58.222	285490	0.64	3,45,6,7,8-hexamethoxyflavone
11	59.045	458478	1.03	2-benzyl-3-phenyl-4-anilinoquinoline
12	59.757	557056	1.26	33-norgorgosta-5,24(28)-dien-3-ol

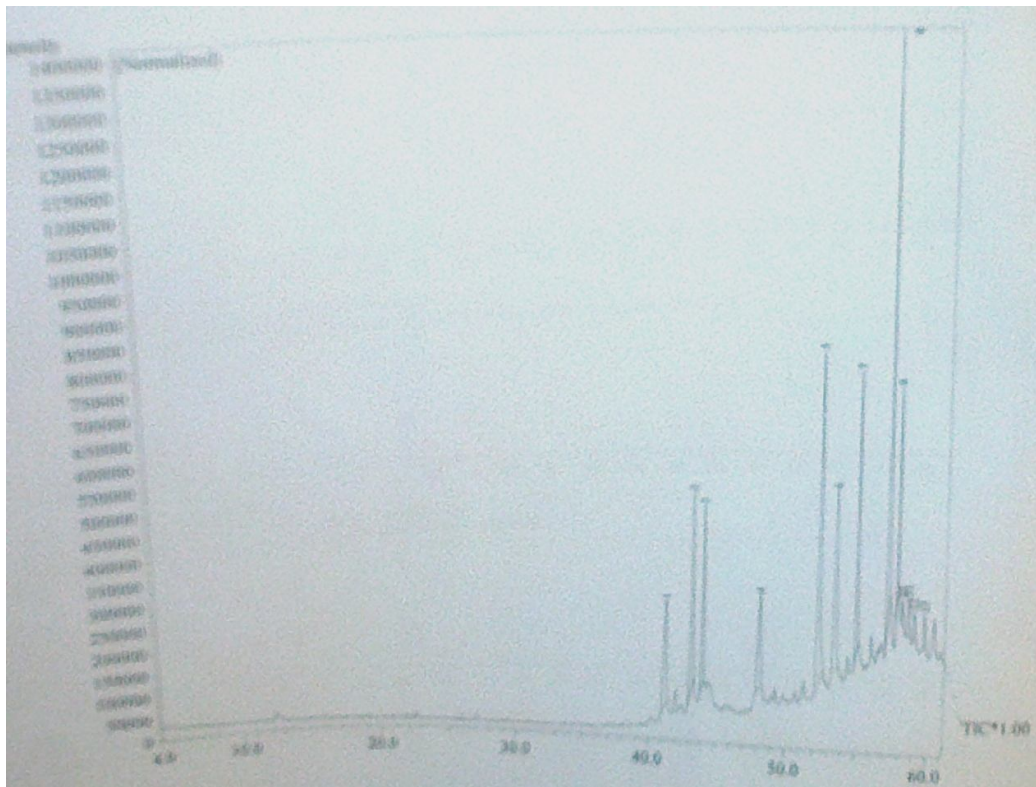


Figure 1. The phytochemical components found in *senna alexandrina*

CHAPTER FIVE

5.1. DISCUSSION

The aim of this study was to investigate the antibacterial activity of medicinal plant (*Cassia Senna (Sanna Makka)* or (*Senna alexanderina*) against isolated pathogenic bacteria. The zone of inhibition produced by the extracts against the test organisms was recorded as a measure of antimicrobial activity of the of the plant extracts, the results showed that, the methanol and chloroform extracts for the tested medicinal plants had moderate activity against all the tested pathogenic bacteria these results agree with those obtained by Hashim *et al.* (2010) and Bonjar *et al.*, (2004). On the other hand our results disagree with results obtained by Shyamala and Thangaraju ., (2012),. This variation may be due to difference in method of sensitivity testing. The methanol extract of *Senna alexanderina* leaves exhibited high antibacterial activity against *E.coli* , *P. aeruginosa* and moderate with *St. aureus* , *K. pneumoniae*, this is come in line with reports of Hashim *et al.*, (2010). However the chloroform extract of *Senna alexanderina* leaves exhibited high antibacterial activity against *St. aureus* , and moderate with *K. pneumoniae* and *P. aeruginosa*. It was observed that the methanol extract exhibited high antibacterial activity against *E.coli* , *P. aeruginosa* more than Amoxicillin . The antibacterial activity of reference drugs against the standard bacteria showed high sensitivity to Ciprofloxacin, Gentamicin , Amoxicillin and Co-trimoxazole , In some exception, *K. pneumoniae* was resistant to Amoxicillin and

also *P. aeruginosa* was resistant to Amoxicillin and Co-trimoxazole, this result similar to Sahar and Hamed Elneel., 2013. The chloroform extract activity was more active than methanolic extract, the best inhibition zone was observed for chloroform extract as 20 mm in diameter against *S. aureus* and 17 mm against *E.coli* at concentration of 100 mg/ml compared with antibiotic tested. Also in methanolic extract best inhibition zone was 18 mm in diameter against *P. aeruginosa* at concentration of 100 mg/ml compared with antibiotic tested. These result agreed with that obtained by Hashim *et al.* (2010) and Bonjar *et al.*, (2004). The variation between plant extracts and standard antimicrobial drugs may due to the mixtures compound in the plant extracts compared to pure compound in standard antibiotics (Gatsing *et al.*, 2010). The plant extracts has been analyzed and revealed the presence of flavonoids, glycosides, phenols, n carbohydrates, saponins, and tannins in most of the selected plants. Similar extracts were also obtained by Sahar (2013) from guava leaves these could be responsible for the observed antimicrobial property. These bioactive compounds are known to act by different mechanism and exert antimicrobial action. The tannins bind to proline rich proteins and interfere with the protein synthesis (Shimada, 2006). Flavonoids are hydroxylated phenol substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective on antimicrobial substances against a wide array of microorganisms.

These activities are probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Marjorie, 1999). Antimicrobial property of saponins is due to its ability to cause leakage of proteins and certain enzymes from the cell (Zablotowicz *et al.*, 1996).

5.2. CONCLUSION:

The chloroform and methanol extracts of *senna alexandrina* leaves are moderate effective against tested bacteria.

Senna alexandrina leaves have activity against infection, which justify their traditional uses as a UTI agent and diarrhea.

Senna alexandrina leaves extracts were not effective than antibiotics in combating the pathogenic bacteria studied.

The efficiency of the antibacterial of the extracts was found to increase by increasing the concentration.

5.3. RECOMMENDATIONS:

1. Based on this study and results, it is recommended that to identify the active ingredients in the extracts responsible for the antibacterial activity using gas chromatography or other techniques.

2. Determination of the minimum inhibitory concentration (MIC) for the active ingredients on each bacterium.

3. Determination of the toxicity of the active ingredients.

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APPENDICES

. Appendix(1):

Kligler Iron Agar (KIA):	Grams/litre:
LAB lemce powder	3.0
Yeast extract	30.0
Peptone	20.0
Sodium chloride	5.0
Lactose	10.0
Dextrose(glucose)	1.0
Ferric citrate	0.3
Phenol red (indicator)	0.05
Sodium thiosulphate	0.3
Agar	12.0

pH: with in the range 7.2 -7.6 at room temperature .

sterilization : in autoclave at 121c for 15 minutes.

koser citatre :-	Gram/Litre
Sodium ammonium phosphate	1.5
Potassium dihydrogen phosphate	1.0
Magnesium sulphate	0.2
Sodium citrate	2.5

Bromothimol blue 0.016

pH : with in the range 7.2-7.6 at room temperature .

sterilization : in autoclave at 121 c for 15 minutes.

(6)Muller Hinton Agar: Grams/litre

Beef dehydrated in fusion from 300.0 casein hydroysate 17.5

Starch 1.5

Agar 17.0

pH: with in range 7.2-7.4

sterilization : in autoclave at 121c for 15 minutes .

Nutrient Agar: Grams/litre

Lab lemco powder 1.0

Yeast extract 2.0

Peptone 5.0

Sodium chlorite 5.0

Agar 15.0

pH: with in the range 7.2-7.6 at room temperature.

Sterilization: in autoclave at 121c for 15 minutes.

Peptone water: Gram/litre

To make about 65 bottles

Peptone 2g

Sodium chlorite	1g
Distilled water	200ml

pH: with in range of 7.0-7.4

sterilization :in autoclave of 121c for 15 minutes.

Urea Medium:	Grams/litre
Peptone	1.0
Glucose	1.0
Sodium chlorite	5.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Phenol red	0.012
Agar	15.0

pH: with in range of 6.6-7.0.

sterilization: in autoclave at 121c for 15 minutes.

Appendix (2):

Reagents:

1-Acetone alcohol decolorizer:

To make 1litre

Acetone	500ml
Ethanol or methanol , absolute	47ml
Distilled water	25ml

Storage : in safe place , (highly flammable).

2-Kovacs indole reagent:

4-dimethyle amino benzaldehyde	2g
150 amyl alcohol (3-methyl 1-butanol)	30ml
Hydrochloric acid concentrated	10ml

Storage: at room temperature.

3-Normal saline:

Normal to make 1litre

Sodium chlorite	8.5g
Distilled water	to 1litre

Storage: at room temperature.

Appendix (3):

1-Crystal violet:

To make 1 litre

Crystal violet	20g
Ammonium oxalate	9g
Ethanol or methanol, absolute	95ml
Distilled water	to 1 litre

Storage: at room temperature.

2-Lugols Iodine:

To make 1 litre

Potassium iodine	20g
Iodine	10g
Distilled water	to 1 litre

Storage: at dark place at room temperature.

3-Safranin:

Safranin	0.5g
Distilled water	100ml

Appendix (4):

Instruments:

1. Sensitive balance	OHAVS	China.
2. Autoclave	Dioxin's surgical Instrument (LTD)	England.
3. Hot air oven	Engineering Widnescheshire	UK.
4. Incubator Torrepicenardicr		Italy
5. Refrigerator		Cold air
6. Microscope	Olympus Optical. LTD	Japan.
7. Water bath	Weston. S. Mares merest	England.

Media:

The Following media were obtained from HI media laboratories PVT. LTD India.

- Cystine Lactose Electrolytes deficient medium (CLED).
- Kligler Iron agar (KIA).
- Peptone water.
- Urea agar Medium.
- Mueller Hinton agar Medium.
- Kosser's citrate broth medium.

Reagent's and Solutions:

1. Distilled water.
2. Normal Saline.
3. 70% alcohol.
4. Kovac's reagent.
5. Lugol's Iodine.
6. Safranin.
7. Crystal Violet
8. MacFarland Standard

Other Materials:

1. Benzen burner.
2. Metal Loops.
3. Test tubes.
4. Oil.
5. Containers.
6. Petridishes.
7. Slides.
8. Pipettes.

Antimicrobial disc:

Ciprofloxacin ,Gentamicin ,Amoxicillin, ,Co- trimoxazol

Gas chromatography of Senna alexanderina (methanol extract)

In gas chromatography , the mobile phase (or moving phase) is a carrier gas, usually any phase inert gas such as helium or an un reactive gas such as nitrogen . The stationary phase is a microscopic layer of liquid or polymer on an inert solid support , inside a piece of glass or metal tubing called a colum (a homage to the fractionating colum used in distillation). The instrument used to perform gas chromatography is called a gas chromatograph (or aerograph, gas separator).

The gaseous compounds being analysed interact with the walls of the colum , which is coated with a stationary phase . This causes each compound to elute at a different time , known as the retention time of the compound. The comparison of retention times is what gives GC its analytical usefulness.

Methylation steps

Two grams from the sample was taken and transferred in a test tube , then 7 ml of alcoholic sodium hydroxide was prepared by {2g (NaOH) to 100 ml (methanol) } , and 7ml of alcoholic sulphuric acid was added which was prepared by {1 ml (H₂SO₄) to 100ml (methanol) }. After that the sample was put for overnight in the room temperature.

Then a suitable volume from normal hexane (n-Hexane) was added, also a suitable amount of super saturated sodium chloride was added. Then it was shaken gently, after that two layers were appeared, the upper one is organic layer and the lower is aqueous layer. Finally a volume of the organic was taken and diluted by diethylene and injected in Gas chromatography Mass spectrometer(GC-MS) by using 1micrometer syringe.

Questionnaire

Sudan University of Science and Technology

College of Medical Laboratory Science

Department of Microbiology

Hospital:

.....

Name:

.....

Age:.....Years

Sex: Male..... Femle.....

Diagnosis UTI

History of antibiotic intake?

Yes: ()

NO: ()

History of chronic disease?

Yes: ()NO: ()

Investigation:

Specimen: Middle stream urine

Specimen NO()

Lab investigation:

Microscopy:.....

Culture:.....

Signature:.....Date / /2012



Figure (2) Senna alexanderina



Figure (3) Senna alexanderina plant



Figure (4) Senna alexanderina pods



Figure (5) Sensitivity test of standard bacteria with antibiotic



Figure (6) Color plate5-Biochemical tests of *E.coli*



Figure (7) Gas chromatography analysis system

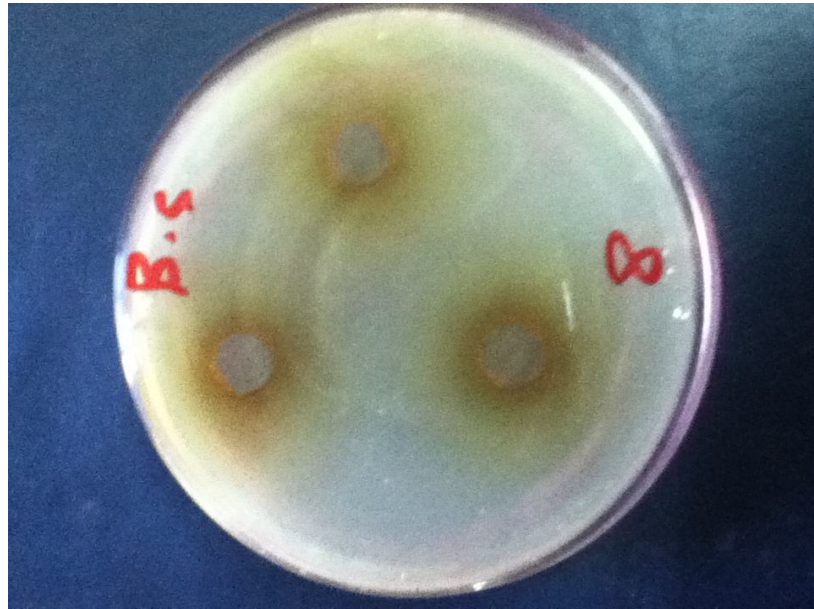


Figure (8) Zone of inhibition of different concentration of plant against Bacteria

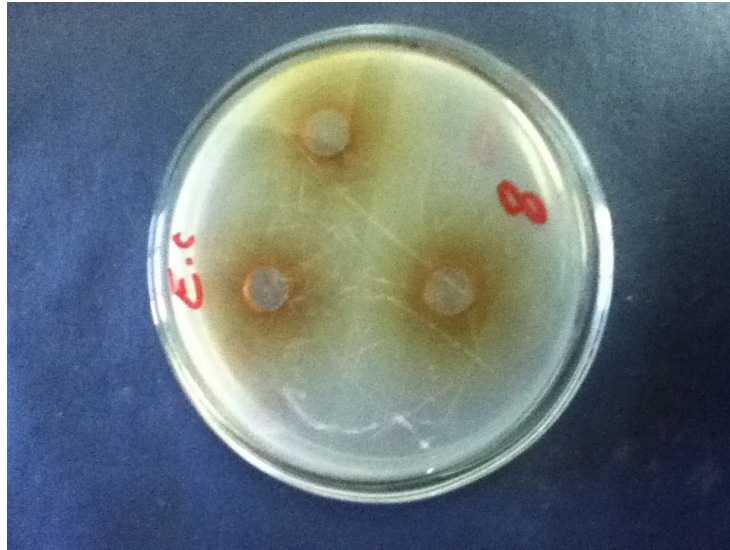


Figure (9) Zone of inhibition of different concentration of plant against Bacteria



Figure (10) Zone of inhibition of different concentration of plant against Bacteria