Sudan University of Science and Technology

College of Graduate Studies

In vitro Antimicrobial Activity of *Acacia nilotica* pods against Selected Reference Bacterial Strains

فعالية القشرة الخارجية لثمرة القرض النيلي كمضاد حيوي ضد بعض البكتريا المرجعية المختارة

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بسم الله الرحمن الرحيم

قال تعالى :

(اقْرَأ بِاسْمِ ربِّكَ الّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانمِنْ عَلَقٍ (2) اقْرَأوربُّكَ الْأَكْرَم (3) الّذِي عَلّمَ بِالْقَلَمِ (4)عَلّمَ الْإِنْسَان مَا لَمْ يَعْلَمْ (5))

سورةالعلق

Dedication

To my father,

Mother,

Brothers,

Sisters,

Friends and Teachers

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Firstly my gratitude's and prayers to the ALMIGHTY ALLAH for the mercy that followed me through the long path of this research. It is pleasure to express my sincere thanks and deep gratitude's to my supervisor Dr. Ahmed Ibrahim Hashim for his guidance during the course of this study and the writing of the thesis.

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Abstract

This was a descriptive experimental study conducted in Sudan University of Science and Technology (College of Medical Laboratory Sciences). The aim of this study was to determine the antimicrobial activity of different concentrations of *Acacia nilotica* pods extracts against selected reference bacterial strains using ethanol, chloroform, and aqueous extracts by agar diffusion method. Six reference bacterial strains were tested [*Escherichia coli* (ATCC 25922), *Klebsiella pnemoniae* (ATCC 27736), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 49461), and *Bacillus cereus* (ATCC 10876)] and the antimicrobial activity was determined against *Acacia nilotica* extracts.The ethanolic extract of *Acacia nilotica* was active against all reference strains. The aqueous extract of *Acacia nilotica* was active against *Staphylococcus aureus and Bacillus cereus* with moderate activity against *Escherichia coli*, *Klebsiella pnemoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis.* All reference strains were resistant to the chloroform extract of *Acacia nilotica*. The minimum inhibitory concentration (MICs) for the tested plant extracts showed that the ethanolic extract was more active than aqueous extract with MICs 6.25 g/ml for 5(6) reference bacterial strains, while the aqueous extract gave MICs ≥ 25 g/ml. Further studies are required to confirm these results

المستخـــلص

هذه دراسة تجريبية وصفية تم انجازها في جامعة السودان للعلوم والتكنولوجيا (كلية علوم المختبرات الطبية) للتعرف على نشاط مستخلص القشرة الخارجية لثمرةالقرض النيلي كمضاد حيوي بتراكيز مختلفة ضد بعض البكتيريا المرجعية المختارةعن طريق بثلاثة أنواع من المـذيب : (الإيثانول، الكلوروفورم، والماء) باستخدام أسلوب تقنية الانتشار المعدل . تم اختبار ستة انواع من البكتيريا المرجعية القياسية (الإشريكية القولونية 25922 ، الكلبسيلة الرئوية 27736 ،الزائفة الزنجارية 27853 ، المكورات العنقودية الذهبية 25923 ، المكورات العنقودية البشروية 49461 ، العصوية الشمعية 10876) وتم اختبارها ضد فعالية القشرة الخارجية لثمرة القرض النيلي .وكان مستخلص الإيثانول الأكثر نشاطا ضد كل أنواع من البكتيريا المختارة. أما المستخلص المائي أظهر نشاطا عاليا ضد المكورات العنقودية الذهبية و العصوية الشمعية واظهر ايضا نشاطا متوسطا ضد الإشريكية القولونية، الكلبيسيلة الرئوية، الزائفة الزنجارية والمكورات العنقودية البشروية. بينما كانت كل البكتيريا المرجعيية مقاومة لمستخلص الكلوروفورمز وأظهرت التركيزات المثبطة الدنيا لمستخلصات النباتات المختبرة أن المستخلص الإيثانولي كان أكثر نشاطا من المستخلص المائي مع تركيز مثبط أدنى بمقدار 6.25غم/مل لخمس سلالاث بكتيرية مرجعية من أصل ستة. في حين أن المستخلص المائي أعطى 25 ≤ غم/مل. مزيد من الابحاث والدراساث مطلوبة للتأكيد على هذه النتائج .

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CHAPTER ONE

INTRODUCTION

1. Introduction

The World Health Organization (WHO) has listed more than 21,000 plants, which are used for many medicinal purposes around the World. They observed that about 74% of 119 plant-derived pharmaceutical medicines are used in modern medicine. It also estimates that 4 billion people (80 percent of the world population) presently use herbal medicine for health care. Over hundreds of years, herbal medicines derived from medicinal plants minerals and organic matter is still the mainstay of about 75–80% of the world's population for health care marketed and gaining popularity in developed and developing countries. Herbs have medicinal property due to presence of different active principles like alkaloids, volatile essential oils, glycosides, resins, oleoresins, steroids, tannins and phenols. In the last few years there is an exponential growth in the field of herbal medicine because of their natural origin, availability, efficacy, safety and less side effects with efficient to cure age-related disorders like memory loss, osteoporosis, and immune disorders for which no modern medicine is available. Medicinal plant researchers pursued with several goals like the development of low cost therapeutic compounds and the discovery of prototypic drugs (Malviya *et al*., 2011). The genus Acacia is the second largest in the family Leguminosae, with about 1350 species. It is distributed throughout tropical and warm temperate areas of the world (Sharma *et al*., 2014). *Acacia nilotica* is also known as Gum Arabic tree, Babul, Egyptian thorn, it is multipurpose nitrogen fixing tree legume. It occurs from sea level to over 2000 m and withstand at extreme temperature (>50 C°) and air dryness but sensitive to

frost when it is young. It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India (Malviya *et al*., 2011). *Acacia nilotica* is a multipurpose plant, it was used for treatment of various diseases; the plant contains a profile of a variety of bioactive components (Singh *et al*., 2009). *Acacia nilotica* is one of the species that has been effectively utilized in folk medicine for the treatment of tuberculosis, leprosy, smallpox, dysentery, cough, opthalmia, toothache, skin cancer as astringent, antispasmodic, and aphrodisiac by rural population (Sharma *et al*., 2014). *Acacia nilotica* leaves are protein rich and highly digestible. Leaves of Acacia plants, in general, possess a significant level of antibacterial activity against a wide range of bacterial pathogens, although the extent of antibacterial activity varies depending upon the type of extract (Sharma *et al*., 2014).

1.1 Rationale

Microbial infections are major public health problems in the world. Antibiotics are used to treat these infections. Due to indiscriminate use of commercial antibiotics, the incidence of multiple antibiotic resistance in human pathogens is increasing. This has forced the scientists to search for new antimicrobial substances from various sources like medicinal plants. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products (Ivanona *et al*., 2005). About 80% of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants (Hema *et al*., 2012). Herbal and natural products have enormous popularity as self-medication products (Greenwald, 1998). Herbal medicines are the oldest remedies known to mankind. Medicinal plants are an important therapeutic aid for various ailments (Gordon and David, 2001). Plants essential oils 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 healthcare.

1.2 Objectives

1.2.1 General objective

To determine the antimicrobial activity of *Acacia nilotica* pods against Selected reference bacterial strains

1.2.2 Specific objectives

1- To confirm the identity of the selected reference bacterial strains through conventional methods

2- To determine the antimicrobial activity of *Acacia nilotica* extracts against selected reference bacterial strains using agar diffusion method

3- To compare the antimicrobial activity of aqueous, ethanol, and chloroform extracts of *Acacia nilotica* against selected reference bacterial strains

4- To determine the Minimum Inhibitory Concentration (MICs) of the selected extracts against reference bacterial strains

CHAPTER TWO

LITERATURE REVIEW

2.1. Medicinal plants and their traditional uses

Medicinal plants have been used as source of medicine in virtually all cultures. During the last decade, the use of traditional medicine (TM) has expanded globally and continues to gain more popularity. It has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in national health care system (Hailu *et al*., 2005). 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 active ingredients isolated from medicinal plants. In the health care up to 40% of modern drugs are derived from natural sources, using either natural substance or synthesized version (Jasim and Naji, 2003).In the early 19th century, when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. Later, chemists began making their own version of plants compounds and over time, the use of herbal medicines declined in favor of drugs. Almost one fourth of pharmaceutical drugs are derived from plant. Herbal medicine is used to treat many conditions, such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome and cancer (Steven and Ehrlich, 2011).In Africa and other developing countries, these traditional medicines derived from plants have continued to form the basis of rural medical care. This is due to the fact that this medicine are easy to get and available in cheap prices (Mohamed, 2012). In Iranian traditional medicine “ITM” the use of

plants in treatment of burns, dermatophytes and infectious disease or as antiseptic and anti-inflammatory was common (Ghahrman and Attar , 1998). Extracts of 13 Brazilian medicinal plants were screened by Holtez *et al.,* (2002) for antimicrobial activity against bacteria and yeast; of these 10 plants extract showed varied levels of antimicrobial activity. Total of 82 Indian medicinal plants traditionally used in medicines were subjected to preliminary antibacterial screening against several pathogenic microorganism the results indicated that 56 exhibited antimicrobial activity (Ahmed *et al.,* 1998). Extracts of 111 Sudanese medicinal plants were subjected to preliminary antibacterial activity where out of 573 extracts screened, 433(76%) exhibited antibacterial activity (Almagboul, 1992).

2.2. Botanical ethno- pharmacological properties of *Acacia nilotica*

Different parts of selected *Acacia nilotica* were recognized as component of the traditional medicine in Sudan. They were arranged with their family, scientific and common name, distribution, botanical description, chemical constituents, antimicrobial activity and medical uses.

2.2.1. *Acacia nilotica*

2.2.1.1. Taxonomical classification

Kingdom: Plantae

Subkingdom: Tracheobinota

Division: Magnoliophyta

Class: Magnoliposida

Subclass: Rosidae

Order: Fabales

Family*:* *Fabaceae*

Genus: *Acacia*

Spices: *nilotica (Malviya et al., 2011).*

Vernacular names Unani Tibbi: Aqaqia , English: Indian gum Arabic, Black babool, Arabic: Um mughilan, Hidi: Kikar, Kannada : Jaali,Gobbi , Latin : Acacia Arabica, Kashmiri : Sac , Punjabi : Kikkar , Bengali : Babla

2.2.1.2. Distribution: The species has wide spread in Africa and Asia. Indian gum Arabic tree is found in well watered Sahelian and Sudanian savannas to the Southern Arabian Peninsula, East Africa and in Gambia, Sudan, Togo, Ghana, and Nigeria and Indian (Malviya *et al*., 2011).

2.2.1.3. Botanical description

Acacia nilotica is a single stemmed plant, grows to 15-18 m in height and 2-3 m in diameter. Pods and seeds: pods are 7-15 cm long green and to mentose (when immature) or greenish black (when mature). Seeds are 8-12 per pods, compressed, ovoid, dark brown shining with hard testa (Malviya *et al*., 2011).

2.2.1.4. Chemical constituents

Acacia species contains secondary metabolites including amines and alkaloids such as cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, nonprotein amino acids, terpenes, flavonoids and condensed tannins (Seigler, 2003).The mature seeds contain crude protein, crude fibre, crude fat, carbohydrates, potassium, phosphorus, magnesium, iron and manganese occurred in high concentration and it richer source of cysteine, methionine, threonine, lysine and tryptophan. Fruit also contains mucilage and saponin (Pande, 1981; Siddhuraju, 1996).

2.2.1.5. Ethopharmacological Studies

The biological activity of *Acacia nilotica* has been reported in various studies *in vitro* as well as *in vivo*. Gilani *et al.,* (1999) reported that the methanolic extract of *Acacia nilotica* pods has led to decrease in arterial blood pressure at dose (3-30 mg/kg). Arora *et al.,* (2003) reported that the acetone extract of *Acacia nilotica* exhibited anti mutagenic activity. Singh and Singh (1972) evaluated crude extract of leave of *Acacia nilotica* extracts and reported *in vitro* antiviral activity against Turnip mosaic virus. Khan (2009) Reported the antimicrobial activity of crude ethanolic extracts of five plants including *Acacia nilotica* can be used against multi drug resistant microbes causing nosocomial and community acquired infections. Wadood *et al.,* (1989) reported that normal rats fed for one week with *Acacia*

nilotica exhibited hypoglycemic effect (blood sugar lowered by 25.05%). Nath *et al.,* (1992) reported that aqueous or 90% ethanol extract of plants were studied in rat orally dose for 10 days and the effect on foetal *Moringa* was 100% abortive while *A*cacia *nilotica* appeared to lack teratologic potential at the doses tested. Eline *et al.,* **(**2004**)** reported that aqueous extract of *A. nilotica* could stimulate milk production in lactating women.

2.2.1.6. Medicinal uses

Tamarind plant has various therapeutic uses such as anticancer, antitumor, astringent, antioxidant, antispasmodial, diuretic, intestinal pain and diarrhea, nerve stimulant, cold, congestion, cough, dysentery, fever, hemorrhages, leucorrhea, ophthalmia and sclerosis. Seed have antimalarial activities, antihypertensive and antispasmodic activities. Leaves & pods are an excellent nutritive source with anti-inflammatory properties; bark is used in the treatment of hemorrhage, cold, diarrhea, tuberculosis and leprosy. Roots were used as anaphrodisiac and the flower for treating syphilis lesions. Gum obtained from the tree pharmaceutically is used as suspending and emulsifying agent and in preparation of many formulations (Malviya *et al*., 2011). The *Acacia nilotica* has been used to treat sore throat, cold, bronchitis, pneumonia, ophthalmia, diarrhea, dysentery, leprosy, venereal disease and hemorrhage. Existing literature reported that Acacia *nilotica* has demonstrated considerable antibacterial & antifungal activity (Abd-elnabi *et al*., 1992).

2.2.2. *In vitro* antimicrobial activity

Antimicrobial activity is measured *in vitro* either to determine the potency of antimicrobial agent in a solution, to evaluate new antimicrobial agents by testing them against a large number of organisms, or to evaluate the sensitivity of a given microorganism to a known concentration of the drug (Bae and Byun, 1987). Measurement of these quantities may be undertaken by diffusion method, using appropriate standard test organism. This method can be employed to estimate either the potency of antimicrobial in sample or the sensitivity of a microorganism. The agar diffusion method could be performed variety of devices such as stainless cylinders or cork borer that is used to make holes in the agar (Stanley and Walton, 1968). The inhibition zones are dependent upon both the dispersion of the agent in the medium and the degree of susceptibility of the organism. The speed of growth and the size of inoculums can influence to marked degree the size of inhibitory zones. The interpretation of the growth inhibition zone was as followed: ≥18 mm was considered sensitive; 14-18 mm considered moderate; < 14 mm was considered resistant (Peter and Plorde, 1963; Kavanagh, 1972).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

This was a descriptive and experimental study

3.2. Study population

Selected reference strains of American Type Culture Collection (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 27736, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 49461, *and Bacillus cereus* ATCC 10876) which were obtained from Omdurman Islamic University and National Health Laboratory Khartoum.

3.3. Confirmation of the identity of the bacteria

The purified isolates were confirmed by traditional conventional methods according to Barrow and Feltham (1993). These include staining reaction, organism morphology growth characteristics, haemolysis on blood agar, and lactose fermentation on MacConkey's agar (Hi Media, India media), motility and biochemical characteristics to confirm the organisms like Catalase test, Coagulase test, DNAase test, Tryptophan hydrolysis test, Citrate utilization test, Urease test, and Gelatin stab culture.

3.4. Extraction of medical plants

3.4.1. Collection of plant samples

The plant extracts were collected and authenticated at the Medicinal and Aromatic plant Research Institute (MAPRI).

3.4.2. Preparation of crude extract

Extraction was carried out according to the method described by Sukhdev *et al.*, (2008). Eighty grams of plant *were* grounded using mortar and pestle and successively extracted with chloroform and ethanol using soxhelt extractor apparatus. Extraction carried out for about four hours for chloroform and eight hours for ethanol. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally extracts allowed to air in petri dishes till complete dryness and the yielded percentages were calculated as followed: Weight of extract obtained / weight of plant sample * 100

3.4.3. Preparation of the aqueous extract

Twenty gram of plant sample were soaked in 500 ml hot distilled water, and left till cooled down with continuous stirring at room temperature. Extract was then filtered and freezed in a deep freezer. Freezed at - 20ºC extract was dried using freeze dryer -40ºC till powdered extract obtained. Yield percentage was calculated.

3.5. Preparation of bacterial suspension

The inoculum density was compared with McFarland standard solution of $BaSO_4$ (0.1ml of 1% $BaCl_2$ + 9.9ml of 1% H_2SO_4). The suspension was stored in the refrigerator at 4ºC until used.

3.6. Data analysis

The Data analyzes was carried out through Microsoft Excel 2007.

3.7 Antimicrobial activity method

In vitro testing for antibacterial activity in the cup plate agar diffusion method according to (Kavangh, 1972) was adapted to assess the antibacterial activity of prepared extracts. One ml of the standardized bacterial stock suspension was thoroughly mixed with 100 ml of Muller-Hinton agar (Hi Media, India) which was maintained at 45ºCand 20 ml aliquots of the inoculated Muller-Hinton agar were distributed into sterile plates. The agar was left to set and in each of these plates, 4 cups (10 mm in diameter) were cut using a sterile cork borer and agar disc were removed. Alternate cups filled with 0.1ml samples of each of the extracts using automatic micro titer pipette, and allowed to diffuse at room temperature for 2hrs. The plates were then incubated in the upright position at 37ºC for 24hrs.

3.8 Determination of Minimum Inhibition Concentration (MICs)

The principle of agar diffusion was the inhibition of the growth on the surface of the agar by the plant extracts. Plates were divided into four holes by core borer. The plant extract was decreasing concentrations in the following order 50, 25, 12.5, 6.25 g/ml. The tested organisms were grown in Muller Hinton agar and pour the plant extract in holes, after that incubated at 37ºC for 24 hours over. The end point (MIC) was the least concentration of antimicrobial agent that completely inhibits the growth. Results were reported as the MIC in g/ml.

CHAPTER FOUR

RESULTS

4.1 Characters and biochemical tests of *Staphylococcus aureus* and *epidermidis*

The biochemical tests of *Staphylococcus aureus* and Staphylococcus *epidermidis* showed in Table 4.1:

Table 4.1 Colonial morphology and selected biochemical tests for Gram positive strains

Bacteria / **Test**	***Staphylococcus aureus***	***Staphylococcus epidermidis***
MacConkey's agar	Yellow colonies	No growth
Blood agar	White creamy colonies	White colonies
Haemolysis	B-haemolysis	Non haemolysis
Catalase	Positive	Positive
DNase	Positive	Negative
Coagulase	Positive	Negative

4.2 Colonial morphology and biochemical test result of *Bacillus cereus*

1- Blood agar: grey-white, irregular colonies with wavy edges. The colonies are haemolytic. 2- Gelatin stab culture: liquefies gelatin stabs

4.3 Colonial morphology and biochemical tests results of Gram negative bacteria

The biochemical tests for Gram Negative bacteria were shown in Table 4.2

Table 4.2 Colonial morphology and selected biochemical tests results used for Gram negative strains

Bacteria / **Media**	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pnemoniae*
MacConkey's agar	pink	Yellow	Pink
Blood agar	Large white, non haemolytic colonies	Large haemolytic colonies	Large mucoid colonies
Shape	Rod	Rod	Rod
Oxidase test	Negative	Positive	Negative
Catalase test	Positive	Positive	Positive
Citrate test	Negative	Positive	Positive
Urease test	Negative	Negative	Positive
KIA Slope/butt	Y/Y	R/R	Y/Y
Gas production	Positive	Negative	Positive
H2S production	Negative	Negative	Negative
Lactose fermentation	Positive	Negative	Positive
Indole test	Positive	Negative	Positive

4.4 Antimicrobial activity results

Every extract was tested in triplicate against the selected bacteria and after incubation the diameters of the resultant growth inhibition zones were measured and the mean values were tabulated (Table 4.3).*Escherichia coli*, *Klebsiella pneumonia*e and *Pseudomonas aeruginosa* sensitive to the ethanol extract, moderate activity to the aqueous extract and resistant to the chloroform extract. *Staphylococcus aureus* and *Bacillus cereus* sensitive to ethanol extract and aqueous extract but resistant to the chloroform extract. *Staphylococcus epidermidis* was sensitive to the ethanol extract and moderate to the aqueous but resistant to chloroform. The result revealed that ethanol extract was the most effective extract followed by aqueous extract and chloroform extract respectively.

Table 4.3 Results of antimicrobial activity of *Acacia nilotica* sub spp nilotica against reference bacterial strains

Reference strains	**Mean diameter of growth inhibition zone (mm)**		
	Aqueous	Ethanol	Chloroform
1. *Escherichia coli*	16	30	12
2. *Klebsiella pneumonia*e	14	22	10
3. *Pseudomonas aeruginosa*	16	26	12
4. *Staphylococcus aureus*	23	30	10
5. *Staphylococcus epidermidis*	17	28	10
6. *Bacillus cereus*	24	29	10

4.5. The Minimum inhibitory concentration of *Acacia nilotica against* selected reference strains

Reference strains	Ethanol	Aqueous
1. *Escherichia coli*	6.25 g/ml (19 mm)	_
2.*Pseudomonas aeruginosa*	25 g/ml (20 mm)	_
3. *Staphylococcus aureus*	6.25 g/ml (19 mm)	25 g/ml (19mm)
4.*Staphylococcus epidermidis*	6.25 g/ml (19 mm)	_
5. *Bacillus cereus*	6.25 g/ml (20 mm)	25 g/ml (19mm)

CHAPTER FIVE

DISCUSSION

There are many reports about usage medicinal plants and their potential as possible therapeutic agent against human pathogen. Many of these studies report the antimicrobial potential based on selected clinical isolates which make the comparison impossible between various studies. Reference strains used for screening antimicrobial activity could minimize or limit the variations between different studies. In vitro studies in this work showed that the essential extract inhibited bacterial growth but in varying degrees. The current study was carried out to screen the antimicrobial activity of *Acacia nilotica* sub spp nilotica. ethanolic, choloroformic and aqueous extract was used against Selected ATCC strains. The results showed high activity of the ethanolic and aqueous extract of *Acacia nilotica* sub spp nilotica but choloroformic extract didn't have activity to all the strains. *Acacia nilotica* ethanol extract was the most active against reference strains. This results agree with these reported by El-kamli and Awad El- karim (2009). The means diameter of growth inhibition zone (MDIZ) of microorganism isolates increases with the increase in drug concentration. This result is in agreement with those reported by Suleiman (2013). In this study the aqueous and ethanol extracts showed high antimicrobial activity against all tested organisms (the aqueous with *Staphylococcus aureus, Bacillus cereus*). In comparison between this study and a study conducted by (Masih *et al*., 2014) it was found this study showed that ethanol extract gave highest inhibition zone with *Staphylococcus aureus* same to this study, and the inhibition zone of *Escherichia coli* larger than *Pseudomonas aeruginosa*. Also when comparing the result of aqueous extract in this study and a study conducted by El-kamli and Awad El- karim (2009) both studies showed large inhibition zone against *Staphylococcus aureus* and same inhibition against

Escherichia coli and *Pseudomonas aeruginosa.* In another study conducted by (Ruttoh, 2009) the antimicrobial potential of *Acacia nilotica* was due to several components such as tannins, terpenoids, cardiac glycosides and alkaloid have been found to have the antibacterial properties, and the results of gas chromatography has shown 33 compounds of *Acacia nilotica* and also these compounds was identified qualitatively by Retention time, and quantitatively by area under the curve. *Acacia nilotica* extracts was more pronounced on the Gram-positive bacteria than the Gram-negative bacteria. The reason for the difference in sensitivity between Gram positive and Gram-negative bacteria might be to the differences in morphological constitutions between these microorganisms, Gram-negative bacteria having an outer phospholipid membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. The Gram-positive bacteria on the other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell wall of Gram-negative organisms which are more complex than the Gram-positive ones act as a diffusional barrier and making them less susceptible to antimicrobial agents than are Gram-positive . In spite of this permeability differences, however, some of the extracts have still exerted some degree of inhibition against Gram-negative organisms as well as E. coli (El-kamli and Awad El- karim (2009).

CHAPTER SIX

Conclusions and Recommendations

6.1 Conclusions

In conclusion *Acacia nilotica* has high activity against some bacteria, which justify it is use in traditional and folk medicine

The ethanolic extract showed high antibacterial activity while the aqueous extract showed medium to high activity. The chloroform extract showed resistant to all bacterial strains. Higher concentrations of *Acacia nilotica* were more effective than lower concentrations which mean that the antimicrobial activity of *Acacia nilotica* extract is concentration dependent. The ethanolic extract was the most potent extract giving MICs of 6.25 g/ml against five reference bacterial strains.

6.2 Recommendations

Further studies with reference strains as well as clinical isolate is required to verify these results and confirm the antimicrobial activities of aqueous, ethanol, chloroform and other extracts of *Acacia nilotica* against clinical bacterial isolates. And identify the active compounds of different extracts and the toxicity of these compounds.

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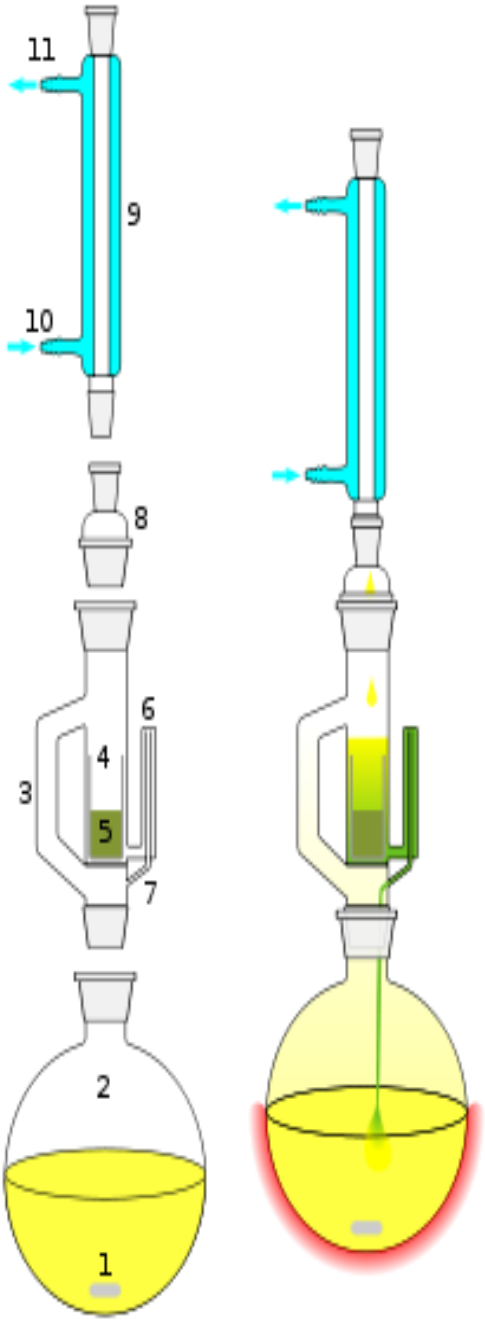
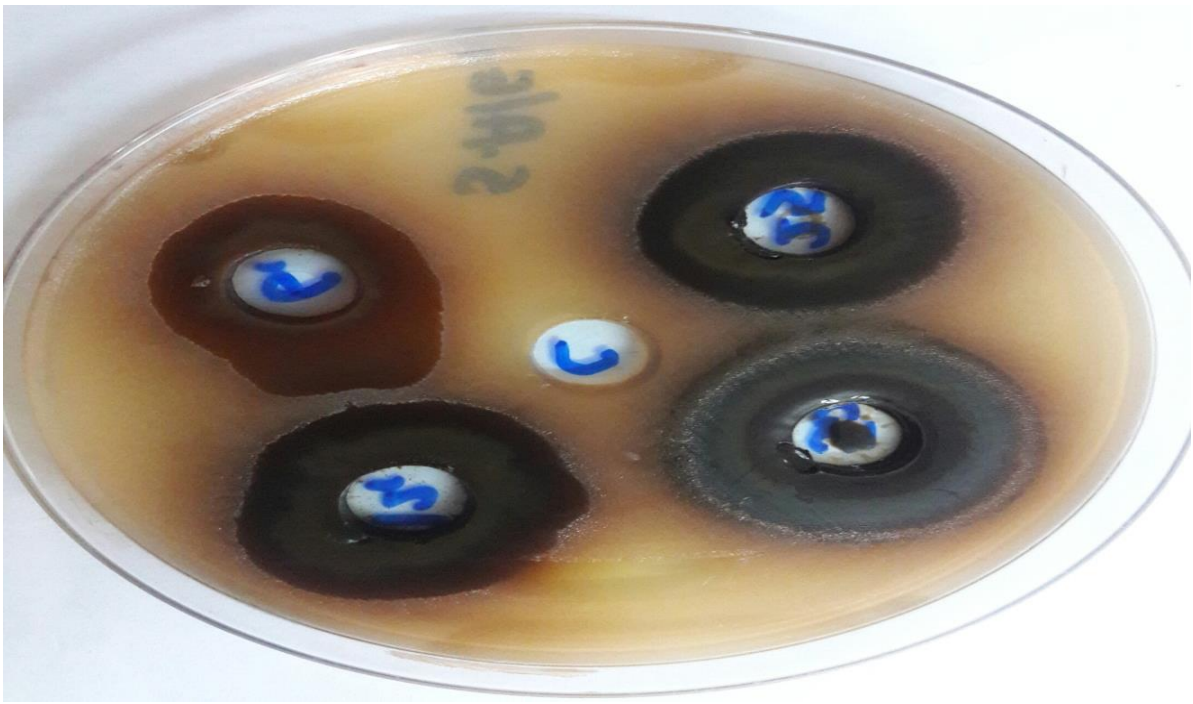
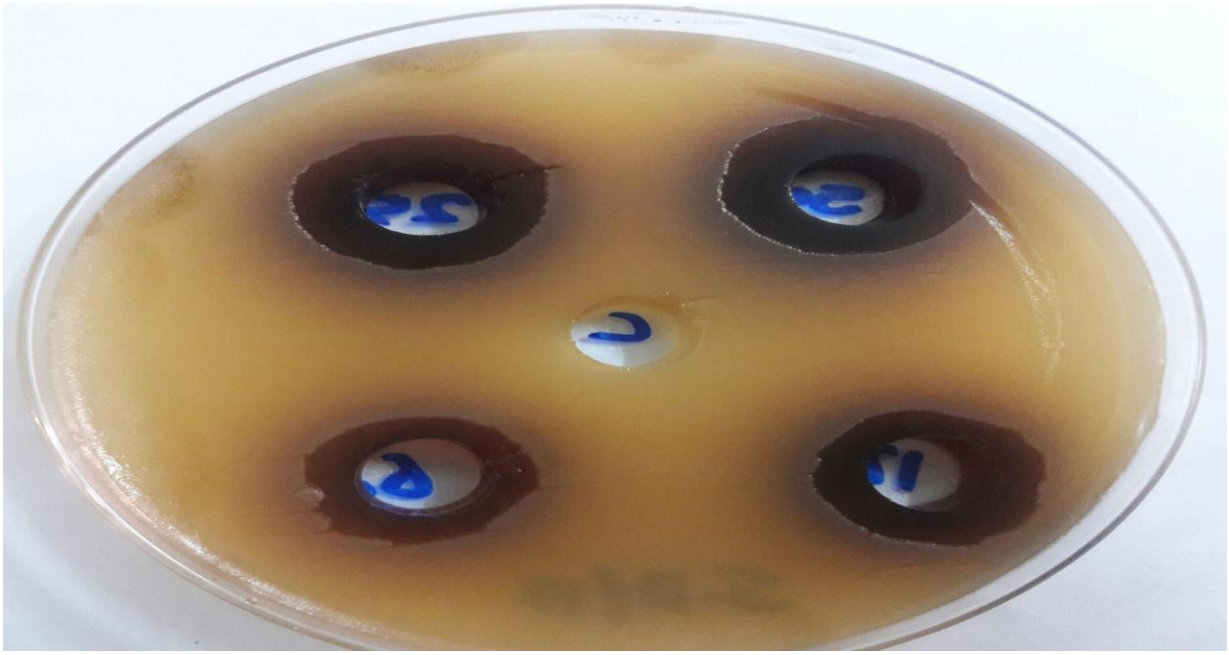


Soxhelt extractor apparatus



Activity of Ethanol extract of *Acacia nilotica* against *S.aureus*



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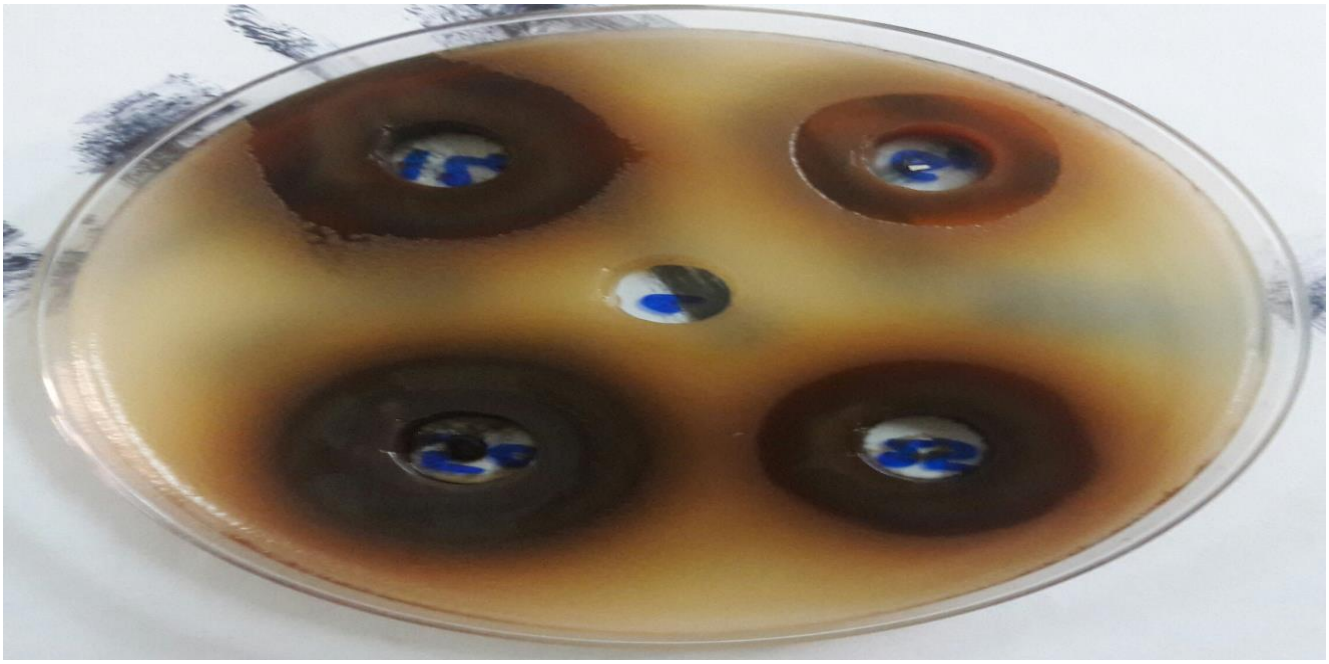
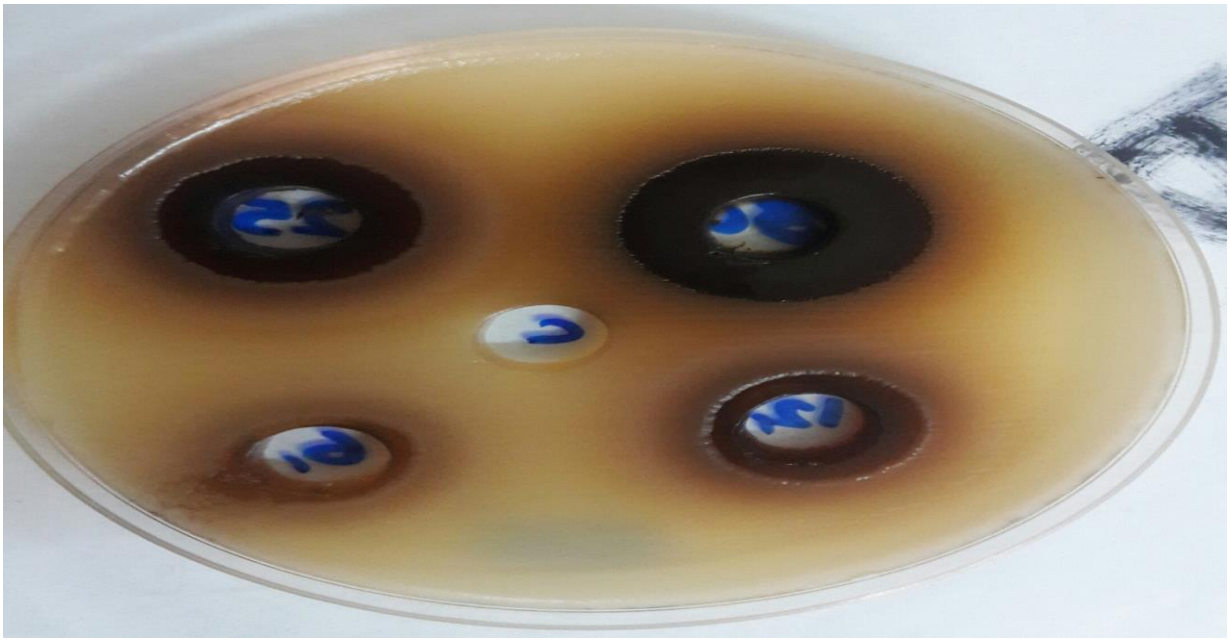
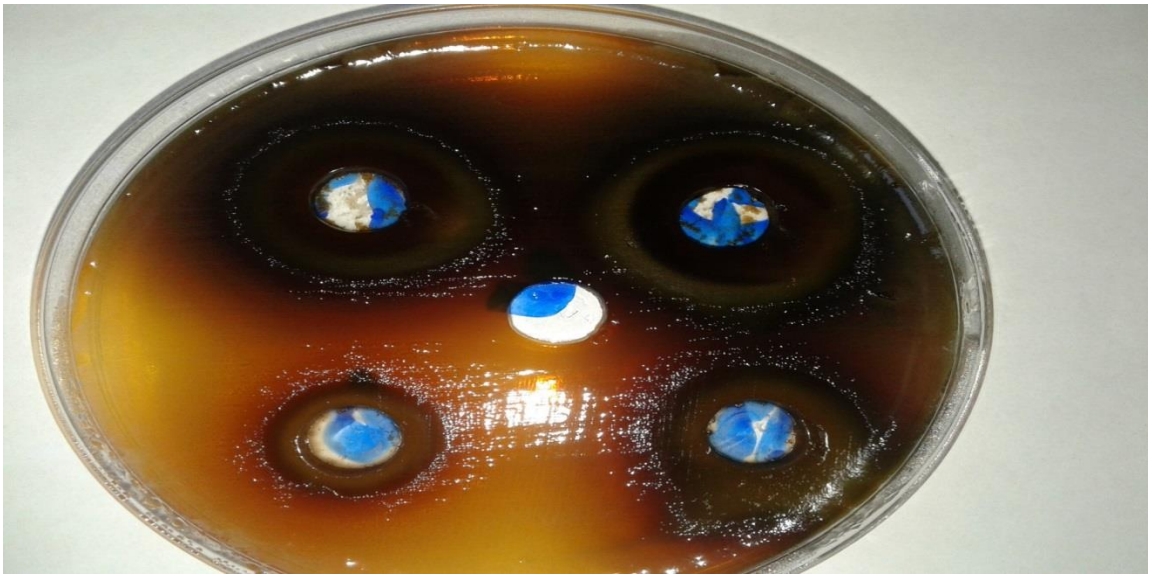


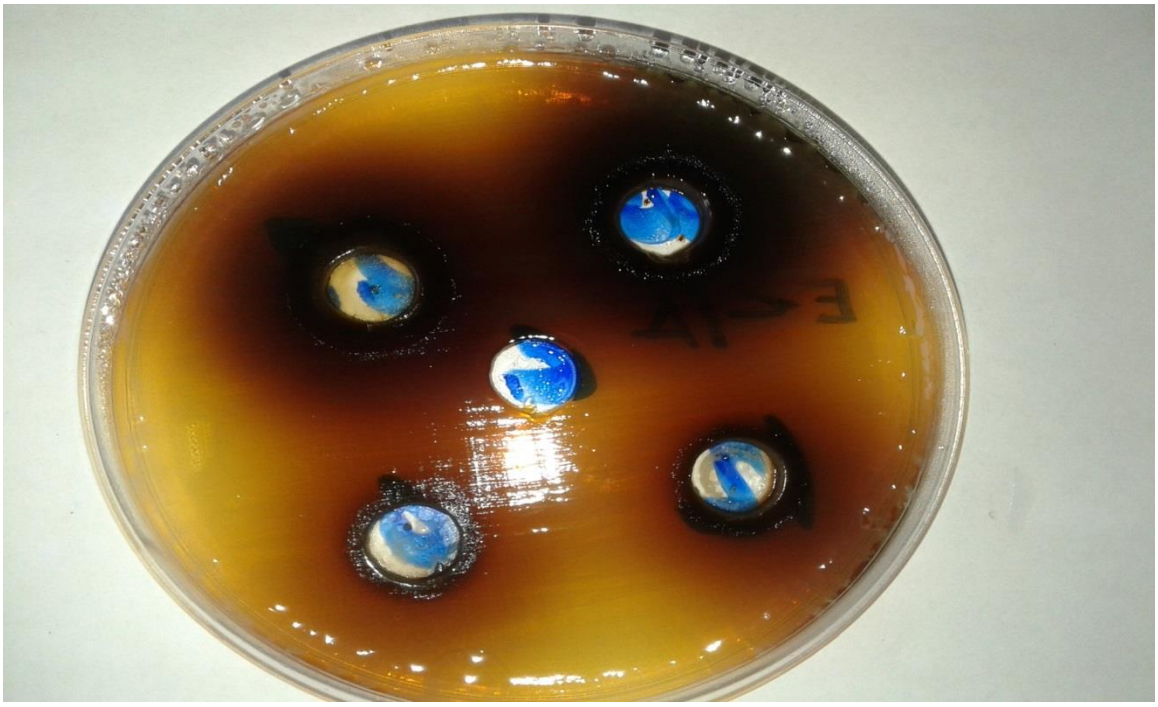
Activity of Ethanol extract of *Acacia nilotica* against Bacillus cereus



Activity of aqueous extract of *Acacia nilotica* against Bacillus cereus



Activity of Ethanol extract of *Acacia niloticaa*gainst *Escherichia coli*



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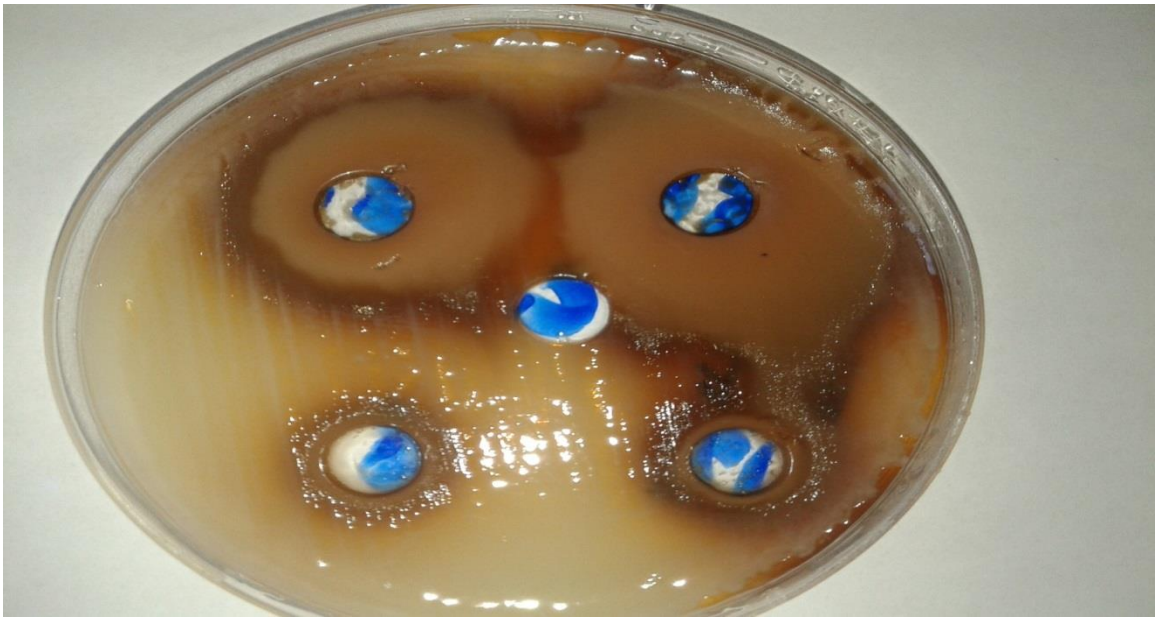
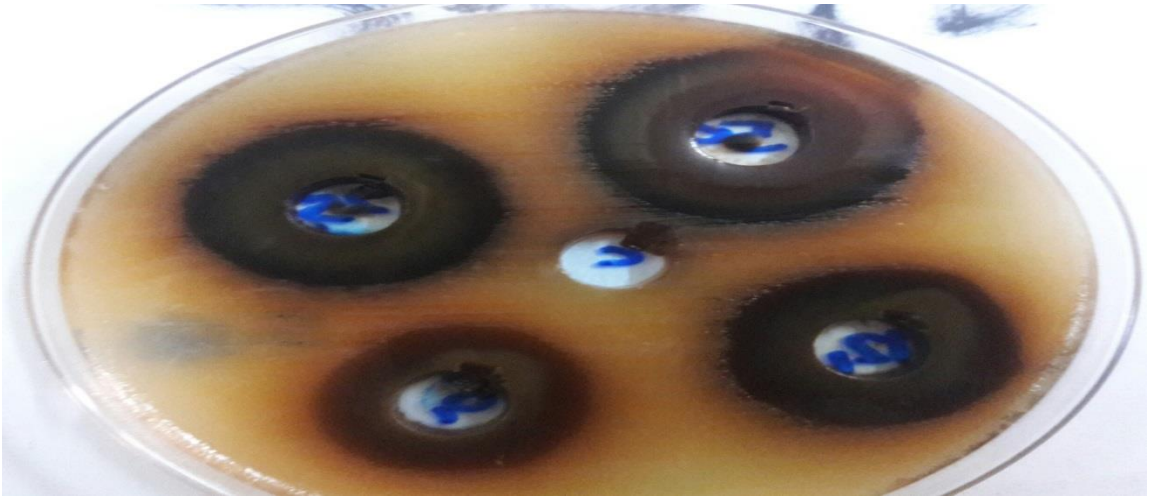


Activity of Ethanol extract of *Acacia nilotica* against *klebsiella pneumoniae*

Activity of Ethanol extract of *Acacia nilotica* against *Pseudomonas aeruginos*



Activity of Ethanol extract of *Acacia nilotica* against *Staphylococcus epidermidis*

Yield percent of extracts:

Weight of sample	**Chloroform**		**Eethanol**	
	Weight of extract	Yield %	Weight of extract	Yield %
80 g	3.253 g	4.066 %	29 g	36.25 %
Weight of sample	**Aqueous**			
	Weight of extract	Yield %		
20 g	3.384 g	16.92%		