


**Sudan University for Science and Technology**

**College of Graduate Studies**

# Antibacterial Activity of Garlic *Allium sativum* Extract Against Bacteria Isolated From Patients with Urinary Tract Infection in Khartoum State

# فعالية مستخلص الثوم ضد البكتريا المعزولة من مرضى المسالك البولية بولاية الخرطوم

A Dissertation Submitted in Partial Fulfillment for the Requirement of M.Sc. Degree in Medical Laboratory Science (Microbiology)

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**بسم الله الرحمن الرحيم**

# الآيـــــــة

**قال تعالى:**

**وَاخْفِضْ لَهُمَا جَنَاحَ الذُّلِّ مِنَ الرَّحْمَةِ وَقُل رَّبِّ ارْحَمْهُمَا كَمَا رَبَّيَانِي صَغِيرًا**

سورة الإسراء الآية (24)

# DEDICATION

I dedicate this thesis to

My father

My mother

My brothers

My teachers

And to all of my friends

# ACKNOWLEDGEMENT

All thanks and praise to ALLAH the lord of all worlds for all givens rewards to me. With sincere thanks and gratefulness, I would like to acknowledg**e** my Supervisor **Prof. Yousif FadlAlla HamedAlneel** for this outstanding, knowledge encouragement, guidance, patience and constructive advice throughout this work.

The compilation of this work could not have been possible without assistance of research laboratory staff **Ust. Omsalama and Rasha**.

I would like to express my thanks and gratitude to my friends **Maison, Amel and Ameera** for their support and kind.

# ABSTRACT

This was a cross sectional study conducted during the period from April to December 2019, aimed to testing the antibacterial activity of of *Allium sativum* ethanolic extract against urinary tract bacterial isolates. A total of one hundred bacterial isolates were collected from from Ribat University Hospital, Albaraha Hospital and Khartoum Army Hospital. These bacterial isolates were inoculated into nutrient agar slope for preservation and then re identified by using different biochemical tests. About 100 bacterial isolates, 35(35%) was *Escherichia coli*, then 25(25%) *Pseudomonas aeruginosa* followed by 20(20%) *Klebsiella pneumonia*, 11(11%) *Enterococcus feacalis* and finally 9(9%) *proteus mirabilis*. After re identification, these isolates were subjected to antibiotics sensitivity by using disc diffusion method, the antibiotics used includes Gentamicin, Ciprofloxacin, Ceftazidime, Cotrimoxazole and Imipinem. The results showed that Ceftazidime (67%) had higher resistant rates. Then test activity of different concentration (100, 50, 25, and 12.5) of *Allium sativum* ethanolic extract against bacterial isolates by using cup plate agar diffusion method. The results showed that *Allium sativum* have antibacterial activity against all pathogenic and reference strains. The results revealed that *Enterococccus faecalis* was more susceptible organism to *Allium sativum* extract *and Pseudomonas aeruginosa* was less susceptible organism to extract with mean of inhibition zones (20.8mm, 17.2mm) respectively at 100 (%w/v). Also the result showed that MIC of Garlic extract for all tested bacteria was 12.5 (%w/v).

# الخلاصة

هذه كانت دراسة مقطعية تم اجرائها في الفترة ما بين ابريل حتى ديسمبر(2019)' تهدف الى اختبار نشاط المضاد البكتيري مستخلص نبات الثوم ضد العزل البكتيري الاحادي. اجمالي مئة عزلات بكتيرية تم جمعها من مستشفى جامعة الرباط ' مستشفى البراحة ومستشفى السلاح الطبي ' هذه العزلات البكتيريه تم تلقيحها داخل المغذيات اجار المنحدر للحفظ ' ومن ثم التعرف عليها وفرزها باستخدام اختبارات بيوكيميائية. من (100) عزلة بكتيريه حوالي 35( 35%) كانت الاشريكيه القولونيه ' 25 (25% ) الزائفة الزنجارية '20 (20% ) الكلبسيله الرئويه ' 11(11%) المكوره المعوية البرازية و9(9%) المتقلبة الرائعة. بعد التعرف على هذه العزلات البكتيرية تم تعريضها للمضادات الحيوية باستخدام طريقة الانتشار الطبقي. المضادات الحيوية التي تم استخدامها احتوت على الجنتامايسين ' السيفتازيديم ' السبروفلوكساسين' الكوتراموكزازول و الايميبينيم . واظهرت النتيجة ان السيفتازيديم له المقاومة الاعلى بمعدل ( 67%). ومن ثم تم اختبار عدة تراكيز (12.5'25'50'100) من المستخلص الايثانولي لنبات الثوم ضد العزلات البكتيرية باستخدام طريقة الانتشار بالاغار. اظهرت النتيجة ان مستخلص نبات الثوم يملك نشاط بكتيري ضد البكتيريا المسببة للامراض والبكتيريا المرجعية. كما اظهرت النتائج ان المكوره المعوية البرازية كانت الاكثر تاثرا بمستخلص نبات الثوم والزائفة الزنجارية كانت الاقل تاثرا بوسيط منطقة منع (20.8و17.2) على التوالي في التركيز (100). وايضا اظهرت الدراسة ان المثبط الادنى لكل البكتيريا التي تم اختبارها كان (12.5).

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# LIST OF ABBEVIATIONS

| Abbreviation | Full name |
|---|---|
| EHEC | Enterohemorrhagic *Escherichia coli* |
| KIA | Kligler iron Agar |
| MHA | Muller Hinton Agar |
| MIC | Minimum inhibitory concentration |
| NCCLS | National culture collection laboratories |
| UPEC | Uropathogenic *Escherichia coli* |
| UTI | Urinary tract infection |
| VUR | Vesico-ureteric reflux |
| WHO | World Health Organization |

# Chapter One

# 1. INTRODUCTION

## 1.1 Introduction

According to World Health Organization (WHO) about 80% of people worldwide are currently depending on traditional medicine for their primary health care needs (Khalil *et al.,* 2017). Herbal drugs have found wide spread use in many countries because they are easily, available, cheaper, and safer than synthetics drug (Retnam and De-Britto, 2007; Prusti*et al.,* 2008). Antimicrobial resistance is a major and increasing problem, a large number of bacteria have responded to the use of antibiotics with their ability to evolve and transmit antibacterial resistance to other species (Nerino *et al.*, 2013).The increase consumption of antimicrobial agents and inappropriate use accelerates this phenomenon. Also the continuous migration of people plays an important role on acquisition and spread of multidrug resistant strains (Nerino *et al.,* 2013).

Urinary tract infection (UTI) is the second most common infectious presenting in community practice. Almost 95% of cases of UTI are caused by bacteria (Bishop *et al.,* 2007). UTI causing bacteria become more resistant to available antibiotics, there is urgency to explore new strategies for managing UTIs (Foxman, 2003). Development of resistance in microorganisms to antibiotics and emergence of new infectious disease create urgent need to discover novel safe and effective antimicrobial compounds (Rajas *et al.,* 2003).

Natural products are major sources of new natural drugs and alternative medicine for treatment of various diseases has been increased in the last few decades. In comparison to the formulated drugs the herbs and spices have fewer side effects. They are also inexpensive, show better patient tolerance and are ready available for low socioeconomic population. In recent years, in view of their beneficial effects, use of spice or herbs is gradually increasing not only in developing countries but also in developed countries (Iram*et al.*, 2012).

*Allium sativum (A.sativum)* commonly known as Garlic which belongs to a family of *Alliaceae*, has more than 500 species in 30 genera and the family is taxonomically intermediate between the *Liliaceae* and *Amaryllidaceae* (Divya*et al.*, 2017). Garlic is widely used in culinary and medicine; it has been utilized to fight infection such as cough, cold asthma, diarrhea, flu, headache sore throat, abdominal discomfort and respiratory infection (Bandna, 2013). Garlic is probably one of the earliest known medicinal plants, which used for ancient time to cure different disease condition in human. Garlic's principal medicinal uses are to lower blood pressure and cholesterol, fight infection and prevent cancer (Gavasane *et al.*, 2011).

## 1.2. Rationale

Recently, modern societies face serious problems with using of the synthetic chemotherapeutic agents, in order to their multiple disadvantages such as harmful side effects, high cost and development of multiple drug resistant due to recurrent usage. The most common UTI causing bacteria become more resistant to available antibiotics, this phenomenon led to explore new strategies to managing UTI and find novel alternatives (Foxman, 2010).So use of herbs and spice are very important because of their antimicrobial properties and useful as therapeutic agent against many pathological infections. Also play an important role as a safer cheaper alternative solution (Iram *et al.*, 2012).In Sudanese culture and as a part of traditional medicine Garlic is used for the treatment of many infections such as wound infections and urinary tract infections, therefore it is of interest to test and prove this actively scientifically using standard microbiological techniques.

## 1.3. Objectives

### 1.3.1. General objective

To find out the antibacterial activity of *Allium sativum* (Garlic) ethanolic extract against clinical isolates from urinary tract infections in Khartoum State.

### 1.3.2. Specific objectives

1. To re identify bacterial isolates by using different biochemical tests.

2. To detect antibacterial activity of commonly used antibiotics against UTI pathogens.

3. To test antibacterial activity of *Allium sativum* ethanolic extract using cup-plate agar diffusion method against the urinary bacterial isolates.

4. To determine minimum inhibitory concentration (MIC) of *Allium sativum* ethanolic extract.

# Chapter Two

# 2. LITERATRE REVIEW

## 2.1. *Allium sativum* Garlic)

*Allium sativum (A.sativum)* belongs to a family of *Alliaceae*, it has more than 500 species in 30 genera and the family is taxonomically intermediate between the *Liliaceae* and *Amaryllidaceae* (Divya *et al.*,2017).

## 2.2. Scientific classification of *A.sativum*

Kingdom:*plantae*

Division: *Angiosperms*

Class: *Monocotyledoneae*

Order: *Asparagales*

Family:*AlliaceaeorLiliaceae*

Genus:*Allium*

Species: *A.sativum*(Divya*et al.*,2017).

## 2.3. Origin and history

A member of the *liliaceae* family, garlic (*Allium sativum*) is a cultivated food highly regarded throughout the world. Originally from central Asia, garlic is one of the earliest of cultivated plants (Gavasane *et al.*, 2011).Garlic is one of the earliest document examples of plants employed for treatment of disease and maintenance of health. It was in use at the beginning of recorded

history and was found in Egyptian pyramids and ancient Greek temples. Medical applications of garlic have been documented in ancient medical texts from Egypt, Greek, Rome, China and India (Prasan, 2012).A bulb of Garlic itself presented a whole pharmacy industry due to broad spectrum of effects at the time when antibiotics and other pharmacy products did not exist. The garlic was given different names that are still in use such as "Russian penicillin", "natural antibiotic", "vegetable Viagra", "plant talisman", "rustics' theriac" and "snake grass (Petrovska and Cekovska, 2010).

## 2.4. Common names

Garlic, Lasuna, Rasonam, lasan, Vellulli, Valli-pundu, Seer, Ullippoondu and Maharu (Divya*et al.,* 2017).

## 2.5. Geographical distribution

Garlic is one of the earliest documented examples of plants employed for treatment of disease and maintenance of health; it is used in medicine and foodstuff for almost three thousand years as evidence by ancient writings from China, Egypt, Greece, and India (Cardelle *et al*., 2010).

## 2.6. Chemical compounds

There are more than two hundred chemical compounds in the garlic bulb, of which, contain volatile oil with sulfur containing compounds abundantly like Ajoene (4,5.9-trithiadodeca-1,6,11-triene-9-oxide), Alliin and Allicin, enzymes like peroxidase, allinase, myrosinase and other compounds like α-phallandrene, β-phallandrene, linalool, citral and geraniol (Eiaz *et al.*, 2003). Garlic contains at least 33sulfur compounds and minerals like germanium,

calcium, copper, iron, potassium, magnesium, selenium and zinc; vitamins A, B1 and C, fiber and water (Gebreselema and Mebrahtu, 2013). It also contain17 amino acids like lysine, histidine, arginine, aspartic acid, threonine, swine, glutamine, proline , glycine, alanine, cysteine, valine, methionine, isoleucin,leucine, tryptophan and phenylealanine (Josling, 2005). Approximately 1% Alliin (S-allyl cysteine sulfoxide) is present dried, powdered garlic. Allicin (diallylthiosulfinate or diallyl disulfide), which is the most biologically active compound in garlic, does not exist until garlic is crushed or cut.Enzyme allinase, which is activated upon injuring the garlic bulb, metabolisesaliin to allicin, Allicin is subsequently metabolized to vinyldithiines, This process requires hours at room temperature and minutes during cooking, also Allicin, which has antimicrobial effects against many viruses, bacteria, fungi and parasites (Prasan, 2012).

## 2.7. Medicinal and therapeutic use

The importance of garlic is due to it is use not only for culinary but also for therapeutic and medicinal purposes in both traditional and modern medicine (Lanzotti*et al.*, 2014). Garlic constituents scavenge free radicals, protect membrane from damage and maintain cell integrity, also play role in the reduction of cholesterol, blood pressure, anti-platelet activities, and inhibition of thromboxane formation prevents atherosclerosis-related disorder, thus conffering cardioprotective benefits (prasan, 2012).

Table1. Uses of garlic plant for treatment of different diseases

| **Part of garlic** | **Preparation** | **Treatment for** |
|---|---|---|
| Leaves | Hot concoction | Common cold |
| Leaves | Tea | Reduce serum cholesterol |
| bulbs | Crushed bulbs | Defense against flu |

(Ravi, 2016)

## 2.8. Internal uses

The broad spectrum activities of garlic include anti-microbial, anti-helminthic, anti-protozoal, anti-fungal, insecticidal, anti-tumor, anti-thrombotic, anti-cancer, anti-arthritic, hypolipidemic, and hypoglycemic proprieties (Mohammad *et al.*, 2006).

Table2.The medicinal spectra of garlic compounds

| **Pharmacologic Activity** | **Chemical components of garlic contributed to activity** |
|---|---|
| Anticoagulation | Ajoene |
| Antihypertensive | Selenium, germanium |
| Antimicrobial | Selenium, germanium |
| Antiparasitic | Allicin- alliin |
| Antibiotic | Allicin- alliin |
| Antimycotic | Allicin- alliin,Ajoene |
| Antiviral | Allicin, Ajoene |
| Hypolipemic | Diallyl disulfide |
| Detoxification of heavy metals | Selenium, allylmercaptangermimation |
| Antitumour | Selenium, germanium |
| Vitamins | Thaimine, vitamin A and C |
| Antioxidant | Selenium, germanium |
| Antiaging | Selenium, diallyl disulfide |
| Natural Killer Cell activity | Selenium, germanium |
| Humoral immunity | Germanium, allicin |
| Complement activity | Magnesium, calcium |

(Divya *et al.*, 2017).

## 2.9. Urinary tract infection (UTI)

Urinary tract infection (UTI) is an infection that begins in urinary system; it is the second most common disease after respiratory infection. The urinary tract consists of the kidneys, ureters, bladder and urethra (Betty *et al.*, 2007). It is associated with multiplication of organisms in the urinary tract and is

defined by the presence of more than a hundred thousand organisms per ml of midstream sample of urine. UTI is common in women more than in men (Mohamed, 2015). UTI causing by different microorganisms, including fungi, viruses, and bacteria are major causative organisms and are responsible for more than 95% of UTIs cases (Banado *et al.*, 2001). Bacteria most often involved in UTIs acquired in a community are *Escherichia coli* and *Staphylococcus saprophyticus*, responsible for more than 80% and 10% to 15% of UTI respectively, occasionally other microorganisms such as *Klebsiella* spp, *Proteus mirabilis*, and *Enterococcus faecalis* (Banfitebiya *et al*., 2018).

## 2.10. Etiological factors

The microbial etiology of urinary infections has been regarded as well established and reasonably consistent. *Escherichia coli* remains the predominant uropathogen (80%) isolated in acute community-acquired uncomplicated infections, followed by *Staphylococcus saprophyticus* (10% to 15%). *Klebsiella*, *Enteribacter*, *Proteus species*, and *Enterococci* infrequently cause uncomplicated cystitis and pyelonephritis. The etiology of UTI is affected by underlying host factors that complicate UTI, such as age, diabetes or catheterization. The majority of community-acquired symptomatic UTI in elderly women are caused by *E.coli*. Etiologic pathogens associated with diabetes include *Klebsiella* spp, Group B *streptococci*, and *Enterococcus* spp (Allan, 2002).

## 2.11. Most common bacteria that cause urinary tract infection

### 2.11.1. *Escherichia coli (E.coli)*

*E.coli* is Gram-negative rods, facultative anaerobic, Lipopolysaccaride consists of outer somatic O polysaccharide, core polysaccharide (common antigen), lipid A (endotoxin) and Virulence factors (Parrick *et al*., 2009).It is the best known and most important species of the genus *Escherichia*, one of the most prevalent members of *Enterobacteriaceae*, and the most common opportunistic pathogen that lives in human and animal gut (Irving *et al.*, 2006). *E.coli* is the most common cause of urinary tract infection and gram-negative sepsis. It is one of two important causes of neonatal meningitis and the agent most frequently associated with traveler's diarrhea. Some strains of *E.coli* are enterohemorrhagic (EHEC) can cause bloody diarrhea (Levension, 2012). Treatment of *E.coli* has been increasingly complicated by the emergence of resistance to most first-line antimicrobial agents (Sabate *et al.*, 2008). Antibiotic resistance rate in *E.coli* are rapidly rising, especially with regard to third and fourth generation cephalosporins and extended-spectrum B- Lactams (Laupland *et al.*, 2008).

### 2.11.2. *Pseudomonas aeruginosa (P.aeruginosa)*

*P.aeruginosa* are Gram-negative, aerobic, non-motile, rod-shaped bacteria with widespread occurrence in nature, especially in damp biotopes (Fritz *et al*., 2005). It is usually recognize by by pigment production including pyocyanin a blue- green pigment and pyoverdin a yellow- green fluorescent pigment. *P.aeruginosa* can be found in the intestinal tract, water, soil and sewage. It frequently found in moist environment in hospital and able to grow in some eye drops, saline and aqueous solution. Many infections with

*P.aeruginosa* are opportunistic hospital acquired and often difficult to eradicate (Cheesbrough, 2006). It has become increasingly clear that resistance development in *P.aeruginosa* multifactorial, with mutations in genes encoding protein, efflux pump, Pencillin-binding protein and chromosoma β- lactamase, all contributing to resistance to β-lactamase, carbapenems, fluroquinone and aminoglcosides (Ozer *et al.*, 2009).

### 2.11.3. *Klebsiellapneumoniae (K.pneumoniae)*

*K. pneumonia* is a Gram negative, non motile, usually capsulated rods, aerobes and facultative anaerobes. *K. pneumonia* causes chest infection and occasionally severebronchopneumoniae with lung abscesses and urinary tract infection (Cheesbrough, 2006). *K. pneumoniais*the most frequently encountered carbapenemase-producing *Enterobacteraceae* (Won *et al.*, 2011).

### 2.11.4. *Proteus mirabilis (P.mirabilis)*

Member of *enterobactericea* that are non lactose fermenting, non motile and produce phenylalanine deaminase. There are several species of *proteus* but *proteus mirabilis* and *proteus vulgaris* account for the vast majority of clinical isolates in this genus, both produce urease and member of this genus also produce hydrogen sulphide. These bacteria are capable of swarming motility as they differentiate. *Proteus* spp are common cause of UTIs, occasionally in normal hosts and very commonly in those with indwelling catheter or functional abnormalities of urinary tract (Parrick *et al*., 2009).

### 2.11.5. *Enterococcus faecalis*(*E.faecalis*)

*E.faecalis* is Gram-positive cocci, occurring in pairs or short chains. They are non-capsulated and the majorities are non-motile. *Enterococci* are part of normal intestinal flora, although they are capable of producing disease in many settings such as opportunistic urinary tract infection and occasionally wound infection (James *et al*., 2004).

## 2.12. Previous studies

Many researches to date have addressed *Allium sativum* (Garlic) antibacterial properties and its effect on many infections. The following Laboratory studies and clinical trials show that *Allium sativum* is effective broad spectrum antibacterial agents. Previous study carried out by (Kumar., Sharma, 2009) in India to determine antibacterial activity of Allicin from *Allium sativum* against antibiotic resistant Uropathogenic bacteria, the results showed that *A.sativum* remarkably sensitive agent against antibiotics resistant Gram positive and Gram negative bacteria.

In Togo,(Banfitebiyi *et al.,* 2018) study the antimicrobial activity of aqueous garlic extract against Uropathogenic bacteria isolated from female which includes (*E.coli, K.pneumoniae, P.aeruginosa, E.faecalis, S.aureus*, *S.saprophyticus* and *Citobacter freudii*), the result showed that diameter of inhibition ranged from20 +_ 3 (*Klebsiellapeumoniae*), 45+_ 1 (*E.faecalis)*, 32+_ 4 (*E.coli*), 25+_ 4(*Proteus mirabilis)*, 27+_3 (*Pseudomonas aeruginosa*).Previous authors described the antibacterial activity of garlic extract against *streptococcus mutans* (Ohara *et al.,* 2008) and against *staphylococcus aureus* (Silva and Fernandes, 2010;Daka, 2011). In addition,garlic was shown antimicrobial activity against *E.coli*, *Salmonella*

*typhi, Shigella flexineri, Proteus mirabilis* (Shobana *et al.*, 2009) and *Vibrio parahaemolyticus* (Vuddhakul *et al.*, 2007).

In Sudan,(Emad, 2017), study antimicrobial activity of garlic against *S.aureus, P.aeruginosa* and *E.coli*, The result showed that gram positive bacteria more susceptible to garlic extract than gram negative bacteria. Also (Shaimaa *et al*., 2014), described Antimicrobial activity of garlic extract on bacteria isolated from teeth, the result showed that garlic have broad spectrum activity against isolated bacteria. Other study in Sudan carried out by (Rashid; Omer, 2018) described in vitro relation of antimicrobial activity of garlic and onion extracts on some clinical isolates.

# Chapter Three

# 3. MATERIALS and METHODS

## 3.1. Study design

This was prospective cross sectional study.

## 3.2. Study area

This study was conducted in Ribat University Hospital, Albaraha hospital and Khartoum Army Hospital.

## 3.3. Study duration

This study was conducted from April to December, 2019.

## 3.4. Study subject

Different clinical pathogens isolated from patients with urinary tract infection.

## 3.5. Inclusion criteria

Most common pathogenic urinary tract bacterial isolates.

## 3.6. Exclusion criteria

Non pathogenic urinary tract bacterial isolates.

## 3.7. Sample size

One hundred of different clinical bacterial isolates that already isolated from urine specimens received during study duration.

## 3.8. Ethical consideration

Permission to carry out the study was taken from the College of Medical Laboratory Science in Sudan University for Science and Technology.

## 3.9. Identification of clinical isolates

All the 100 collected clinical isolates were subjected to purification processes followed by re identification procedure based on microscopic examination, culture characteristics and biochemical reactions.

### 3.9.1. Gram stain

The Gram stain differentiates bacteria into two fundamental varieties of cells. Bacteria that retain the initial crystal violet stain (purple) are said to be Gram-positive, whereas those that are decolorized and stain red with safranine are said to be Gram-negative. This staining response is based on chemical and structural makeup of the cell walls of both varieties of bacteria. Gram-positives have a thick, relatively impermeable wall that resists decolorization and is composed of peptidoglycan and secondary polymers. Gram-negatives have a thin peptidoglycan layer plus an overlying lipid-

protein bilayer known as the outer membrane, which can be disrupted bydecolorization (Beveridge, 2009).

### 3.9.2. Biochemical tests

**A. Identification of Gram positive clinical isolates:**

**1. Litmus milk reduction test**

Litmus milk is a milk-based medium used to distinguish between different species of bacteria. The lactose (milk sugar), litmus (Ph indicator), and casein (milk protein) contained within the medium can all be metabolized by different types of bacteria; the litmus in the medium acts as both a pH indicator. The test itself tells whether the bacterium can ferment lactose, reduce litmus, form clot, form gas, or start peptonization (Schierl *et al*., 2011).

**B. Identification of Gram negative clinical isolates**

**1**. **Oxidase test**

The Oxidase test is used to detect the presence of the cytochrome oxidase, in the presence of an organism that contain cytochrome oxidase enzyme, the released color less reagent become an oxidized purpule colored product (Patrica *et al*, 2010).

**2. Kligler's iron agar test (KIA)**

The tested organism was inoculated in KIA medium, using a straight wire loop, agar butt was stabed, the opening was closed and then the top slope was streaked (as Zigzag). The medium was incubated at 37C for 24hr,

glucose fermentation, lactose fermentation, hydrogen sulphide production; gas production was looked for (Cheesbrough, 2006).

**3. Indole test**

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole, it is used as part of IMVic procedure a battery of tests designed to distinguish between a members of the family *Enterobacteriaceae* (Maria, 2009).

### 3.10. Antibacterial susceptibility testing

**Modified Kirby-Bauer Method**

Isolated organisms were tested against disc-diffusion method in which 3-5 selected colonies were touched by sterile standard loop then emulsified into sterile normal saline and adjusted to 0.5 McFarland standards (Cheesbrough, 2006). The following antibiotic discs were used: Ciprofloxacin, Gentamicin, Ceftazidime Cotrimoxazole, and imipinem.

## 3.11. Quality control

### 3.11.1. Control of culture media

The performance of culture media was controlled by testing each patch with control strains *Pseudomonas aeruginosa* ATCC 27853and *Enterococcus faecalis*ATCC29212 to check the quality of the media.

### 3.11.2. Control of susceptibility testing method

The quality control strain *Pseudomonas aeruginosa* ATCC27853 and *Enterococcus faecalis* ATCC29212 were used as described by CCSI document M7-A7 (NCCLS,2000) to assess the antimicrobial disks efficiency. The control strains were brought from National Health Laboratories in Khartoum. Susceptibility test was tested within reference strains to determine if zone diameter obtained within the expected range or not and to check the quality of test**.**

## 3.12. Preparation of *Allium sativum* ethanolic extract

Extraction was carried out according to method described by Melvin *et al*.,(2009)100gm of Garlic bulbs were dried under the shade, then coarsely powdered using mortar and pestle, Coarsely sample were soaked and extracted with ethanol alcohol 95% using soxhlet apparatus, then the solvent was evaporated on a rotary evaporator under reduced pressure and dried by oven then different concentration made by distilled water.

## 3.13. Cup-plate agar diffusion method

The agar well diffusion method was done on Muller Hinton Agar (MHA) medium for the assay of antimicrobial activity of *A.sativum* (Garlic) bulbs ethanolic extracts against different isolates from urine specimens, 3 colonies with the same characteristics were emulsified in 1ml normal saline and adjusted to 0.5Mcfarland turbidity standard. A sterile cotton swab was inserted into the bacterial suspension, rotated and then compressed against wall of the test tube to expel any excess fluid. The swab was then streaked on the surface of MHA plate. To ensure a uniform, confluent growth, the swab streaked three times over entire plate surface (Cheesbrough, 2006). Sterile cork borer was then used to make wells 6mm in diameter) on MHA

medium. Under a septic conditions 100micriliter of four concentration of *A.sativm* extract (100(%w/v),50(%w/v), 25(%w/v) and 12.5(%w/v) were introduced into the wells. The plates were allowed to stand for 1hours in the refrigerator for diffusion of the extract take place and incubated at 37C for 24hrs. Zone of inhibition were measured (in mm) and the mean were calculated (Aneja and Joshi, 2009).

## 3.14. Determination of minimum inhibitory concentration (MIC) of Garlic extract by agar diffusion method

Determination of inhibition zones and MIC of *A.sativum* (Garlic extract were assessed using agar diffusion method as described by NCCLS(2000). One gram from extract was dissolvedin 10ml(100%) distilled water(D.W)and then serially diluted two fold to obtained final concentration of (50 (5%w/v), 25 (%w/v)and 12.5 (%w/v). 100µLeach prepared concentration was added into corresponding well. The plates were left for 1 hour in refrigerator (4C$^{o}$) for diffusion of effective compounds of Garlic in media and then incubated at 37C$^{o}$for 24 hours. Inhibition zone around each well were measured using a ruler in millimeters. MIC considered as the lowest concentration of extract that prevent visible bacterial growth (Anja and Joshi, 2009).

## 3.15. Statistical analysis

Data was computed and analyzed by using Statistical Package for Social Sciences (SPSS) computer software version 20 to check frequency and mean.

# Chapter Four

# 4. RESULTS

## 4.1. Clinical isolates frequency

In this study 100 clinical isolates were tested as follows; 35(35%) *Escherichai coli*, 25(25%) *Pseudomonas aeruginosa*, 20(20%) *Klebsiella pneumonia*, 11(11%) *Enterococcus faecalis* and 9(9%) *Proteus mirabilis*.

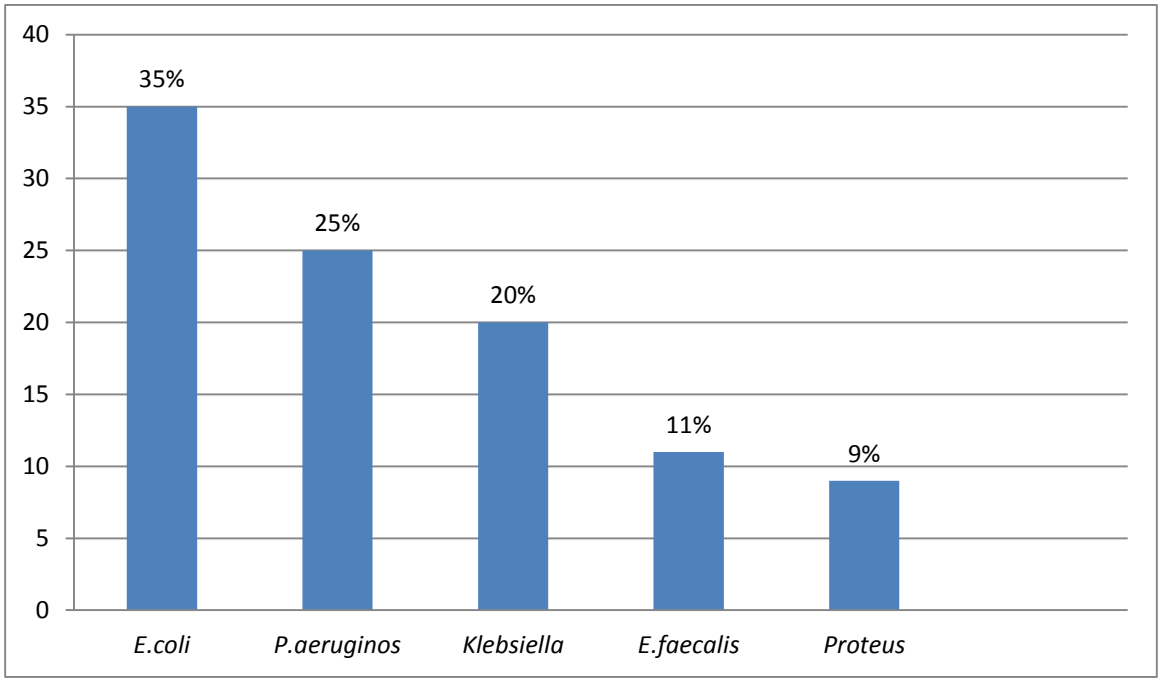


Figure1. **Frequency of clinical isolates**

## 4.2. Susceptibility of Clinical isolate to selected antibiotics

Table3.**Antimicrobial Susceptibility Testing Results of UTIs isolates**

| Antibiotics | IPM | | COT | | CIP | | GEN | | CAZ | |
|---|---|---|---|---|---|---|---|---|---|---|
| Tested organisms | S% | R% | S% | R% | S% | R% | S% | R% | S% | R% |
| *E.coli* N=35 | 60% 21 | 40% 14 | 37.1% 13 | 62.9% 22 | 34.3% 12 | 65.7% 23 | 28.6% 10 | 71.4% 25 | 20% 7 | 80% 28 |
| *Ps.aeruginosa* N=25 | 88% 22 | 12% 3 | 40% 10 | 60% 15 | 36% 9 | 64% 16 | 24% 6 | 76% 19 | 36% 9 | 64% 16 |
| *Klebsiella* N=20 | 85% 17 | 15% 3 | 50% 10 | 50% 10 | 45% 9 | 55% 11 | 50% 10 | 50% 10 | 65% 13 | 35% 7 |
| *E.faecalis* N=11 | 63.6% 7 | 36.4% 4 | 27.3% 3 | 72.7% 8 | 36.4% 4 | 63.6% 7 | 72.7% 8 | 27.3% 3 | 18.2% 2 | 81.8% 9 |
| *Proteus* N=9 | 77.8% 7 | 22.2% 2 | 44.4% 4 | 55.6% 5 | 66.7% 6 | 33.3% 3 | 33.3% 3 | 66.7% 6 | 22.2% 2 | 77.8% 7 |
| Total and% | 74% 74 | 26% 26 | 40% 40 | 60% 60 | 40% 40 | 60% 60 | 37% 37 | 63% 63 | 33% 33 | 67% 67 |

**Key words:**IPM: Imipinem, COT: Co-trimoxazole, CIP: Ciprofloxacin, GEN: Gentamicin, CAZ: Cefatzidime, S: Sensitive, R: Resistant.

## 4.3. Susceptibility of standard isolates to *Allium sativum* ethanolic extract

Antimicrobial activity of *Allium sativum*ethanolic extract was firstly screened against reference strains (*E.faecalis* ATCC29212 and *P.aeruginosa*ATCC27853).

Table4. **Antibacterial activity of *A.sativum* ethanolic extract against standard isolates**

| Standard isolates | Mean of inhibitory zones in mm | | | |
|---|---|---|---|---|
| | Con 100 Mg/ml | Con50 Mg/ml | Con25 Mg/ml | Con 12.5 Mg/ml |
| *E.faecalis*ATCC29212 | 25 VA | 23 VA | 22 VA | 20 VA |
| *Pseudomonas* ATCC27853 | 22 VA | 20 VA | 17 A | 14 A |

**Key words**: VA: Very active, A: Active, PA: Partial active, NA: Non active

**Interpretation of results:-**

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

## 4.4. Susceptibility of clinical isolates to *Allium sativum* extract

Table5.**Antibacterial activity of *A.sativum* ethanolic extract against different isolates**

| Bacterial isolates | Mean of inhibitory zone In mm | | | |
|---|---|---|---|---|
| | Con of 100 Mg/ml | Con of 50 Mg/ml | Con of 25 Mg/ml | Con of 12.5 Mg/ml |
| *E.coli* | 18.4 | 15.8 | 8.4 | 5.4 |
| | VA | A | NA | NA |
| *P.aeruginosa* | 17.2 | 14.9 | 10.9 | 4.3 |
| | A | A | PA | NA |
| *K.pneumoniae* | 18.4 | 15.5 | 11 | 6.2 |
| | VA | A | PA | NA |
| *E.faecalis* | 20.8 | 18 | 13.9 | 11 |
| | VA | A | A | PA |
| *P.mirabilis* | 18.6 | 16 | 9.6 | 8.2 |
| | VA | A | PA | NA |

## 4.5. Activity of *Allium sativum* extract against Gram positive standard and Clinical isolates.

Table6. **Shows comparison between *E.faecalis* ATCC29212 and *E.feacalis***

| **Bacterial isolates** / **Concentration** | ***E.feacalis* ATCC29212** | ***E.feacalis*** |
|---|---|---|
| **Concentration of 100 Mg/ml** | 25(VA) | 20.8(VA) |
| **Concentration of 50 Mg/ml** | 23(VA) | 18(A) |
| **Concentration of 25 Mg/ml** | 22(VA) | 13.9(A) |
| **Concentration of 12.5 Mg/ml** | 20(VA) | 11(PA) |
| **P-value** | 0.216 | 0.216 |

## 4.6. Activity of *Allium sativum* extract against Gram positive standard and Clinical isolate

Table7.**Shows comparison between *Pseudomonas ATCC27853* and other Gram negative clinical isolates**

| **Bcterial isolates** / **Concentration** | ***Pseudomonas ATCC27853*** | ***E.coli*** | ***P.aeroginosa*** | ***K.pneumoniae*** | ***P.mirabilis*** |
|---|---|---|---|---|---|
| **Concentration of 100 mg/ml** | 22(VA) | 18.4(VA) | 17.2(A) | 18.4(VA) | 18.6(VA) |
| **Concentration of 50 mg/ml** | 20(VA) | 15.8(A) | 14.9(A) | 15.5(A) | 16(A) |
| **Concentration of 25 mg/ml** | 17(A) | 8.4(NA) | 10.9(PA) | 11(PA) | 9.6(PA) |
| **Concentration of 12.5 mg/ml** | 14(A) | 5.4(NA) | 4.3(NA) | 6.2(NA) | 8.2(NA) |
| **P-value** | 1.0 | 0.173 | 0.113 | 0.115 | 0.115 |

## 4.7. Minimum inhibitory concentration (MIC) of *Allium sativum* ethanolic extract obtained by using agar diffusion method

Table 8: **MIC of *A.sativum* ethanolic extract**

| **Bacterial isolates** | **MIC mg/ml** |
|---|---|
| *E.coli* | 12.5 |
| *p.aeruginosa* | 12.5 |
| *K.pneumoniae* | 12.5 |
| *E.faecalis* | 12.5 |
| *P.mirabilis* | 12.5 |
| *E.faecalis* ATCC29212 | 12.5 |
| *P.aeruginosa* ATCC27853 | 12.5 |

# Chapter Five

# 5. DISSCUSSION

## 5.1. Discussion

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

This study was carried out to evaluate antibacterial activity of *A.sativum* ethnolic extract against bacterial isolates from urinary tract infections. The most common bacterial isolates were *E.coli* 35 (35%), *P.aeruginosa* 25(25%), *K.pneumniae* 20(20%), *E.faecalis* 11(11%) and *P.mirabilis* 9(9%), this results were in agreement with Agalu*et al*., (2014).

The result showed that Ceftazidime had high resistant rates (67%) followed by Gentamicin (63%) then Cotimoxazole (60%) and Ciprofloxacin (60%) and finally Imipinem (26%), increasing rate of resistance of this antibiotics that due to overuse and misuse of these medication (Gould and Bal, 2013; Viwanathan, 2014 and Michael *et al.*, 2014).

Garlicethanolic extract used in this study had shown antibacterial activity on Gram positive (*E.faecalis*) and Gram negative (*E.coli, P.aeruginosa, K.pneumoniae* and *P.mirabilis)*clinical isolates and also showed activity on standard isolates ( *E.faecalis* ATCC29212 and *P.aeruginosa* ATCC27853), this results agreed with Banfitebiyi , (2018) who demonstrate activity of garlic extract against uropathogenic bacteria isolated from female which

includes (*E.coli, K.pneumoniae, P.aeruginosa, E.faecalis, S.aureus, S.saprophyticus* and *Citobacter freudii*).

The results showed that the *E.faecalis*was more susceptible organisms to garlic extract with inhibition zone (20.8mm) at concentration 100(%w/v) and *P.aeruginosa* was less susceptible organisms to garlic extract with inhibition zone (17.7mm) at the same concentration. This findings in agreement with Kumar, Sharma (2009) whom reported that Gram positive bacteria showed significant higher sensitivity to garlic extract than Gram negative bacteria. In (2006) Indeu *et al.* have also reported that garlic extract possessed low level of antibacterial activity against Gram negative bacteria due to major plant pathogens are Gram negative having an effective permeability barrier such as outer membrane. The antibacterial activity *A.sativum* ethanolic extract has been evaluated *in vitro* against clinical and standard isolates (*P.aeruginosa* ATCC27853 and *E.faecalis* ATCC29212). *E.feacalis* ATCC29212 showed higher sensitivity to garlic extract than *P.aeruginosa* ATCC27853 with inhibition zones (25mm, 22mm) at concentration 100(%w/v) respectively.

The study revealed that ethanolic extract of Garlic bulbs inhibits growth of all clinical and standard strains with MIC 12.5 % (w/v).

## 5.2. Conclusion

The most common bacterial isolates were *E.coli* 35 (35%), *P.aeruginosa* 25(25%), *K.pneumniae* 20(20%), *E.faecalis*11(11%) and *P.mirabilis* 9(9%).

*A.sativum* possesses high antibacterial activities against pathogenic bacteria (*E.coli, P.aeruginosa, K.pneumoniae, E.faecalis* and *P.mirabilis*, that cause UTIs in human), and standard isolates (*P.aeruginosa* ATCC 27853 and *E.faecalis* ATCC 29212). *E.faecalis* was more susceptible isolate to *A.sativum* ethanolic with inhibition zone (20.8mm) at concentration 100(%W/V) and *P.aeruginosa* was less susceptible to garlic extract with inhibition zone (17.7mm).

MIC of ethanolic extract of *A.sativum* against all clinical isolates and standard organisms were 12.5(%w/v).

## 5.3. Recommendations

1. Further studies in *A.sativum* should be done by using large sample size.

2. Examine ethanolic extract of *A.sativum* on different isolates and use different methods and different solvents for extraction process.

3. Determination of Minimum Inhibitory Concentration by using tube dilution method.

4. Determination of active ingredients compounds found in garlic by gas chromatography.

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# APPENDICIES

## Appendix (1): Reagents and media

## A. Reagents

### 1. Crystal violet

**Content**

To make 1 litter

Crystal violet……20g

Ammonium oxalate……9g

Ethanol or methanol, absolute……95ml

Distilled water……..1 litter

**Procedure**

1. Weight the crystal violet on a piece of clean paper. Transferred to a brown bottle pre marked to hold ne litter.

2. Add the absolute ethanol or methanol and mix until the dye is completely dissolved.

3. Weight the ammonium oxalate and dissolve in about 200ml of distilled water. Add the stain, make up to one litter with distilled water and mix well.

4. Label the bottle and store it at room temperature. The stain is stable for several months.

**2. Kovac's reagent**

**Content**

To make 20 ml

4-dimethylaminobenzaldehyde……..1g

Isoamylacohol (3-methyl-1-butanol)……15ml

Concentrated hydrochloric acid…….5ml

**Procedure**

Weight the dimethylaminobenzaldehyde, dissolve in the isoamylalcohol. Add concentrated hydrochloric acid and mix well. Transfer to a clean brown bottle and stored at 2-8C.

**3. Lugol's iodine solution**

**Content**

To make one litter

Potassium iodine solution……..20g

Iodine……..10g

Distilled water…….1 litter

**Procedure**

1. Weight the potassium iodine, and transfer to brown bottle pre marked to hold 1 litter.

2. Add about quarter of the volume of water, and mix until the potassium iodine solution is completely dissolved.

3. Weight the iodine, and add to potassium iodine solution. Mix until the iodine is dissolved.

4. Make up 1litter distilled water, mix well. Label the bottle and marked toxic. Store at dark place.

**4. Turbidity standard Equivalent to 0.5 McFarland (Barium sulphate)**

**Content**

To make 1%v/v

Concentrated sulphuric acid……1ml

Dihydrate barium chloride (BaCl2.H2)……..0.5

Distilled water……150ml

Procedure

1. Prepare 1%v/v solution of sulphuric acid by adding 1 ml of concentrated sulphuric acid to 99ml of distilled water and mix

2. Prepare 1%v/v solution of barium chloride by dissolving 0.5g of dehydrates barium chloride in 50ml of distilled water. Add 0.6ml of barium chloride 99.4mlof sulphuric acid solution and mix well.

## B. Media

### 1. Kligler's Iron Agar

KIA reactions are based on the fermentation of lactose and glucose (dextrose) and the production of hydrogen sulphide.

**Formula in grams per litter**

Peptic digest of animal tissue……15

Yeast extract ……..3

Beef extract…….3

Peptose peptone……..5

Dextrose……..1

Lactose……...10

Ferrous sulphide ……0.20

Sodium chloride…….5

Sodium thiosulphate…….0.3

Phenol red……..0.042

Agar……15

Final PH (at 25 C)…….7.4

## 2. Litmus Milk Medium

Litmus milk medium is used in the rapid litmus milk reduction test to identify enterocooci.

### Formula

To make about 20ml of medium:

Skimmed milk powder……2g

Distilled water…….20ml

Litmus (indicator)……...small amount

### Preparation

Dissolve the milk in the water, add the litmus. Sterilize by autoclaving at 110C for 10 minute and Dispense aseptically in sterile tubes.

## 3. Muller Hinton Agar

### Formula in grams per litter

Beef infusion ……2

Casein acid hydrolysate…….17

Starch soluble……..1.5

Agar……..17

Final PH (at 25C) ……. …….7.3

**Preparations**

Suspended 38g in 1000ml distilled water. Heat to boiling to dissolve medium completely. Sterile by autoclave at 15 ibs pressure (121 C) for 15 min. Mix and pour.

**4. Nutrient agar**

Nutrient agar is used for cultivation of less fastidious organisms, can be enriched with blood or other biological fluids.

**Formula in grams per litter**

Peptone…….10

Beef extract……..10

Sodium chloride……....5

Yeast extract……..1.5

Agar…….15

Final PH (at 25C) ……..7.3

**Preparation**

Suspended 28g in 1000ml distilled water. Heat to boiling to dissolve medium completely.Sterile by autoclave at 15 ibs pressure (121 C) for 15 min. Mix and pour.

**5. Peptone water**

Use for culturing organisms to proceed indole test in the presence of Kovac's or Erlich's reagent that react with the indole to produce a red coloured compound.

**Formula in grams per litter**

Peptic digest of animal tissue…….10

Sodium chloride……5

Final PH (at 25 C) ……7.2

**Preparation**

Suspended 15g in 1000ml distilled water. Heat to boiling to dissolve medium completely.Sterile by autoclave at 15 ibs pressure (121 C) for 15 min. Mix and pour.

**6. Simmons citrate agar**

This test used to assist in identification of *enterobacteria*. The test is based on the ability of an organism to use citrate as its only source of carbon.

**Formula in grams per litter**

Magneseium sulphide……0.02

Ammonium dihydrogen phosphate ……..1

Dipotassium phosphate…….1

Sodium citrate …….2

Sodium chloride……...5

Bromothymol blue …….0.08

Agar…….....15

Final PH (at 25 C)…….6.8

**Preparation**

Suspended 24.28g in 1000ml distilled water. Heat to boiling to dissolve medium completely. Sterile by autoclave at 15 ibs pressure (121 C) for 15 min. Mix and pour. Set as slope.

**7. Urea Agar (Christensen)**

Testing for urease enzyme activity is important in differentiating *enterobacteria*. Especially for *proteus spp.*

**Formula in grams per litter**

Peptic digest of animal tissue…….1

Dextrose …….1

Disodium phosphate …….1.20

Mono potassium phosphate…….0.80

Sodium chloride ……..5

Phenol red ……0.012

Agar……15

Final PH (at 25 C)……6.8

**Preparation**

Suspended 24g in 950 ml distilled water. Heat to boiling to dissolve medium completely. Sterile by autoclave at 15 ibs pressure (121 C) for 15 min. Cool to 50C and aseptically add 50ml of sterile 40% of urea solution mix and pour. Set as slope.

## Appendix (2): Interpretation chart for antimicrobial susceptibility testing

| Antibiotic disc | Sensitive | Resistance |
|---|---|---|
| Imipinem (30mcg) | ≥ 23mm | ≤ 19mm |
| Co-trimoxazole(25mcg) | ≥ 16mm | ≤ 10mm |
| Ciprofloxacin (5mcg) | ≥ 21mm | ≤ 15mm |
| Gentamicin (30mcg) | ≥ 17mm | ≤ 14mm |
| Ceftazidime (25mcg) | ≥ 21mm | ≤ 17mm |

## Appendix (3) culture media and *Allium sativum* plant

**Figure (3): *Allium sativum* plant**

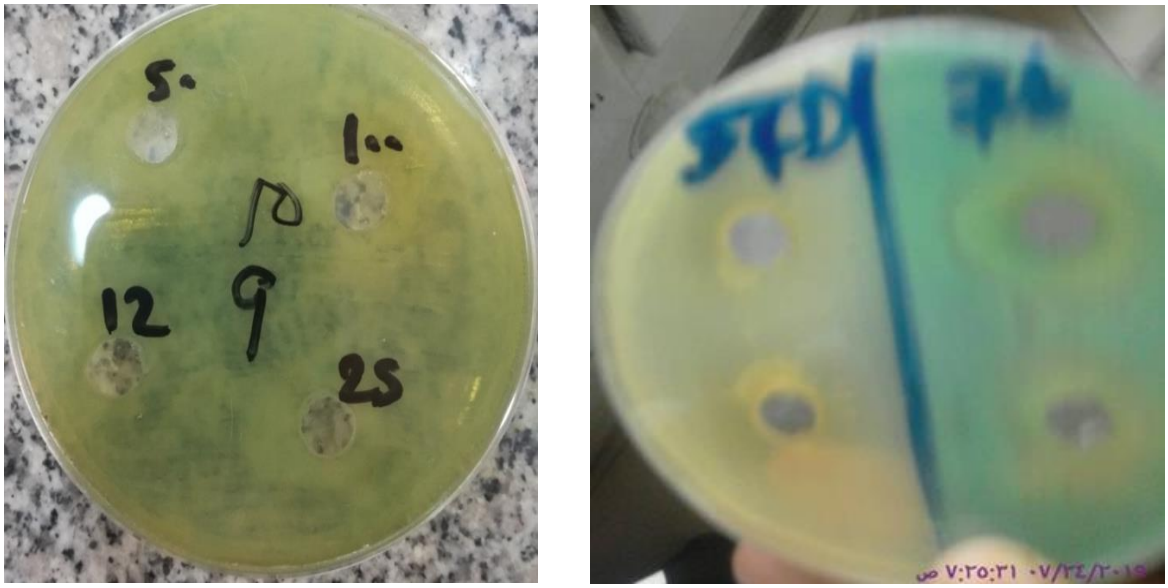


**Figure (4): The inhibition zone of *Allium sativum* extract against *Pseudomonas aeruginosa***


**Figure (5): The inhibition zone of *Allium sativum* extract against *Escherichia coli***

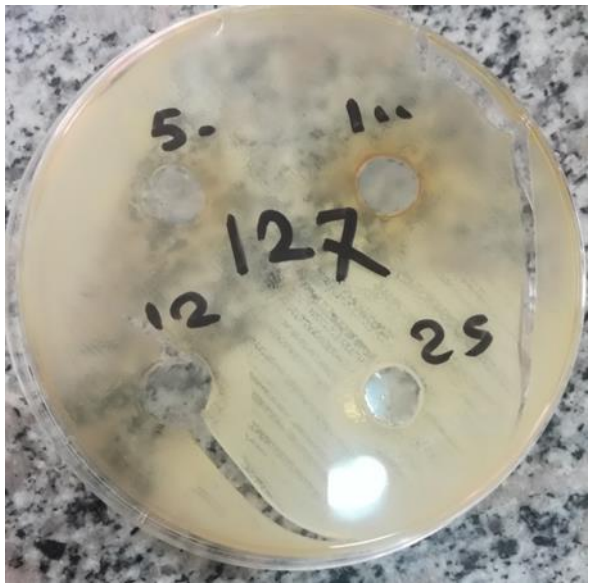


**Figure (6): The inhibition zone of *Allium sativum*extract against *klebsiellapneumonia***