CHAPTER ONE

1-Introduction :

1-1 Antibacterial:

Antibacterial anything that destroy bacteria or suppresses their growth or their ability to reproduce. Heat, chemical such as chlorine and antibiotic drug all have antibacterial properties (1)

Mechanism of action of antibacterial is inhibition of cell wall synthesis, inhibition of cell membrane function. Inhibition of protein

Synthesis. inhibition of nucleic acid synthesis, inhibition of other metabolic process such as aminoglycosides,cephalosporin,macrolides,pleuromutilns,lincosamides,penicillin,quinolone,sulphanamides,tetracyclin(1).

1-2-Acacia Nilotica:

1-2-1 scientific classifications:

Kingdom :plantae

Division : magnoliophyte

Class :magnolias

Order :Fabless

Family :Fabaceae

Genus :acacia

Species :Nilotica

1-2-2- Botanical Description: -

it gum Arabic tree 5-20m high with a dense aspheric crown stems and branches usually dark to black colored, fissured bark, grey-pinkish slash, exuding a reddish low quality gum the tree has thin, straight, light, grey spines in axillary pairs, usually in 3to12 pairs,5to 7.5(3in) long in young tree, mature trees commonly without thorns. The leave is pinnate with 3-6 pairs of pinnate. flowers inglobulous

head 1.2-1.5cm in diameter of a bright golden yellow color, set up either axillary or whorl on peduncles 2-3cm long located at the end of the branches .Pods are strongly constricted, hairy, white- grey, thick and softly tomatoes it is seed number approximately 8000/kg. (2)

1-2-3-Distribution: -

acacia Nilotica, scented thorn acacia is native from Egypt across the Maghreb and Sahel, south to Mozambique and natal, and east through Arabian Peninsula to Pakistan, India, Burma and Australia. acacia Nilotica is restricted to river in habitats and seasonally folded areas within its native range, however in its introduce range its spread by livestock and growth outside riparian areas (2).

1-2-4-Traditional uses: -

1-2-4-1-forageandfodder

In part of its range small stock consume the pods and leaves, but elsewhere it is also very popular with cattle. Pods are used as a supplement to poultry rations in India. Dried Pods are particularly sought out by animals on range lands .in India branches are commonly lopped for fodder. Pod are best fed dry as a supplement, not as green fodder (3).

1-2-4-2-Hedges

Acacia Nilotica make good protective hedge because of its thorns (3).

1-2-4-3-Medicine

Acacia Nilotica may be also used for medicine purposes, as demulcent or for conditions such as gonorrhea leucorrhoea, diarrhea, dysentery or diabetes. it is styptic and astringent. in siddha medicine, the gum is use to consolidate otherwise watery semen (3).

1-2-4-4-Bark

To Hartwell, African Zulu take bark for cough. it acts as an astringent and its use to treat diarrhea, dysentery and leprosy (3).

1-2-4-5-Barkandroot

Maasai are intoxicated by the bark and root decoction, said to impart courage, even aphrodisiac, and the root is said to cure impotence (3).

1-2-4-6-Bark or gum

In west Africa, the bark or gum is used to treat indurations of liver and spleen, condylomas and excess flesh. sap or bark, leaves and young pods are strongly astringent due to tannin and are chewed in Senegal as an antiscorbutic (3).

1-2-4-7-Leaves

The bruised leaves are politicked and use to treat ulcers (3).

1-2-4-8-Resin

In Lebanon, the resin is mixed with orange-flower infusion for typhoid convalescence.

1-2-4-9-Root

The chip uses the root for tuberculosis. in Tonga, the root is used to treat tuberculosis

1-2-4-10-Wood

In Italian Africa, the wood is used to treat smallpox. In Ethiopia, certain part of the tree is used as a lactagogue.

1-2-4-11-Lumber

The tree’s wood very durable if water seasoned” and its uses include tool handles and lumber for boats. The wood has a density of about 1170kg\m.

1-2-4-12-Twige

In most parts of Indian sub-continent, thin twigs are chewed and use as tooth brush.

1-2-4-13-Seed pods

Egyptian Nubians believe that diabetics may eat un limited carbohydrate as long as they also consume powdered pods.

1-2-5-Constituents: -

The stem bark extract of the plant possessed the active compound (terpenoids, tannins, alkaloids, saponins glycosides phenol resin oleosin steroids terpenesphalobataninGallic acid protocatecuicacidpytrocatcacid epi-gallacactechin-7gallate epi gallocatechin-5-7 digallae (3)

1-3 Rational and Justification:

Recently, modern a societies face serious problems with using of the synthetic chemotherapeutic agent in order to their multiple disadvantage such as: harm full side effect, height cost and development of multi resistance strains due to recurrent usage. So the traditional medication –especially in the middle and Far East societies started to play and important role as a safer and cheaper alternative solution.

In Sudanese culture and as part traditional medication, acacia Nilotica are used for treatment of many infections are respiratory tract and uro -genital tract infection

1-4-Objectves:

1-4-1-General objective:

To detect anti-bacterial acacia Nilotica on the certain bacteria.

1-4-2-Specific objective:

1-To detect ant bacterial acacia Nilotica on staphylococcus aureus, pseudomonas aeruginosa and Escherichia coli.

2-To determine the minimal bacterial concentration(MBC)

1-5-Literature review:

Organisms:

Staphylococcus aureus:

Staphylococcus aureus are gram positive cocci of uniform size, occurring characteristically in group but also single and in pair, non-motile ,non-capsulated (4)

Description:

It is gram positive cocci , non-motile , non-capsulated (4).

Normal habitat:

Staphylococci are distributed in environment. this form part of normal microbial flora of the skin, upper respiratory tract and intestinal tract (4)

Pathogenicity :

S. aureus cause boils, styles, pustules, impetigo, infections of wounds (cross-infections), ulcers and burn , osteomyelites , mastitis, septicemia, meningitis, pneumonia, and pleural empyema. also toxic food poisoning (rapid onset, no fever), toxic shock syndrome and toxic skin exfoliation.

S. aureus is carried in the nose and on the skin of many healthy people. It is easily spread in hospital, particularly on surgical wards. (4).

Extra cellular enzymes and toxins produced by strains of S, aureus that contribute to its invasiveness and pathogenicity

- Coagulase: clots plasma, interferes with phagocytosis , facilities spread in the tissues
- haemolysins: lyse red cells
- leucocidins: kill leucocyte
- fibrinolysis: digest fibrin.
- Lipase: breaks down fat.
- hyaluronidase: facilitates spread in tissue by destroying hyaluronic acid (component of connective tissue).

- Protein a: antiphagocytic (prevent complement activation).
- enterotoxins (heatstable): cause food poisoning (particularly vomiting).
- Toxic chock syndrometoxin-1: Chock rash, desquamation of skin.
- Chemotaxis inhibitory protein: inhibit migration and activation of neutrophils.
- Epidermolysistoxins A and B:generalized peeling of the skin.

Laboratory features:

Specimen: pus and swab from infected sites ,sputum, cerebrospinal fluid, blood for culture, feces, vomit and the remains of food when food poisoning is suspected.

Culture: staphylococci grow well aerobically and in a carbon dioxide enrich atmosphere. Most strains also grow an aerobically, but less well.

Temperature range for growth is 10 – 42 C with an optimum of 35-37 c.

Blood agar, chocolate (heated blood) agar: S. aureus produce yellow to cream or occasionally white 1-2 mm in diameter colonies after overnight incubation (see color plates 22 and 23. pigment is less pronounced in young colonies. some strains are beta – hemolytic when grown aerobically. colonies are slightly raised and easily emulsified.

macConkey agar: smaller (0.1- 0.5 mm) colonies are produced after overnight incubation at 35-37 c. most strains are lactose fermenting.

mannitol salt agar: A useful selective medium for recovering. S. aureus from fecal specimens when investigating staphylococcal food – poisoning. it can also be used to screen for nasal carriers. S. aureus ferments mannitol and is able to grow on agar containing 70-100 g/l sodium chloride (plus 4 mg /l methicillin) is recommended, particularly for is isolating MRSA strains.

Biochemical tests: - S. aureus is:

- coagulase positive
- DNA-ase positive
- Catalase positive

Commercially produced test kits to identify S. aureus:

Several latex agglutination test kits are available to identify S. aureus based on detection of clumping factor and, or, protein A (latex particles are sensitized with fibrinogen and immunoglobulin G). the manufacturers literature must be read carefully, particularly regarding specificity and sensitivity and whether the test detects MRSA strains.

Pastorex staph plus test:

This latex agglutination test kits are available from Bio-Rad laboratories. it detects all strains of S. aureus, including up to 95% MRSA strains (reagent contain Abs to the capsular polysaccharides found in MRSA as well as fibrinogen and protein A). A test kits of 50 test has a shelf – life of 12-18 months when stored 2-8 c.

Dry spot staph test plus:

This latex agglutination test is available from oxoid uses reagent that has been dried on a reaction card. it detects up to 97% of S. aureus strains. including most MRSA. Colonies of S. aureus are emulsified in saline and mixed with the dry reagent. agglutination of the blue latex particles indicates a positive test. test cards can be stored at room temperature (up 25 c). each pack contains 120 tests.

Other pathogenic staphylococcus species:

- Staphylococcus saprophytic: causes UTI in sexually active women.
- Staphylococcus epidermidis: causes endocarditis and bacteremia following infection in cannula , indwelling catheters, shunts or other appliances positioned in the body. infection is difficult to treat due to resistance of S. epidermidis to many antimicrobials.

Culturally the colonies S. epidermidis are white and usually non hemolytic. the colony of S. saprophytic us may be white or yellow. they are non-hemolytic. growth may not occur in macConkey agar. staphylococcus saprophytic us and staphylococcus epidermidis are coagulase negative

Escherichia coli:

Description:

It is one of the most important members of enterobacteriaceae. gram negative rod, non-spore , some strain are capsulated , lactose fermenting ,some strain are gas produce (4)

Normal habitat:

Escherichia coli organism form part of normal microbial flora of human and animal. Also found in environment, water, vegetation and soil. (4)

Pathogenicity :

Urinary tract infections .E. coli is commonest pathogen isolated from patients with cystitis. recurring infection are common in women.

Infections of wounds, peritonitis, sepsis and endotoxin induce shock.

Meningitis and bacteremia in neonates. E. coli capsular type K1 is associated with neonatal meningitis.

Diarrheal disease: infantile gastroenteritis ,travelers' diarrhea , dysentery, and hemorrhagic diarrhea which may progress to hemolytic uremic syndrome.

E. coli strains associated with diarrheal disease

- ETEC (Enter toxogenic E. coli) Causes watery
- (secretory)diarrhea due to the production of plasm mediated toxin (LT, ST) in infants and adults, particularly in developing countries. It is often referred to as travelers' diarrhea. Many serogroups are involved.
- EPEC(Entero pathogenic E. coli): Causes vomiting, fever, and prolonged diarrhea mainly in infants (less than 2y). Due to bacteria adhering to epithelial cells, multiplying and causing lesion. many serogroups are involved. EPEC is major problem in developing countries.
- EIEC(Entero invasive E. coli): cause dysentery (similar to shigellosis), fever and colitis, with blood, mucus, and many pus cells in fecal specimens. Due to bacteria invading and multiplying in epithelial cells. Many serogroups are involved.
- EHEC(Entero hemorrhagic E. coli): Causes life-threatening hemorrhagic diarrhea (colitis)in all ages, without pus cells, and often without fever. It can progress to hemolytic been uremic syndrome with renal failure. EHEC has been reported mainly from Europe and north America. Outbreak have also occurred in refugee camps in Mozambique. Swaziland and malwi. EHEC is duo to cytotoxins damaging vascular endothelial cell, and it is mainly associated with serogroup0157:H7. It is sometimes referred to VTEC

(verocytotoxin- producing E, coli, because it is toxic to Veromonkey cell in culture). infection occurs by ingestion contemnedmeat produce, un-pasteurized milk and dairy products.

- Egg Ec (Entero aggregative E. coli): cause chronic watery diarrhea and vomiting, mainly in children. due to bacterial adhering to tissue cell often in stacks (aggregates).

Laboratory features

Specimen: depend on the site of infection, specimen include urine, pus, feces, cerebrospinal fluid(infant), and blood for culture.

Culture

E. coli is an aerobe and facultative an aerobe. Optimum temperature for growth is 36-37C with most stains growing over the range 18-44C.

Blood agar:

E. coli produce 1-4mm diameter colonies after overnight incubation. The colonies may appear mucoid. Some strains are hemolytic.

MacConkey agar and CLED agar:

E. coli ferment lactose, producing smooth pink colonies on macConkey agar and yellow colonies on CLED agar. Some strains(eg .in active strains) are late or non-lactose fermenting.

Sorbitol macConkey agar:

E. coli (VETC)0157 is non sorbitol–fermenting, producing color less colonies. Most their E. coli strains and other Enterobacteria ferment sorbitol. E. coli(VTEC)0175can be identify by testing the colonies using 0157 latex reagent.

XLD and DCA agar:

Yellow colonies are produced on XLD agar. Growth of E. coli is usually inhibiting on DCA agar.

KIA agar (kliglar iron agar):

Most strains of E. coli produce an acid deep and acid slope with gas production and no H2S blackening (similar to other lactose fermenting coli forms).

Biochemical reaction:

Most strains of E .coli

- Indole positive.
- Lysine decarboxylase(LDC)positive.
- Beta-glucuronides(PGUA)positive (E .coli 0157 is PGUA negative).
- Reduce nitrite to nitrate ,giving appositive urine nitrate test.
- Citrate and H2s negative.

Identification of E .coli strains and toxin testing

E. coli and when required, testing for toxin. when infection with EHEC0157is suspected (hemorrhagic colitis), a presumptive diagnosis can be made by isolating sorbitol non- fermenting E. coli on sorbitol maccokany agar .The colonies can be identified as E .coli 0157 by testing with specific 0157 anti-sera, available latex agglutination test from oxide.

Pseudomonas aeruginosa:

Description:

Gram negative rod. aerobic.non fermentative, motile with polar flagella monotrochoids (4).

Normal habitat

can be found in the intestinal tract, water, soil, sewage and is frequently found in moist environments in hospital (sinks, cleaning buckets, drains humidifiers). It is able to grow in some eye drops (especially quaternary ammonium compounds), saline and other aqueous solution. Because of this, many infection of P.Aeruginosa are opportunistic hospital-acquired, affecting whose already in poor health and immunosuppressed infection are very difficult eradicate duo to p. aeruginosa being resistant to many antimicrobials.

Infection cause by P. aeruginosa include:

- Skin infections, specially burn sites, wound, pressure sore,and ulcer (often as secondary invader). Septicemia may develop.
- Urinary infection, usually following catheterization or associated with chronic urinary disease.
- Respiratory infection especially in patient with cystic fibrosis or condition that cause immune suppression.

- External ear infection (otitis externa)and eye infection often secondary to trauma or surgery
  laboratory feature:

Specimen: depend on the site of infection, specimen include pus, urine, sputum, effusion, and blood for culture.

Culture:

P. aeruginosa is an obligatory aerobe .it is usually recognized by pigment. it produces including pyocyanin a blue- green pigment, and pyrivdine (fluorescein) a yellow –green fluorescent pigment. Aminority of strains are non-pigment producing. Culture have a distinctive smell duo to production of 2-aminoacetophenone. P. aeruginosa grows over a wide temperature rang 6-42C with an optimum of 35-37C.

Blood agar:

P. aeruginosa produce large, flat, spreading colonies which are often hemolyticand usually (90 of strains) pigment –producing.

The pigment diffuses into medium giving it a dark greenish –blue color. some strains produce small colonies or mucouid colonies. When the culture is left at room temperature, pigment color becomes more intense.

MacConkey agar and CLED medium:

P. aeruginosa produce pale colored colonies on macConkey agar and green colonies on CLED medium. compared with blood agar, pigment production is less marked.

KIA medium:

characteristic pink-red slope (often with metallic appearance),and pink-red butt are produced. no gas is formed and no H2s is produced.

Biochemical reaction:

P. aeruginosa is oxidase positive and produce acid only from glucose (no gas).these feature together with typical pigment produce by some strains and distinctive smell of culture are usually sufficient to identify the organism growth as 42C differentiates p. aeruginosa from less commonly isolated pseudomonas, P. putida and P. florescence

Antibiotics: -

drug used to treat bacterial infections. antibiotics have no effect on viral infections. originally, an antibiotic was substance produce by one microorganism that selectively inhibits the growth of another synthetic antibiotic, usually chemically related to natural antibiotics, have since been produced that accomplish comparable tasks (1).

Antimicrobial susceptibility: -

Antibiotics with activity against S. aureus include penicillin, vancomycin, macrolide, cephalosporin and ludic acid(4).

MRSA(methicillin resistant s. aureus):

these strains are resistant to methicillin and relate penicillin and are particularly difficult to treat because they are also resistant to most other common antibiotics. Vancomycin is often needed to treat MRSA infection. culture is usually mixture of sensitive and resistant organism. MRSA strains cause hospital- acquired infections, particularly wound infections and septicemia. improved control measures and surveillance are required to combat increase in the isolation rates of multi-drug resistant MRSA in hospital environment (4).

Antimicrobial that are used to treat E. coli and other infections include those with activity against gram negative organism such as sulphonamides , trimethoprim , cotrimoxazole , nalidixic acid, nitrofurantoin, tetracycline, ampicillin , amoxicillin, cephalosporin's and aminoglycosides (4).

Plasmid-mediated antibiotics resistance, however, is common. in the treatment of E. coli diarrhea, the use of antibiotics in general only of minor importance. rehydration of the patient is always the most important measure taken(4).

P. aeruginosa is resistant to most of the commonly used antibiotics. antimicrobials that usually show activity against pseudomonas include aminoglycosides ,polymyxin , and some penicillin and cephalosporin (4).

anti-bactericidal resistant to penicillin may occur due to beta-lactamase production cell membrane alterations reducing antibiotics uptake (gram negative bacteria), or changes in penicillin –binding proteins are occur with MRSA (4).

Cephalosporin:

like penicillin, are bactericidal and also have a beta-lactam ring. they are, however, stable to staphylococcus penicillin's and have broad spectrum activity they are less likely to cause hypersensitivity than penicillin. they are mainly used to treat sever systemic infections caused by aerobic gram negative organisms. they are expensive drug. some cephalosporin can damage the kidney. (4) .

Aminoglycoside:

bactericidal, showing synergy with beta-lactam agents. Mainly reserved for the treatment of sever sepsis due to coli forms and other gram negative aerobic bacilli. With beta-lactams, they are sometimes used to treat endocarditis caused by staphylococci and streptococci (4).

vancomycin:

bactericidal is used to treat serious infection such as endocarditis and septicemia caused by gram positive bacteria, particularly multi-resistant strains. treatment requires monitoring due to auto toxicity and nephrotoxicity. (4)

Carbapenems:

these beta-lactam antibiotics have potent activity against a wide range of gram positive and gram negative bacteria and are resistant to hydrolysis by lactamases. (4)

Macrolides:

Useful bacteriostatic agent (may be bactericidal at high concentration) mainly used to treat staphylococcalinfections, respiratory infections, non- specific arthritis, and when indicated, campylobacterenteritis. they are useful second – line drugs for treating patient with penicillin hypersensitivity. resistant may occur with S. aureus, S. pyogen and S.Pneumonia. side effect include gastrointestinal upset and rash(4) .

Lincosamide:

useful in treating staphylococcal bone and joint infections and an aerobic infection, but lincosamide have been associated with pseudomembranous colitis. (4)

Chloramphenicol:

bacteriostatic broad spectrum drug, used in treating typhoid fever, meningitis, rickettsia and chlamydial infection and also eye infection. It can cause aplastic anemia and is toxic in neonate. (4)

Quinolones:

bacteriostatic or bactericidal agent nalidixic acid is used to treat lower gram negative urinary infection. ciprofloxacin is active against pseudomonas and is also used to treat serious systemic infection (4).

Tetracycline:

Bacteriostatic widely used broad spectrum antibiotic is activity against gram positive and some gram negative bacteria and also Borelli, Rickettsia , Chlamydia , and mycoplasma . side effect includes gastrointestinal disturbances, kidney damage and staining of teeth in children. it should not be used in pregnancy. resistant to tetracycline is common .e.g. with H. influenza, S. pneumonia, and S. pyogen (4).

Sulphonamides and trimethoprim:

bacteriostatic agent with activity against gram positive and gram negative organism. is used to treat urinary and respiratory tract infection pneumocystis pneumonia and invasive salmonellosis. Many enterobacteriaceae are resistant. side effect include nausea and vomiting, rashes, mouse ulceration and occasionally thrombocytopenia and leukopenia. side effect is less with trimethoprim (4).

Gentamycin:

Sold under brand names Gentamycin among other, is an antibiotic used to treat several type of bacterial infection. This may include bone infection ,endocarditis , pelvic inflammatory disease, meningitis, pneumonia, urinary tract infection, and sepsis among other. It is not effective for gonorrhea or Chlamydia infection. It can be given intravenously, by injection into muscle or topically. topical formulation may be used in burns or for infections of the outside of the eye. In the developed

world it is often only used for two days until bacterial culture determine what antibiotic the infection is sensitive to. The dose required should be monitored by blood testing. (5)

CHAPTER TWO

2-1-Materials and Methods:

2-1-1-Study design:

Cross sectional study

2-1-2-Study area:

Laboratory of AL sheikh Abdallah Albadri university

2-1-3-Study population:

Known isolate bacteria

2-1-4-Sample size:

10 samples

2-1-5-Inclustion:

Known species of organism E. coli,P. aeruginosa and S. aureus

2-1-6-Exclustion:

Other species of micro organism

2-1-7-Ethical consideration

Approve of university of AL sheikh Abdallah albadri

2-2-Materials:

2-2-1-Equipments:

1-Autoclave
2-Incubator
3-Hot air oven
4-Referigator
5-Sensitive balance
6-Wire loops with handles
7-cork borer (0.5 cm in diameter)
8-Bunsen burner
9-Rack
10-syrings
11-filter paper
Glassware:
1-Petri dishes
2-Flasks with different size
3-Measuring cylinder
4-Beakers
5-Sterile containers (bijou bottles)
6-Test tubes
2-2-2-Disposable materials:
1-Disposable syringes
2-Swabs

2-2-3-Methods:

Plant material: -

Plant were collected from the market.

Preparation of cured extracts: -

1- Methanolic extract of the stem bark of the plant: -

Methanolic extract of the stem bark of the plant was extracted according to the method described by Okogun (2000) with slight modifications. A 250 g sample of the stem bark of the plant was air-dried, ground into powder using an electric blender. The blended material was transferred into a beaker and 100 ml of 95% methanol was added at ambient temperature (28 ± 2°C). The mixture was extracted by agitation by hand for 3 hours. after waiting 3 days' filtration using filter paper (4)

Extraction was allowed to proceed for removed by evaporation at room temperature (28 ±2°C) to obtain the extract.

Preparation of serial dilution of acacia Nilotica:

10mcg_____0.01g of extraction in 1ml of D.W

100mcg _____0.1g of extraction in 1 ml of D.W

1000mcg _____ 1g of extraction in 1 ml of D.W

Preparation of standard bacteria suspension: -

Ten ml normal saline were placed in test tubes and sterilized in autoclave at 121 C for 15 minute, a loop full of purified bacteria were inoculated in sterile normal saline and compare with McFarland standard (4)

Disc diffusion susceptibility tests:

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility.

A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism.

The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organism.
Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc.
For clinical and surveillance purposes and to promote reproducibility and comparability of results between laboratories.

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CHAPTER THREE

Result:

The acacia Nilotica were screened for antimicrobial activity against two grams negative (Escherichia coli, Pseudomonas aeroginosa) and one gram positive (Staphylococcus aureus). used the methanolic extract. the extract obtained from acacia Nilotica exerted pronounced activity against several bacteria strain tested as indicated by diameter of growth inhibition zones that varied from (17-34) mm.

Out of the nine cultures tested it showed best activity against pseudomonas aeroginosa different concentration (34-30-25) mm and then Escherchia coli (33-30-20) mm , and finaly Staphylococcus aureus (30-20-17) mm. for each test control positive Gentamycin in concentration (10mcg \ disc) with zone of inhibition 15 mm against all bacteria strained tested .

Interpretation of zone sizes:

Using the Interpretative Chart, interpret the zones sizes of each antimicrobial, reporting the organism as 'Resistant', 'Intermediate/Moderately susceptible', 'Susceptible'.

Zone of inhibition in mm:

	10mcg	100mcg	1000mcg	Gentamycin 10mcg
p. aeroginosa	25mm	30mm	34mm	25mm
E. coli	20mm	30mm	33mm	25mm
S. aureus	17mm	20mm	30mm	25mm

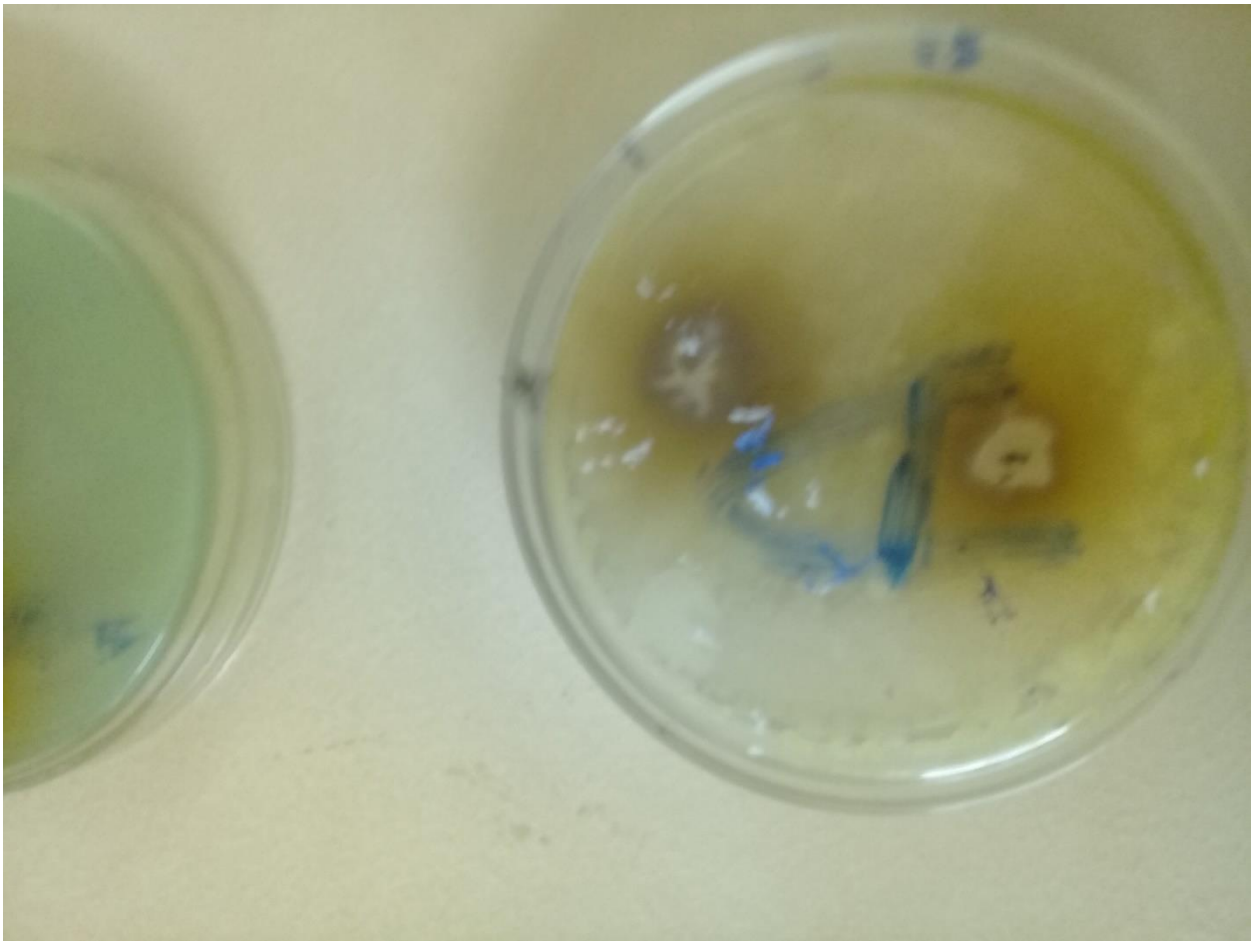

Figure (1): the inhibition zone of acacia Nilotica againstS. aureus in 10 mcg

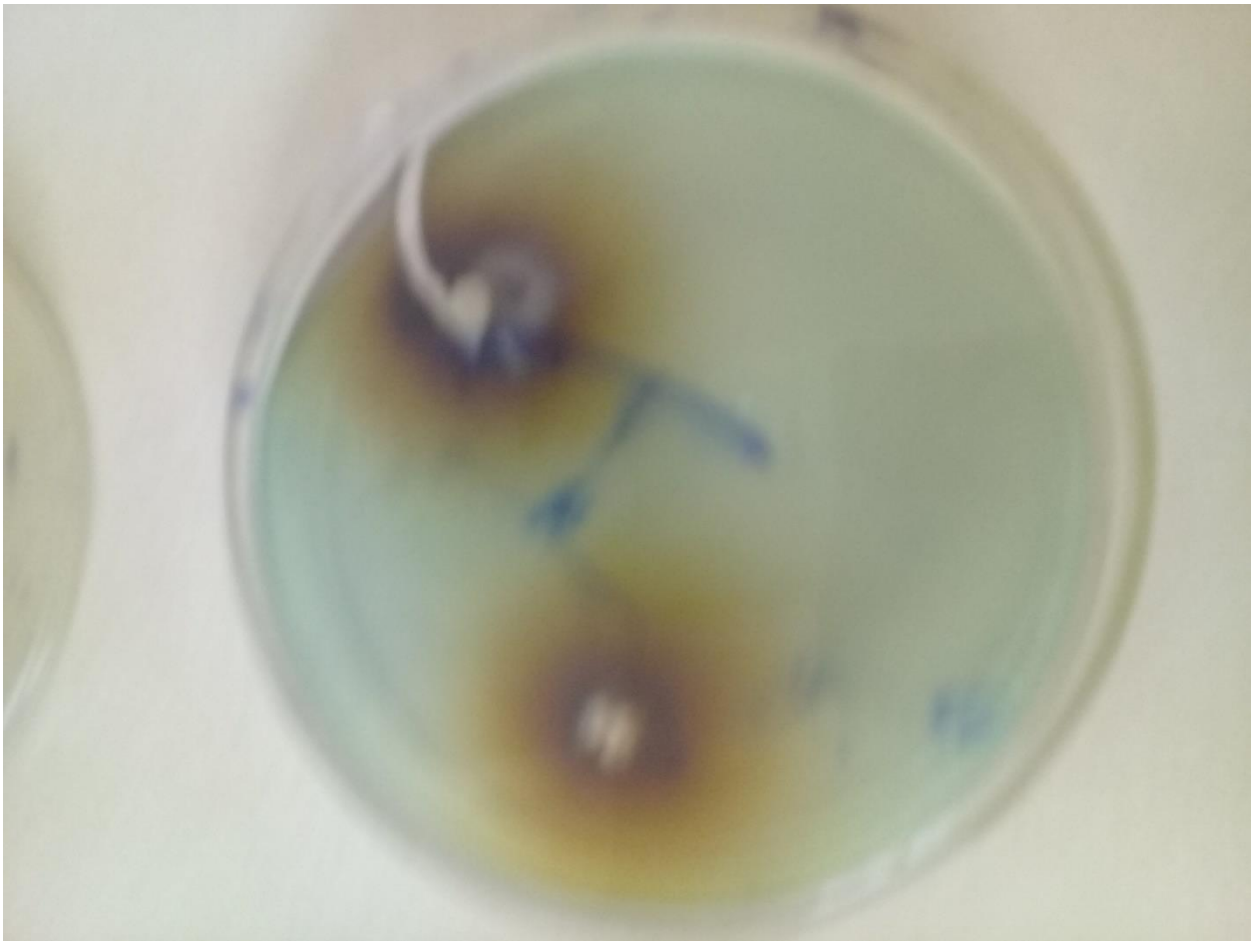

Figure (2): the inhibition zone of acacia nilotica against P. aeruginosa in 10 mcg

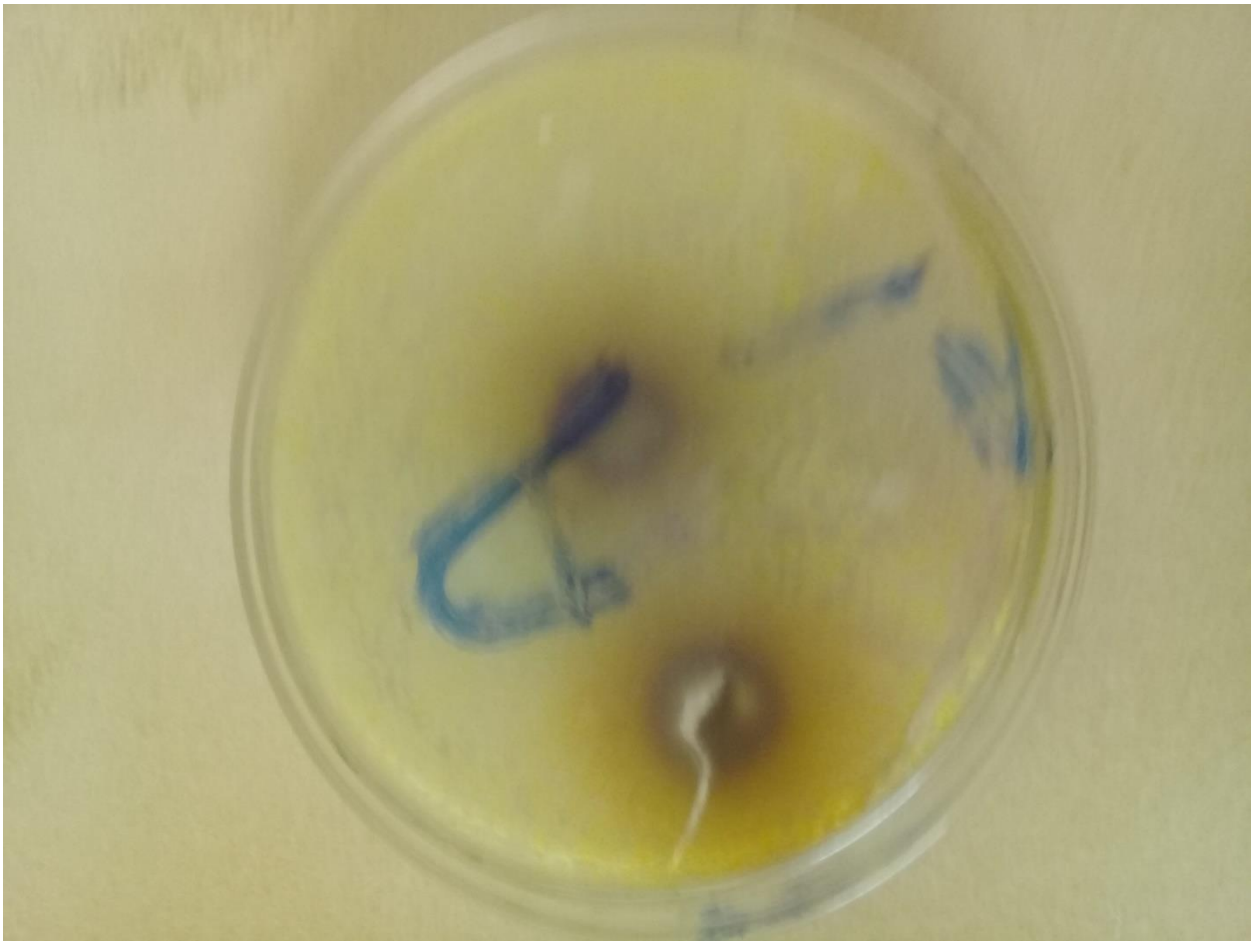

Figure (3) the inhibition zone of acacia nilotica against E. coli in 10 mcg

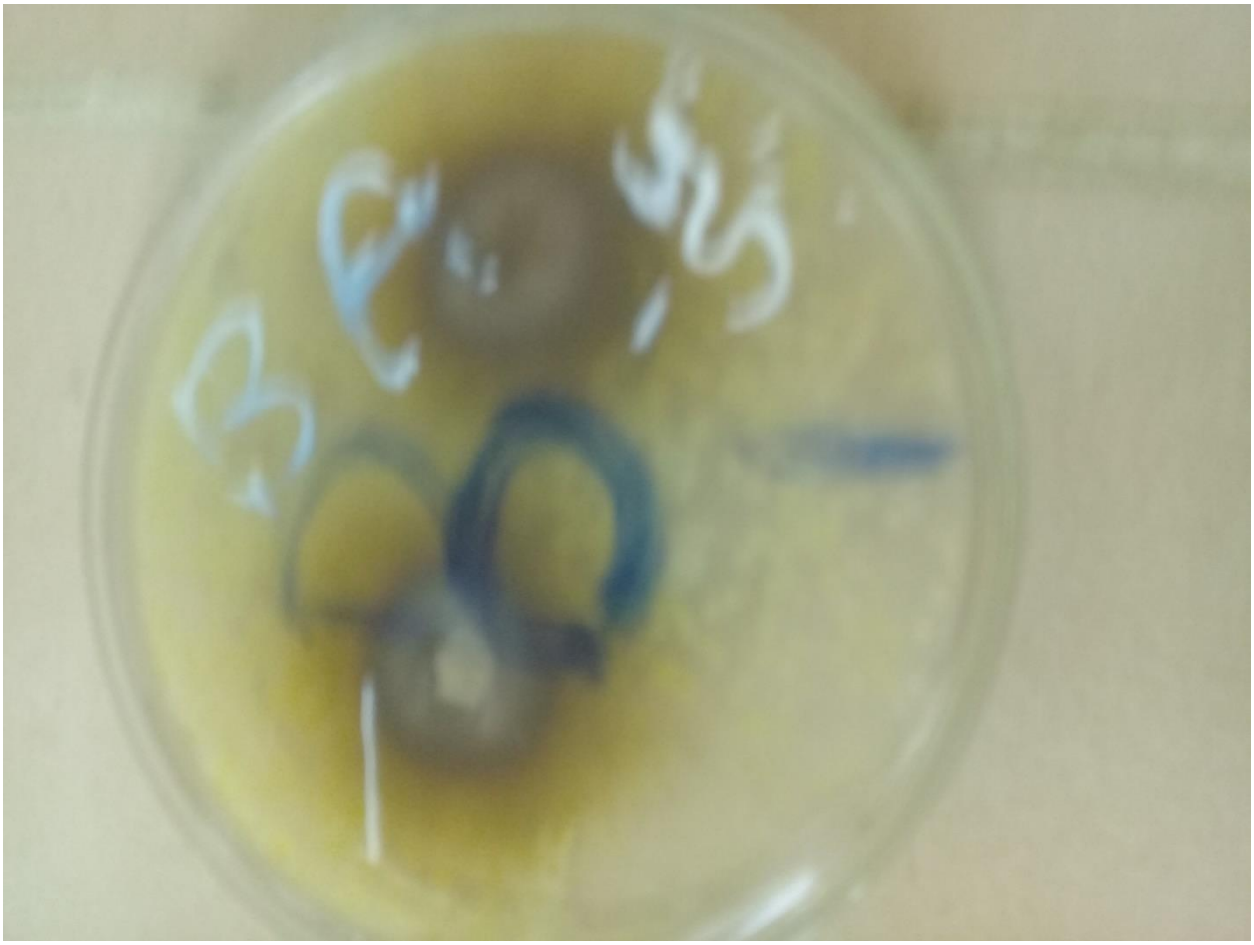

Figure (4) the inhibition zone of acacia nilotica against S. aureus in 100 mcg

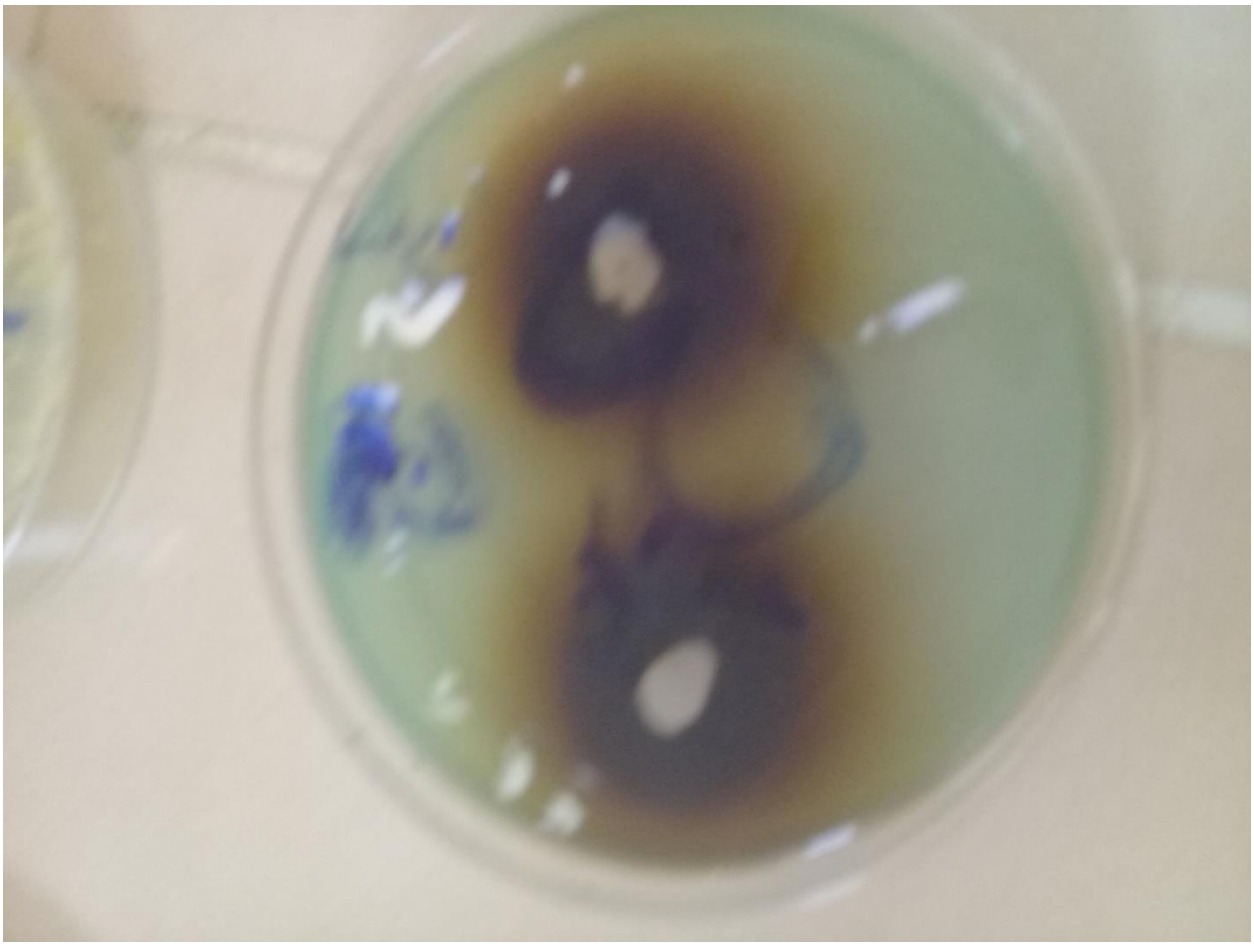

Figure (5) the inhibition zone of acacia nilotica against P. aeruginosa in 100 mcg

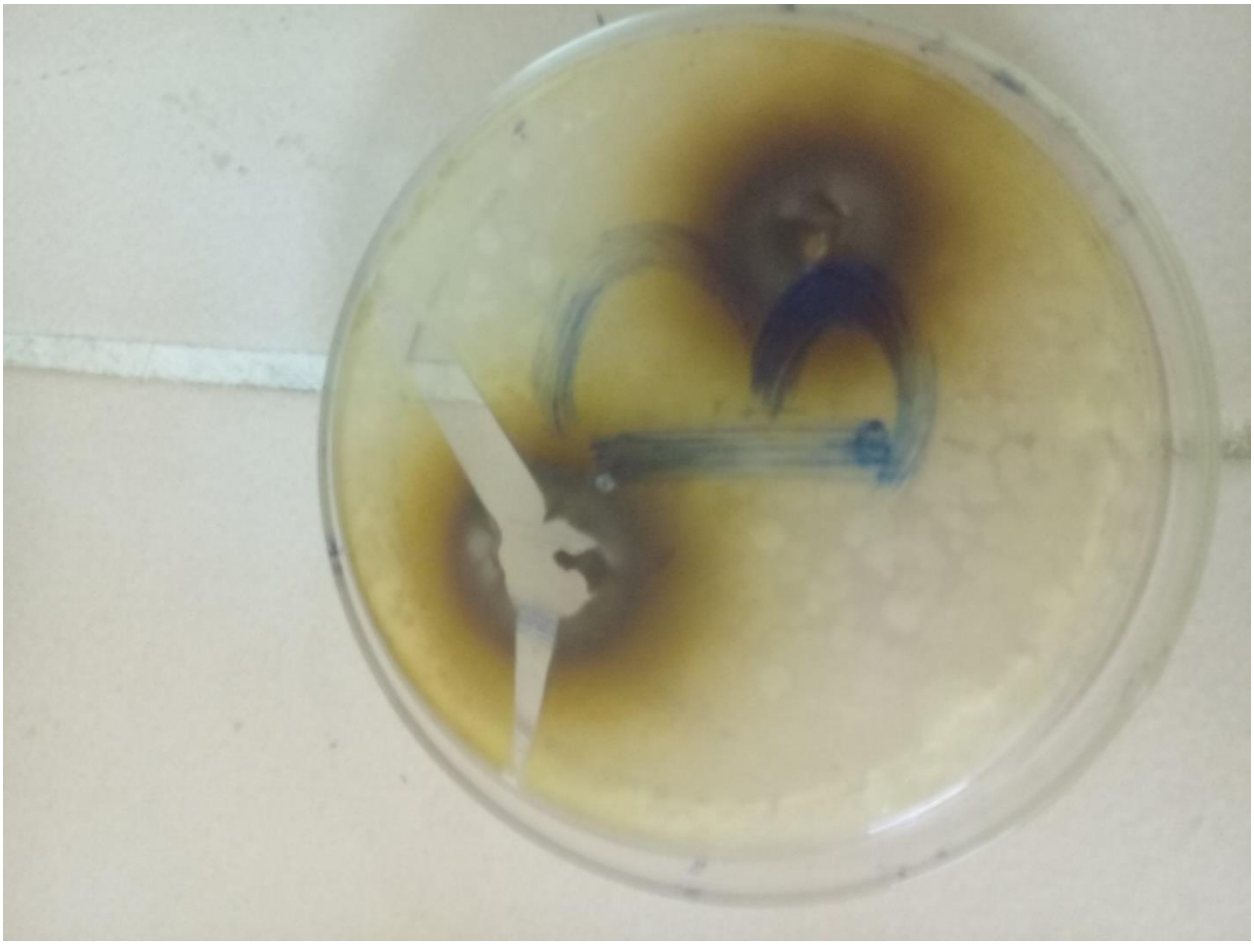

Figure (6) the inhibition zone of acacia nilotica against E. coli in 100 mcg

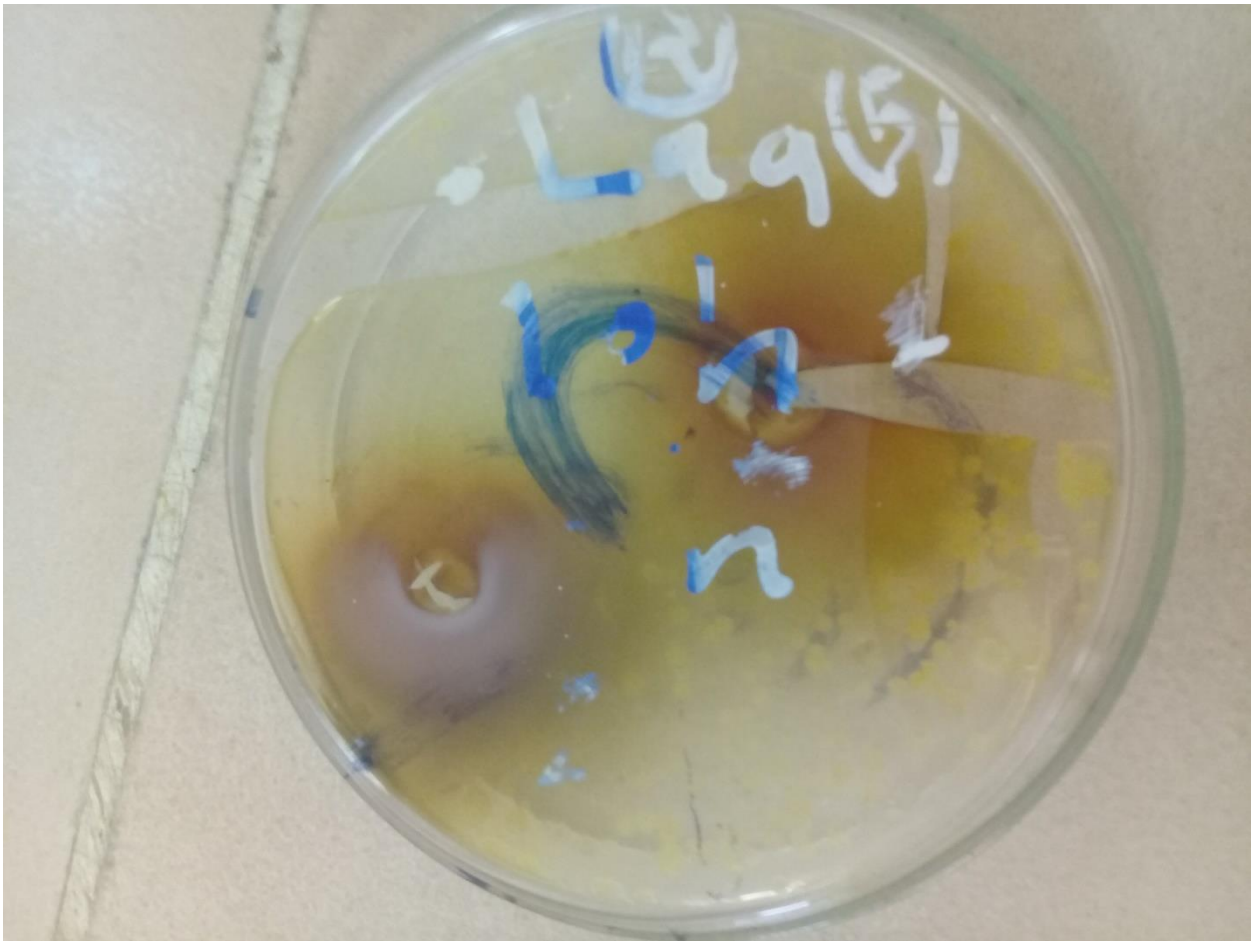

Figure (7) : the inhibition zone of acacia nilotica against S .aureus in 1000 mcg

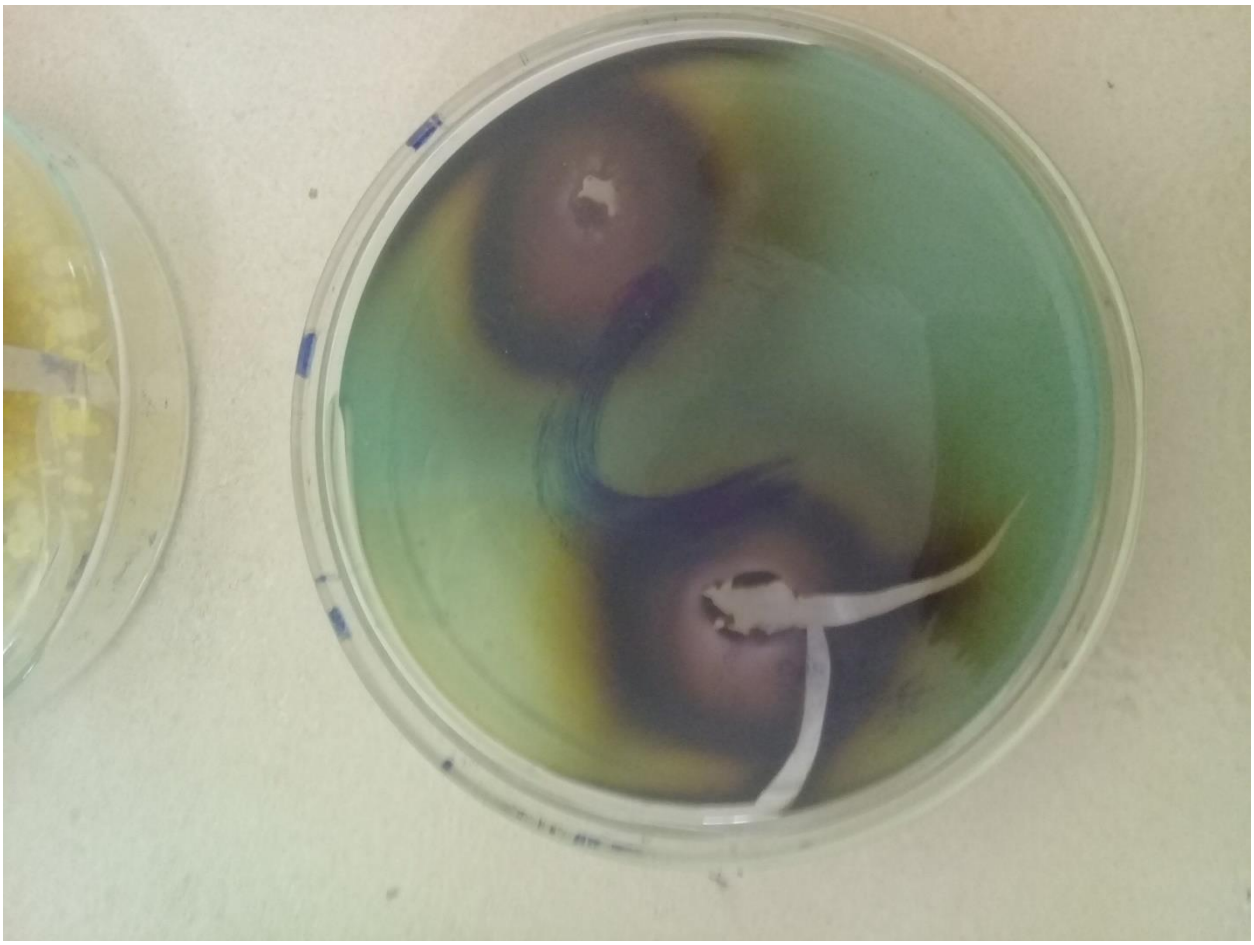

Figure (8): the inhibition zone of acacia nilotica against P. aeruginosa in 1000 mcg

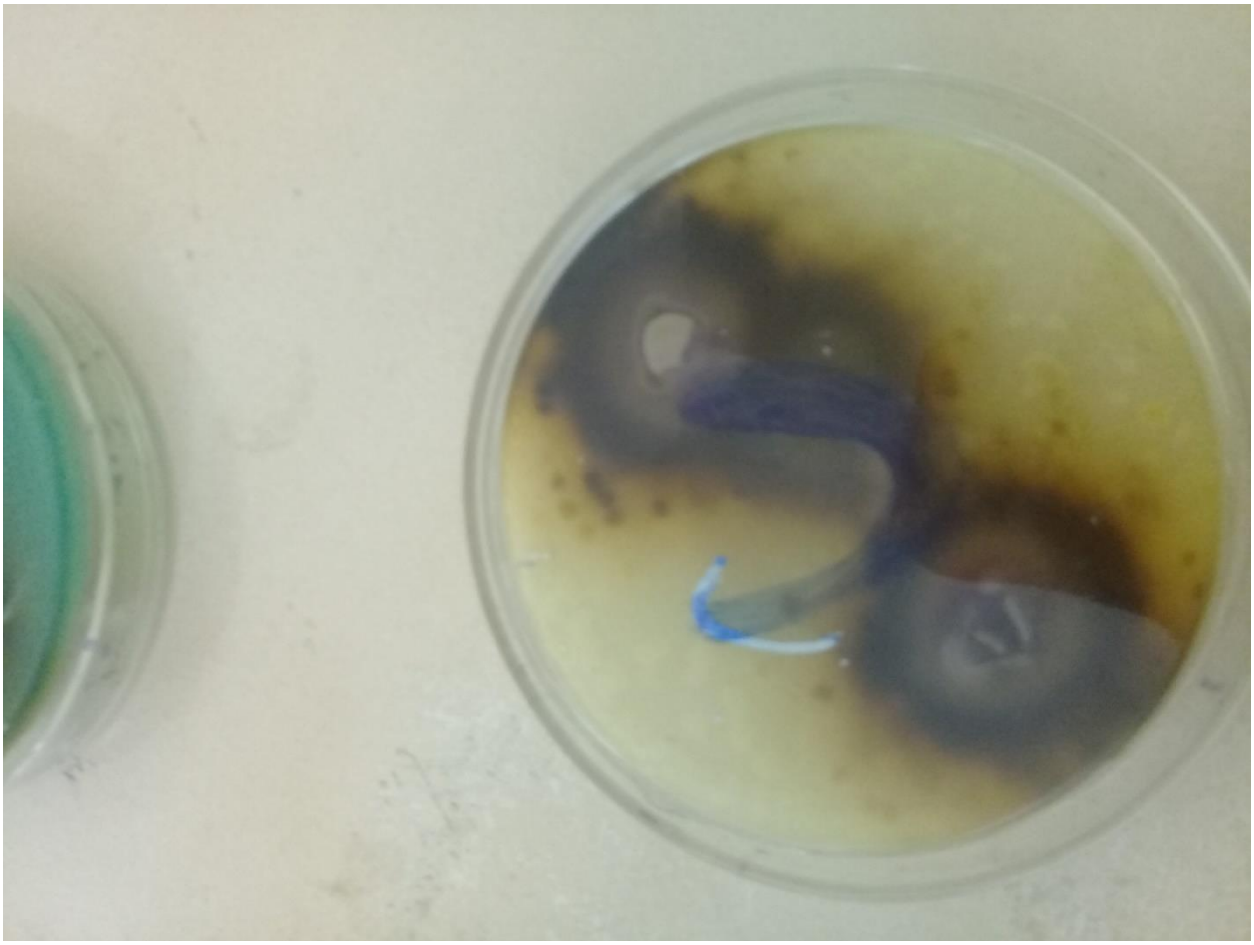

Figure (9): the inhibition zone of acacia nilotica against E. coli in1000 mcg

Figure (10): the inhibition zone of gentamycin (control) in 10 mcg

CHAPTER FOUR

Discussion:

Plants essential oil and extracts have been used for thousands of years, in food preservation,
pharmaceuticals, alternative medicine and natural therapies. Therefore, it is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of health care
In our study, *Acacia Nilotica* extracts exhibited activity against the selected Bacterial strain. Methanol extracts of *Acacia Nilotica* produced significant diameter of zone inhibition due to its major components. This is because methanol is an organic compound and liberate active component required for antimicrobial activity. comparing standard organisms with reference antibiotics tested resulted in that all *Acacia Nilotica extracts* had more effectivity against P.aeruginosa than *Gentamycin in all concentration, While in S. aureus and E. coli tested* less effectivity than gentamycin.

The Study agree with studies :

Study done in 2008 the results proved the broad spectrum inhibition activity of acacia Nilotica against clinical isolates of citrobacter species, klebsiella species, Escherichia coli, proteus mirabilis, pseudomonas aeruginosa, staphylococcus aureus, streptococcus facials, salmonella typhi, salmonella typhimurium, salmonella para typhoid, salmonella para typhi B, shigella flexneri, shigella sonnies, shigella boddiy (6).

study done in 2009 on the antibacterial activity of the stem park extract of acacia Nilotica. The antimicrobial activity of the extract was assayed against streptococcus viridians, staphylococcus aureus, Escherichia-coli, bacillus subtilis and shigella sonnies' using the agar diffusion method the most susceptible to plant extract while candida albicans was the most resistant the minimum inhibitory concentration 35-50gl ml, while the minimum bactericidal concentration 35-60glml (7).

study done in 2010 by using methanolic extraction of acacia Nilotic and chloroform and ethyl acetate and water showed antimicrobial activity in clinical isolates. by disc diffusion method the metabolic extraction inhibited klebsiella pneumonia, shigella dysentery and staphylococcus aureus water extraction inhibited isolates with big zone (9)

study done in 2010 on candida albicans cured extract of acacia Nilotica 30ML showed zone with dimeter 22mm on four different candida (8).

study done in 2011 cured extract of methanol -ethyl water by sox helt shown antimicrobial activity on staphylococcus aureus and Escherichia coli methanol extraction of acacia Nilotica showed maximum zone on both 29 mm while either 13 in Escherichia coli 15 mm in staphylococcus aureus while water extraction 20mm (10).

conclusion:

It was concluded that: Acacia *Nilotica* have antimicrobial activities, but with varying degrees of effectiveness. We believe that this investigation with previous studies provided support to the antimicrobial properties of *Acacia Nilotica* .Traditional medicinal practice could provide a source for new drugs and therefore efforts should be directed to evaluate traditional medicinal practice based on scientific methodologies available. Resort new source of antimicrobial agents to treat antibiotic resistant microbes in order to avoid the high cost and the side effects of medications. These results justify the use of some plants as folk medicine.

Recommendations:

• Further advanced non-cost extraction techniques to determine the active components responsible for the antimicrobial activity. (for example using instrument that is powerful way to extract the active gradients of any natural herbal material)

• Determination of the Minimum Inhibitory Concentration (MIC) using tube dilution method.

• Applied other way to gradient of acacia nilotica because easy and not long time and uses other bacteria and fungi.

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Appendix:

Muller Hinton agar (Oxoid code CM 337):
Typical formula g/l
Contents:
Beef, dehydrated infusion form
Gasienhydrolysate 17.5
Starch 1.5
Agar 17.0
PH 7.4 0.2
70
Direction:
Suspend 38g in 1 liter of distilled water. Bring to boil to dissolve completely. Sterilize by
autoclaving at 121C for 15 minutes. Cool to 50C, and pour into sterile Petri dishes. Dry the
surface of the medium before inoculation.
Preparation of McFarland turbidity standard:
1-prepare 1% (v/v) solution of sulphuric acid by adding 1ml of concentrated sulphuric acid to 99
ml of water and mix well.
2-Prepare 1.175% (w/v) solution of barium chloride by dissolving 2.35g of di-hydrate barium
chloride (Bacl2.2H2O) in 200ml of distilled water.
3-Add .5ml of barium chloride solution to 99.5 ml of sulphuric acid solution and mix