CHAPTER ONE

INTRODUCTION

1.1. Introduction

Diabetes Mellitus (DM) is a progressive disease wide-reaching and remains an important cause of morbidity, mortality and major lower extremity amputation at some stage of life (Caputo *et al.*, 1997). The number of diabetic patients (DP) worldwide in 2007, about 246 million infected, and is expected to amount to more than 350 million in 2025 (Rubaiaan, 2008).

Diabetic foot infections (DFI) are in general caused by microorganisms. They are usually poorly managed due to misunderstanding of microbial prevalence and therapeutic approaches, so most of these microorganisms have developed resistance to many antibiotics because of indiscriminate use of antimicrobial drugs that create a big problem in the treatment of infectious diseases .With the increase in resistance of many microorganisms to the currently used antimicrobials and the high cost of production of synthetic compounds; in addition to many side effects; there is a need to look for the alternatives. Plants have provided a good source of anti-infective agents; emetine, quinine, berberine, tannins, terpenoids, alkaloids and flavonoids remain highly effective instruments in the fight against microbial infections (Tariq et *al*., 2011).

Multiple drug resistance has developed due to excessive use of existing antimicrobial drugs in the treatment of infectious diseases. Antimicrobial resistance is harmful to mankind, because most of the infectious microbes become multiple drug resistant (Dnyaneshwar *et al*., 2011). In concern to drawbacks of convectional medicine, the use of natural products as an alternate

to the convectional treatment in healing and treatment of various diseases has been rise in the last few decade (Manik *et al.*, 2013).

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases(Manik *et al*., 2013).

The importance of natural product in the treatment of disease has been increased because of its natural source and comparatively lesser side effects as compared to the complexity in formulating chemical based drugs as well as uprising cost has led worldwide researchers to focus on the medicinal plant research (Manoj, 2017).

Essential oils obtained from aromatic plants have recently gained popularity and scientific interest. Many plants are used for different industrial purposes such as Food, drugs, and perfumery manufacturing .These compounds possess a wide spectrum of pharmacological activities .They also do not enhance the "antibiotic resistance", a phenomenon caused by long-term use of synthetic antibiotics. However, due to an increasing use of herbal products, a special care should be given to their safety, effectiveness, and drug interactions (Nicholas *et al.,* 2015)

Plants in several families of the plant kingdom have been used for centuries in medicine. One of these plants is *Ricinus communis* which have a good antimicrobial activity against different organism and others medicinal use (Bentley and Trimen, 2007)

1.2. Rationale

Pathogenic bacteria have always been considered as a major cause of death worldwide especially in developing countries (Adel *et al.*, 2011). Though over the past century, antimicrobial drugs effectively neutralized the bad effect of microorganisms but their therapeutic potentiality now under great risk as many frequently used antibiotics have already became less effective against certain organisms. The recurrent ineffectiveness of the conventional therapeutic agents has occurred not only due to the secretion of novel microbial toxins but also for the emergence of multidrug resistant strains of bacteria, showing negligible susceptibility to current antibiotics (Nikaido, 2009).

Due to increasing resistance phenomenon to antimicrobial agents a numerous of studies must be done to search for alternative substances to acts against the resistant's strains. The different types of medicinal plants in many previous studies have been seen to posses high activities against several microorganisms, so, more clinical studies and trials must be carried out, especially for herbal extract that proved to possessing antimicrobial properties, that's finally provide new lead in the development of new drugs for the therapy of infectious disease.

1.3. Objectives

1.3.1. General objective

To determine the antimicrobial activates *Ricinus communis* ethanolic extract against selected isolates from diabetic foot infection

1.3.2. Specific objectives

1. To collect, isolate and identify the common bacterial strain found in diabetic foot infections by conventional methods.

2. To determine the effect of selected standard antibiotics on isolated microorganisms.

3. To determine the antimicrobial activity of ethanolic extract of *Ricinus communis* on isolated microorganisms by using disc diffusion method .

CHAPTER TWO

2. LITRETURE REVIEW

2.1. Drug resistance

The number of multi-drug resistance microbial strain and the appearance of strain with reduced susceptibility to antibiotic are continuously increasing; This increase has been attributed to indiscriminate use of broad spectrum antibiotic, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Graybill, 1988 and Gonzales et *al*., 1996). In addition in developing countries, synthetic antibiotic are not only expensive and inadequate for the treatment of the diseases) but often with adulteration and side effects, Therefore there is need to search new infection fighting strategies to control microbial infection (Sieradzki *et al*., 1999).

2.2. Diabetic disease

Diabetes Mellitus (DM) is a progressive disease worldwide and remains an important cause of morbidity, mortality and major lower extremity amputation at some stage of life (Caputo *et al.*, 1997). The number of diabetic patients (DP) worldwide in 2007, about 246 million infected, and is expected to amount to more than 350 million in 2025 (Rubaiaan, 2008).

Diabetes is a chronic disease that affects about 14 million Americans and increasing in population to the point where public health authorities are calling diabetes an" epidemic" that requires argent attention. (Bennett, 1999). Diabetes drain 33% of health budgets in European countries and cost the United State of Americans (USA) 115 billion dollars ($) annually. In kingdom Saudi Arabia, the rate of infection 24.7% a year and annual growth rate of DP are about 1% (Rubaiaan, 2008).

The term ‘diabetic foot’ includes any pathology that results directly from diabetes mellitus or its long term complications. Foot ulceration in diabetes mellitus is common. Foot problems remain the commonest cause of hospital admission amongst patients with diabetes in many countries. The lifetime risk of a patient with diabetes developing an ulcer is 25%, and up to 85% of all lower limb amputations in diabetes are preceded by foot ulcers. As many as 50% of older patients with type 2 diabetes have risk factors for foot problems and regular screening by careful clinical examination is essential; those found to be at risk should attend more regular follow-up together with education in foot self-care. The key to management of diabetic neuropathic foot ulceration is aggressive debridement with removal of callus and dead tissue, followed by application of some form of cast to offload the ulcer area. Most ulcers will heal if pressure is removed from the ulcer site, if the arterial circulation is sufficient and if infection is managed and treated aggressively (Boulton *et al.,* 2005).

2.3. Diabetic foot infection

Diabetic foot infection (DFI) defined as the disease caused by a microbial pathogen that occurs when the presence of replicating organisms is associated with tissue damage (White *et al*., 2001). Other definition, DFI is the presence of multiplying bacteria in body tissues, resulting in spreading cellular injury due to competitive metabolism, toxins, intracellular replication, or antigen-antibody response (Ayton, 1985).

DFI causes a major public health problem and impose a heave burden on health services and own family. It is responsible on patient hospital admission, drain of future earning power, major foot amputations, disability, loss work, family fragmentation, and eventually death (Caputo *et al*,, 1997). In USA in 2001, over one million amputations per year, every 30 seconds a leg is lost, 50% amputees

undergo second amputation, and 50% amputees die 2-5 years after amputation (Johonson and Citron, 2002).

In1990, over 54000 lower extremity amputations accrued among diabetics (8.3 per 1000). Medical cost of treatment of the DF $85 billion (average of $ 45,000 per patient) (Josef and Nesbit, 1998).

In kingdom Saudi Arabia, the direct cost of DM is expected (5.9) billion riyals a year. DM is first cause for the foot amputations and depression, 20% of the mothers after the pregnancy are development of diabetes and 9% of patients die annually (Rubaiaan, 2008).

Microorganisms can cause the ulcers of DM patients to become very inflamed, sore and delay healing include: aerobes bacteria such as *Streptococcus pyogens*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli* and *Klebsiella pneumonia*. Anaerobes bacteria are *Clostridium perfringens*, *Bacteriodes fragilis* and yeast is *Candida albicans*. Infections in patients with diabetes are difficult to treat because these patients have weakness in microvascular circulation, which limits the access of phagocytic cells to the infected area and results a poor concentration of antibiotics in the infected tissues (Sharma et *al*., 2013).

Medicinal plants have a vital role to preserve the human healthy life. The large family Euphorbiaceae contains nearly about 300 genera and 7,500 species. Generally they are the flowering plants. Amongst all, these plants such as is *Ricinus communis* has high traditional and medicinal value for maintain the disease free healthy life. Traditionally the plant is used as laxative, purgative, fertilizer and fungicide etc. whereas the plant possess beneficial effects such as antioxidant, antihistamic, Antinociceptive, antiasthmatic, antiulcer, immunemodul, Antidiabetic, hepatoprotective, Antifertility, anti-inflammatory,

antimicrobial, central nervous system stimulant, lipolytic, wound healing, insecticidal and Larvicidal and many other medicinal properties.

This activity of the plant possess due to the important phytochemical constituents like flavonoids, saponins, glycosides, alkaloids and steroids etc (Jena and Gupta, 2012).

2.4. Ricinus communis

Ricinus communis L. [Family: Euphorbiaceae, popularly known as 'castor plant' and commonly known as 'Palm of Christ', *Verenda* (Bengali), *Arand*, *Erand*, *Andi* (Hindi), *Errandi* (Marathi), *Diveli* (Guajarati), Urdu (Be danjir, Arand) and Punjabi (Arind**)]** (Manoj, 2017).

Table (1).Scientific Taxonomic Classification OF *Ricinus communis*

Kingdom	Plantae
Order	Malpighiales
Family:	Euphorbiaceae
Sub Family:	Acalyphoideae
Tribe:	Acalypheae
Sub Tribe:	Ricininae
Genus:	*Ricinus*
Species:	*Ricinus communis*

2.4.1. Geographical Distribution

The plant *R. communis* is probably native to Africa, and is cultivated in many tropical and subtropical areas of the world, commonly appearing spontaneously.

It is found in India, cultivated as well as wild, up to 2400 meters. It has escaped cultivation and become naturalized as weed almost everywhere in the world that has a tropical or subtropical climate (Manoj, 2017).

2.4.2. Phytochemical constituents

The Preliminary Phytochemical study of *R. communis* revealed the presence of steroids, saponins, alkaloids, flavonoids, and glycosides. The dried leaves of R. communis showed the presence of two alkaloids, ricinine(0.55%) and N-demethylricinine (0.016%), and six flavones glycosides kaempferol-3-O - β-D-xylopyranoside, kaempferol-3-O-β-D-glucopyranoside, quercetin_ 3- 3-O-β-D xylopyranoside, quercetin -3-O-β-D-glucopyranoside, kaempferol - 3- O-β-rutinoside and quercetin-3-O β- rutinoside7. The monoterpenoids (1, 8-cineole, camphor and α -pinene) and a sesquiterpenoid (β-caryophyllene), gallic acid, quercetin, gentisic acid, rutin, epicatechin and ellagic acid are the major phenolic compounds isolated from leaves. Indole-3-acetic acid has been extracted from the roots (Darmanin *et al.*, 2009, Singh and Ambika, 2009).

The seeds contain 45% of fixed oil, which consist glycosides of ricinoleic, isoricinoleic, stearic and dihydroxystearic acids and also lipases and a crystalline alkaloid ricinine10. The GLC study of castor oil showed the presence of ester form of palmitic (1.2%), stearic (0.7%), arachidic (0.3%), hexadecenoic (0.2%), oleic (3.2%), linoleic (3.4%), linolenic (0.2%), ricinoleic (89.4%) and dihydroxy stearic acids 11. The stem also contains ricinine. The ergost_ 5-en-3-ol, stigmasterol, Y-sitosterol fucosterol; and one probucol isolated from ether extract of seeds. The GC-MS analyses of *R. communis* essential oil using capillary columns are identified compounds like α-thujone (31.71%) and 1,8-cineole (30.98%), α-pinene (16.88%), campho (12.92%) and camphene (7.48%)12. Lupeol and 30 - Norlupan-3β-ol-20-one are obtained from coat of castor bean. (Malcolm *et al.*,1968).

2.4.3. Ancient uses of *Ricinus Communis*

The castor beans are known for their high toxicity for centuries. In ancient times, farmers knew to keep their livestock away from the castor plant or else they would risk losing them. Their seeds have been used in folk medicine against a wide variety of diseases. The use of the castor bean seed proteins has been reviewed for medical treatments since ancient times. Later, their important roles in the early days of immunological research and some of the fundamental principles of immunology were discovered. During the last three decades, the mechanism of action of the toxins was elucidated. This led to a major effort to target the toxins to malignant cells. Ricin has been used in bioterrorism also. Recently, the toxins have played important roles as experimental models to elucidate the intracellular trafficking of endocytosed proteins18. Castor oil is still produced in large quantities throughout the world and the toxin which remains in the castor meal after the oil has been extracted with hexane or carbon tetrachloride is easily removed through a simple salting-out procedure. There are versatile uses of this plant (oil, leaf, seed and fruit) in different aspects of life. The treated oil can also be used as paints, enamels and varnishes, oiled fabrics, linoleum, patent leather, flypaper, typewriting, printing inks, greases and special lubricants (Bhakta and Das, 2015)

2.4.4. Pharmacological Activities

The various pharmacological activities of *R. communis* L. is described below:

2.4.4.1. Wound healing activity

The *R. communis* possess wound healing activity due to the active constituent of castor oil, which produces antioxidant activity by inhibiting lipid peroxidation. The study of wound healing activity of castor oil was in terms of the scar area, percentage closure of scar areas and epithelization in the excision wound model. Due to the astringent and antimicrobial property the tannins, flavonoids,

triterpenoids and sesquiterpenes present in the castor oil, promote the wound healing process, which are responsible for wound contraction and increased rate of epithelialisation. The study resulted that the castor oil showed wound healing activity by reducing the scar area and also the epithelialisation time in the excision wound model (Manoj, 2017).

2.4.4.2. Antimicrobial activity

The *R.communis* plant extract shows the antimicrobial potential against a wide variety of microorganisms. The petroleum ether and acetone extracts exhibited higher zone of inhibition than ethanol extract. The different solvents root extracts of *R.communis* possess antimicrobial activity by using the well diffusion method against pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Proteus vulgaris*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger*. The hexane and methanol extracts showed maximum antimicrobial activity whereas the aqueous extracts have no significant antimicrobial activity (Mathur *et al*., 2011).

2.4.4.3. Antihistaminic Activity

The ethanolic root extract of *R. communis* L. has the antihistaminic activity at the dose 100, 125, and 150 mg/kg body weight when inserted into the body intraperitoneally by using clonidine induced catalepsy in mice (Manoj, 2017).

2.4.4.4. Antiasthmatic activity

Ethanol root extract of *R. communis* is effective in the treatment of asthma because of its antiallergic and mast cell stabilizing potential activity. Saponins has mast cell stabilizing effect and the flavonoids possess smooth muscle relaxant and bronchodilator activity; the apigenin and luteolin like flavonoids generally inhibit basophil from histamine release and neutrophils from beta glucuronidase release, and finally shows *in vivo* antiallergic activity. The

ethanolic extract of *R. communis* decreases milk induced leucocytosis and eosinophilia and possess antiasthmatic activity due to the presence of flavonoids or saponins (Taur *et al.*, 2011).

2.4.4.5. Anti-inflammatory activity

Ilavarasan et al. reported the antiinflammatory activity of the leaves and root extract of *R. communis* in rats42. The 250 and 500 mg/kg dose of *R. communis* methanol leaves extract possess protective effect in the prevention of cellular events during edema formation and in all the stages of acute inflammation43. The antiinflammatory potential of the *R. communis* methanolic extract was due to the presence of flavonoids against carragennan-induced paw edema in rats (Saini *et al*., 2010).

2.4.4.6. Anti-diabetic activity

Ethanol roots extract of *R. communis* possess significant effects on fasting blood glucose, total lipid profile, liver and kidney functions and no significant difference on alkaline phosphatase, serum bilirubin, creatinine, serum glutamate oxaloacetate transaminases, serum glutamate pyruvate transaminases and total protein which was observed even after the administration of the extract at a dose of 10 g/kg body weight (Manoj, 2017).

2.4.4.7. Hepatoprotective activity

Prince et al. studied the hepatoprotective effect of ethanol leaves extract of *R. communis* at different doses, the presence of flavonoids and tannins exhibited an inhibitory effect on the activities of serum transaminases, liver lipid peroxidation level and the activities of acid and alkaline phosphatase in liver induced by carbon tetrachloride29. N-demethyl ricinine showed anticholestatic and hepatoprotective potential in paracetamol-induced hepatic damage (Manoj, 2017).

2.4.4.8. Lipolytic activity

The ricin produces the lipolytic activity by using the various substratesricin from *R. communis* act as a lipase and has the capability of hydrolyzing different lipid classes. The action of ricin on membrane phospholipids could occur through a phospholipase activity which is very often as a minor activity of lipases (Lombard et *al*., 2001)

2.4.4.9. Antioxidant activity

The high antioxidant activity of the *R. communis* seed at low concentration shows that it could be very useful for the treatment of disease resulting from oxidative stress. The responsible chemical constituent of *R. communis*, which produces antioxidant activity, is methyl ricinoleate, ricinoleic acid, 12-octadecadienoic acid and methyl ester. *R. communis* stem and leaf extracts also produce antioxidant activity due to the presence of flavonoids in their extracts (Gupta et *al*., 2006).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study design

This was a descriptive and experimental study

3.2. Study area

This study was carry out in Zienam, and Jabir abu-aleiz specialized diabetic center in Khartoum city then the specimens were transported to Sudan University of science and Technology Collage of Graduate Studies laboratory for processing of antimicrobial test.

3.3. Study population

Diabetic patients with diabetic foot infection of both gender of different age groups range from 50 to 69 years old.

3.4 Sample size

A total of 100 patients suffered from diabetic foot infection (DFI) were enrolled in this study.

3.5. Collection and identification of plant material

The *Ricinus communis* leave were collected from Northern state of Sudan, Merowe town, and Dewam Wadhaj village during Marsh 2018 and authenticated on Medicinal and Aromatic Plants Research Institute.

3.6.Method of preparation of the *Ricinus communis* extract

Extraction was carried out according to method described by Sukhedev *et al*., (2008): as followed: 100 grams of Ricinus Communis leave was grinded using mortar and pestle, the powder plant were dissolved with 200 ml ethanol(98%) and extracted by shaking at 60ºCfor six hours After that extract was dried by evaporator at 60°Cunder reduced pressure using rotary evaporator apparatus .

At last the extract allowed to dry in Petri dish and the yield percentage was calculated as followed:

Weigh of extract obtained/Weight of plant sample*100

The yield of the extract was presented in Table 1.1

Thirty disks were Prepared (5 mm diameter) of whatman filter paper by used borer. Put each 30 disk in test tub then closed it and sterilized by dry temperature at 140 ºCto 1h. Dissolved 100mg of extracted material in 1 ml of solvent (ethanol), and then taken 0.5 ml of solution to another two tubes t which contains 0.5 mal to make serial dilution as following 100mg/ml, 50mg/ml and 25mg/ml.

3.7. Collection of wound swab Samples

A total of 100 samples were collected from patients. Pus **s**amples were collected by using sterile cotton swabs which are moistened with sterile saline to prevent drying. The swabs were brought to the laboratory in a sterile container labelled with the date, time of collection, the patient's name, number of sample within one hour after the collection and processed then the specimens were cultivated as soon as possible. Swab was inoculated on MacConkey agar and blood agar plates for isolating the pathogens. The inoculated plates were incubated at 37ºC overnight (Abdullah, 2014 and Slater *et al*., 2004).

3.8. Identification

3.8.1.Colonial morphology

Colonial morphology was considered first step in the identification of the clinical isolates depending on the size. Edges, pigments and fermentation of sugar and other characteristics on MacConkey agar and Blood agar.

3.8.2. Microscopic examination

Dried and fixed smear were prepared from culture media .Gram stain were applied crystal violet stain for 1 minute ,washed with tape water and decolorized by alcohol for few second ,washed immediately with tape water and covered with safranin for 2 minutes then washed again, allowed to dry and examined microscopically using oil immersion lens (X100) .(Beveridge, 2001)

3.8.3. Biochemical test

Biochemical tests that were used in this study was KIA, Indole, Urease, Citrate utilization and Motility test for Gram negative isolates as well as inoculation on differential selected media such as Mannitol salt agar (MSA) and also catalase test for Gram positive isolates.(Smith, 1981)

3.8. 4. Susceptibility test of isolates against selected antibiotics discs used

Antibiotic susceptibility pattern of the isolates was studied by Kirby Bauer s disc diffusion method. Both broad spectrum and narrow spectrum antibiotics were used. The antibiotics tested were Penicillin (10 units), (Methicilin 10 mcg), Ciprofloxacin (5 mcg), (30 mc Imipenem g), Gentamicin (30 mcg), Tetracycline (30 mcg), Erythromycin (15 mcg), Co-Trimoxazole (25 mcg) and Cefixime (30 mcg).

The plates were left at room temperature then incubated at 37ºC for 18-24 hours and the inhibition zones were measured, results interpreted according to CLSI chart and recorded in chapter four (Bauer, *et al*., 1966).

3.9.Statically analysis: Analysis of the data was performed by SSPS version 16.

4. CHPTER FOUR

RESULT

4.1. Percentage yield of extracts

Percentage yield of *Ricinus communis* leave extracts was determined by following formula, and has mentioned in Table (2).

4.2. Result of pathogenic microorganisms isolated from the samples

Out of 100 samples, the Gram-negative bacteria was present in 56 (58.3%) isolates, followed by gram-positive bacteria 40 (41.7%), and in the rest samples 4 of the 100 samples was no growth .

A total of 96 pathogenic microorganisms were isolated from 100 sample. The organisms that was isolated from diabetic patients suffering from diabetic foot. The most of them were *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Staphylococcus epidermis* and *Citrobacter species* were isolated in low frequency.

Table (2). Percentage yield of plant extracts obtained

Solvents used	**Weight of raw plant material (g)**	**Weight of extracts obtained (g)**	**Percentage Yield (%)**
Ethanol	70	7.7	11%

4.3. Effects of some standard antimicrobial agents on isolated microorganism

According to this study showed that micro-organisms were tested against the several antimicrobial agents showed high resistance rate to many of these agents used, the result record as following:

Most antimicrobial agents used against chosen organisms (*Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella species, Proteus species, Escherichia coli, Citrobacter species and Staphylococcus epidermis*) show high percentage of resistance to ciprofloxacin, Gentamicin, co-trimoxazole ,tetracycline and penicillin, but imipenem showing higher powerful activity against all tested organism also methiciln have a good result against chosen *staphylococcus species.* The result for *Staphylococcus aureus and Staphylococcus epidermis, Pseudomonas aeruginosa, Klebsiella species, Proteus species, Escherichia coli and Citrobacter species are* shown at Table (3) and Table (4). at respectively

Table (3). Effects of some standard antimicrobial agents against *Gram positive organisms*

Antibiotics	*Staphylococcus aureus*			*Staphylococcus epidermis*		
	R (%)	*I (%)*	*S (%)*	*R (%)*	*I (%)*	*S (%)*
Penicillin	88.9	_	11.1	100	_	_
Methicillin	33.3	_	66,7	33.3	_	66.7
Ciprofloxacin	77.8	_	22.2	33.3	_	66.7
co-trimoxazole	44.4	_	55.6	100	_	_
Erythromycin	66.7	_	33,6	100	_	_

Penicillin : Sensitive > 12 R: Resistance <11

Erythromycin: Sensitive > 21 R: Resistance <19

Methicillin : Sensitive > 19 R: Resistance <16

Table (5). Effects of some standard antimicrobial agents against Gram negative bacteria

Antibiotics	Imipenem		Tetracycline		Ciprofloxacin		Co_trimoxazole		Gentamicin	
~~Tested organisms~~	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
Ps. auer	60	40	20	80	40	60	_	_		80
K.pneu	100	_	_	**100**	50	**50**	_	**100**	75	**25**
Pr.mir	_	100	100	_	100	_	_	100	100	_
E.coli	100	-	20	80	20	80	-	100	80	20
Citro	_		_	_	_	_	_	_	_	_

Keywords :

Ps. aeur: *Pseudomonas aeurginosa* K.pneu: *Klebsiella species*

Pr.mir: *Proteus mirabilis* E.coli*: Escherichia coli*

Citro*: Citrobacter species*

Ciprofloxacin.Resistant(R) < or = 15 mm Sensitive(S) > 21

Gentamycin: Resistant < or = 14 mm Sensitive > 17

Cotrimoxazole: Resistant < or = 10 mm Sensitive > 16

Tetracycline: Resistant < or = 17 mm Sensitive > 21

Imipenem: Resistant < or = 19 mm Sensitive > 23

4.4 Effects of *Ricinus communis* extract on isolated microorganisms

The antibacterial assay was performed for the *Ricinus communis* extract by agar disc diffusion method the disc (0.5cm, Hi-Media) was saturated with 20 µl of the test extract, allowed to dry, and introduced on the agar plate. The plates were incubated overnight at 37ºCMicrobial growth was determined by measuring the diameter of the zone of inhibition.

The effects of *Ricinus communis* extract were studied against common isolated bacteria from DFI patients which it's have resistance to more than three antimicrobial agents used in this study .The present study showed that extract had powerful activity against all tested bacteria (inhibition zone was surrounded discs and measured in mm) when plant extracts were at concentration 100mg/ml, at concentration 50mg/ml and 25mg/ml the extract have a moderate activity except 20% of *Pseudomonas aeruginosa* and *Klebsiella pneumonia* which they are inactive at concentration 25mg/ml .

The percentages of antibacterial activities of the extracts against tested organism was presented for *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis, Escherichia coli and Staphylococcus epidermis* shown at Table (10).

Table (10) Effects of *Ricinus communis* extract on isolated microorganism

Tested organisms	Percentage of extracts								
	100 mg/ml			50 mg/ml			25 mg/ml		
	ACT	INT	INA	ACT	INT	INA	ACT	INT	INA
S.aur	100%	_	_	44.4%	55.6%	_	22.2	88.9%	_
P. aer	80%	20%	_	60%	40%	_	20%	60%	20%
K. pneu	100%	_	_	50%	50%	_	25%	75%	_
P.mir	100%		_	66.7%	33.3%	_	_	100%	_
E.coli	80%	20	_	40%	60%	_	20%	60%	20%
S.epid	66.7%	33.3%	_	66.7%	33.3%	_	_	100%	_

ACT: Active

INT: Intermediate

INA: In active

(-): No activity

CHAPTER FIVE

5. DISCUSSION

5.1 Discussion

Now a day's large members of microorganisms develop resistance to many antibiotics due to many reason one of them was prescription of antibiotics without sensitivity testing (Kushwah and Singh, 2012). In addition many of the synthetic antibiotics have side effects, therefore there is a need to search for anew safe and effective agents to solve these problem, one of these solution is screen the antibacterial activity of medicinal plants acts against these microorganisms (Afolayan, 2003).

In this study microorganisms that cause diabetic foot infection were detected, then the sensitivity to plant extract and selected antimicrobial disc were studied and the results showed that all patients samples have a pathogenic microorganisms except in four samples. Similar finding were also reported in Saudi Arabia and Nepal (El Tahawy, 2000 and Sharma et *al*., 2006).

This study showed that Gram negative bacteria is most frequently isolated pathogens (58.3%) and Gram positive bacteria accounted as (41.7%) *Staphylococcus aureus* was predominant strain among gram positive isolates, the isolated bacteria were identified as were *Staphylococcus aureus* 40 (37%), *Pseudomonas aeruginosa* (15%), *Escherichia coli* (12%), *Klebsiella pneumonia* (13%), *Proteus mirabilis* (13%) *Staphylococcus epidermis* and *Citrobacter species* were isolated in low frequency (3. % for each).This finding is in agreement with Raj, (2007) who found the same result (Gram positive bacteria 48%, Staphylococcus aureus is predominant among Gram positive 44%, and the percentage of Gram negative bacteria was 52% including *Proteus spp*25%,

Pseudomonas auerginosa 28%, *Klebsiella pneumonia* 15% and *Escherichia coli* 9%)

Detection of the effect of chosen standard antibacterial agents against selected isolates was done. For the Gram positive shown that *staphylococcus aureus* strains have highly resistance rate to penicillin G, erythromycin and ciprofloxacin, Staphylococcus epidermis also its more resistance to co-trimoxazole. Similar result was reported by Shen *et al*., (2014).

Among 66 strains of Gram negative bacteria tested in this study showed that highly percentage of resistance to Ciprofloxacin, Tetracycline, Co-Trimoxazole and Gentamicin except *Escherichia coli* which it's more susceptible to Gentamicin, Imipenem was most effective agent against gram negative bacteria (Sharma *et al.*, 2006). The activity of imipenem my be due to their high affinity to binding to penicillin binding protein and unaffected by strains that produce plasmid-mediated beta-lactamase (Zhanel *et al*, 1998)

Isolates that reveal multiple drugs resistance were exposure to ethanol extract of *Ricinus communis* in different concentration to determine the activity of the ethanolic extract of leaves of *Ricinus communis* against the isolates. Significant susceptibility was recorded by most of the organisms tested (*Staphylococcus aureus, Klebsiella pneumonia, Proteus mirabilis, Staphylococcus epidermis, Pseudomonas aeruginosa,* and *Escherichia coli*) with concentration 100mg/ml, 50mg/ml and moderate activity with 25mg/ml except *Proteus mirabilis* which showed no susceptibility to extract with concentration 50% and moderate activity at 25%. The findings from this study agree with that of an earlier study on extracts of the same plant where *Klebsiella pneumonia, Escherichia coli, Proteus mirabilis* and *Staphylococcus aureus* were found to be appreciably susceptible (Jombo and Enenebeaku, 2008).

The findings from the present study show R. *communis* extract to have a wide spectrum of activity involving several gram negative as well as gram positive with different concentration from the plant extract showed powerful activity at higher concentrations(100mg/ml) compared to low concentrations (25mg/ml) (Jombo and Enenebeaku, 2008**)** .

Ethanolic extract of *Ricinus communis* showed high activity against most tested strains this may be due to hot extraction method induce chemical change in the active components of the plant resulting in increasing biomolocules extracted from plant that's may be more active than that found in cold extract also ethanol have low polarity that determine solubility of active molecules found in the leaves of Ricinus *communis* **(**Jeyaseelan *et al*., 2012).

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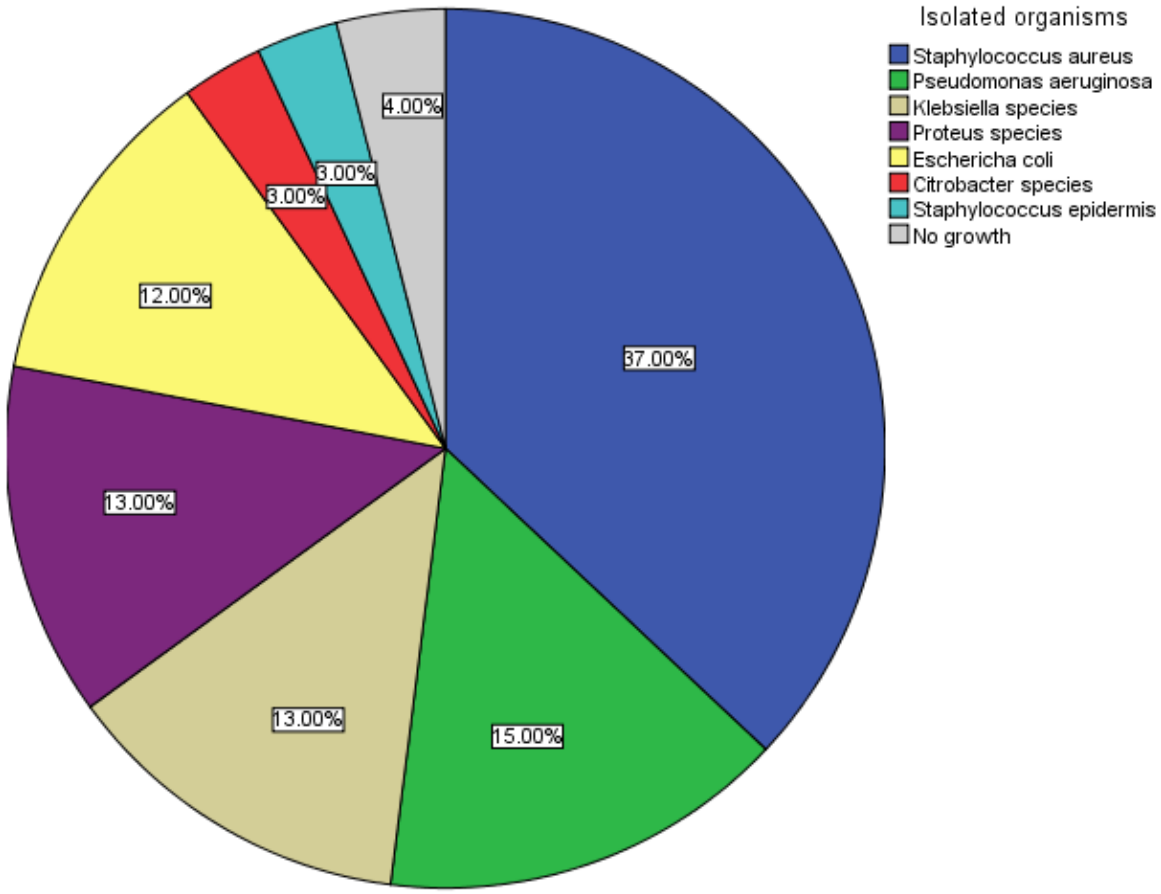
6.1 Conclusion

The result of present study indicate that plant extract showing positive antimicrobial activity provide the scientific base to include the traditional practices in modern system of medicines. Therefore, provide new leads in the development of new antimicrobial drugs for therapy of infection

6.2 Recommendations

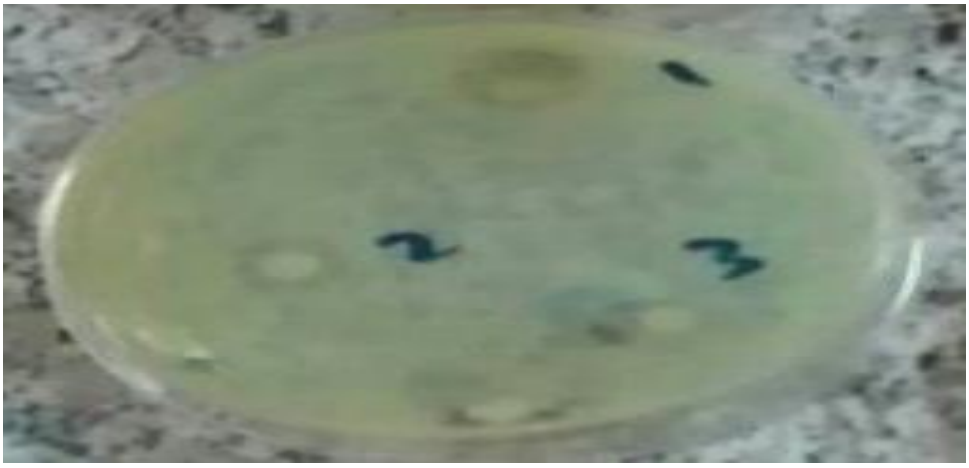
More clinical studies and trails must be carried out, especially for herbal extract that proved to possessing antimicrobial properties. Precise photochemical investigations and pharmacological studies were need to isolate, purify and characterize the effective molecules present in this plants

Appendix(1)



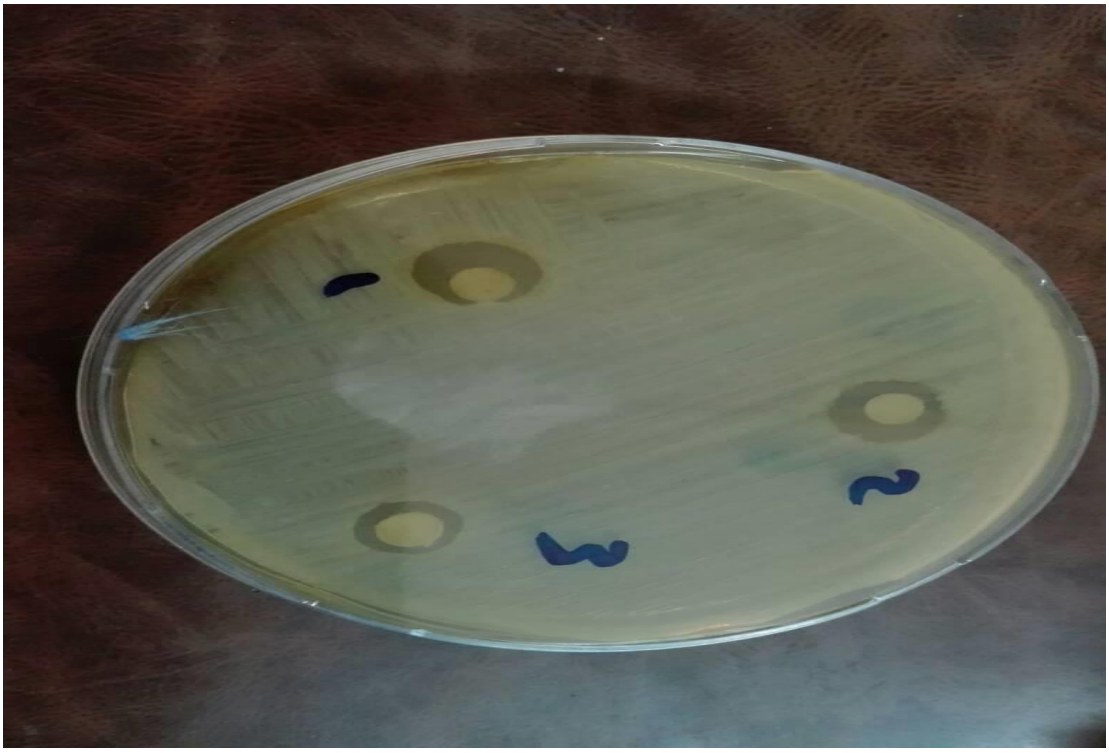
Figure(1) The percent of isolated pathogens

Appendix(2)



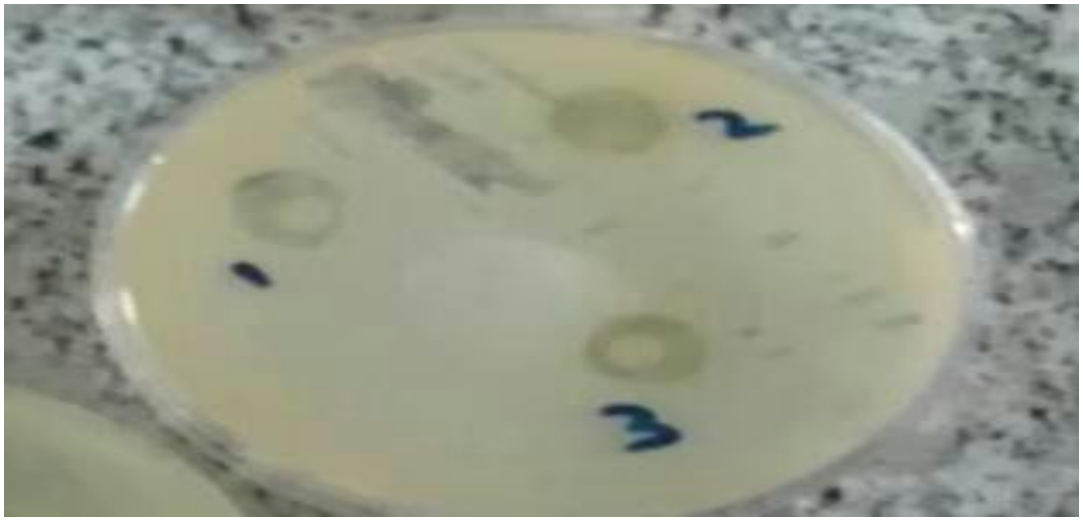
Figure(2) The inhibition zone of *Ricinus communis* extract against *Pseudomonas aeruginosa*

Appendix(3)



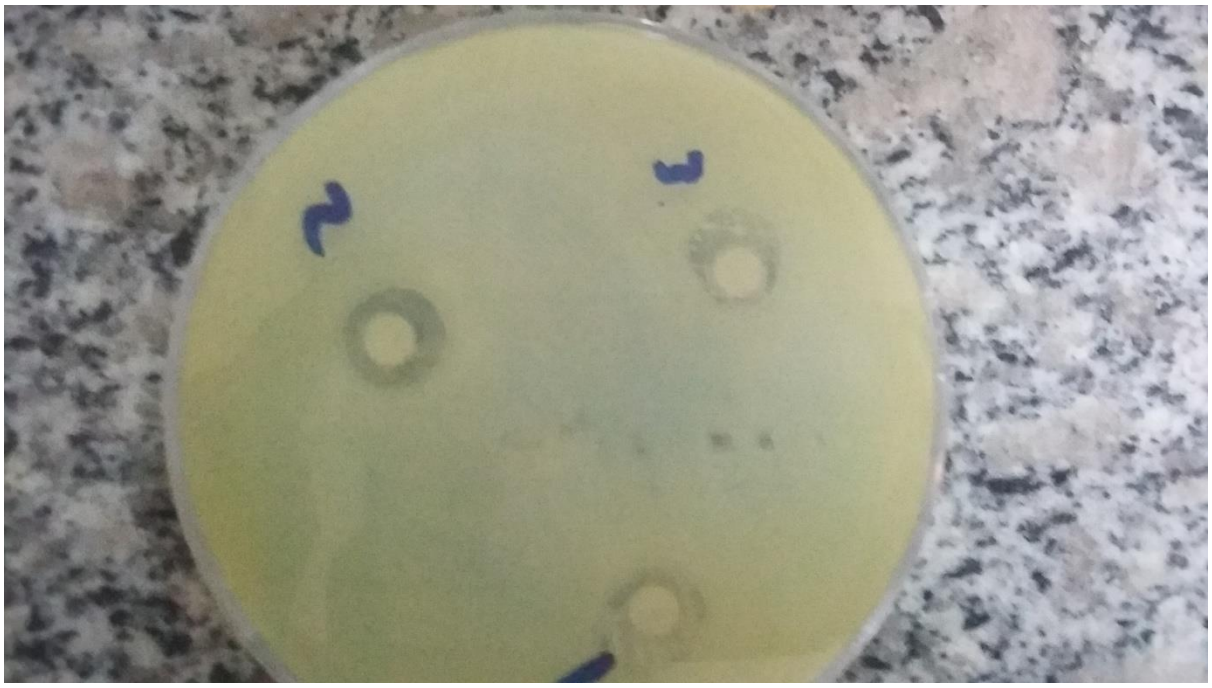
Figure(3)The inhibition zone of Ricinus communis extract against *Klebsiella pneumonia*

Appendix(4)



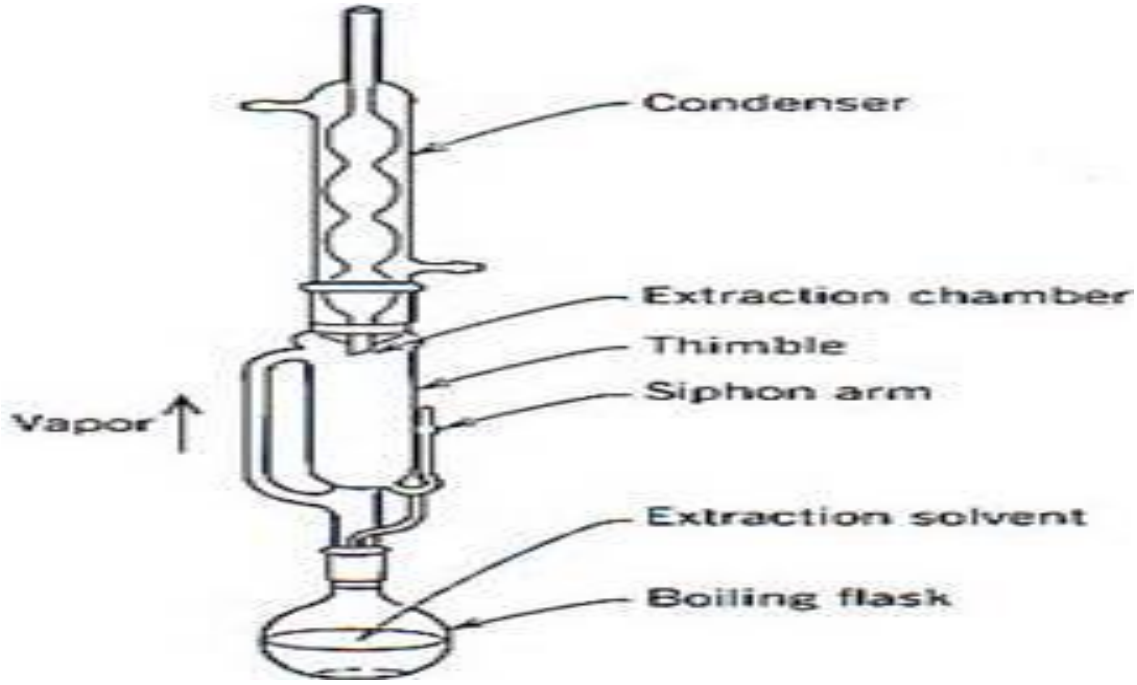
Figure(4) The inhibition zone of Ricinus communis extract against *Staphylococcus aureus*

Appendix(5)



Figure(5) The inhibition zone of Ricinus communis extract against *Escherichia coli*

Appendix(6)



Soxlet (Duran UK**)**

Appendix(6)

Rotary evaporator (Buchi switzerland**)**

Appendix(7) :DNase Test Agar

Formula in grams per liter

Casein Peptone 15,00

Soy Peptone.....5,00

Sodium Chloride..... 5,00

Deoxyribonucleic Acid.....2,00

Bacteriological Agar..... 15,00

Final pH 7,3 ± 0,2 at 25ºC

Preparation

Suspend 42 grams of the medium in one litre of distilled water. Mix well to obtain a homogeneous suspension.

Heat with frequent agitation and boil for one minute. Sterilize in an autoclave at 118-121°C (15 lbs. sp.) for 15

minutes. Cool to 45-50°C and pour into sterile Petri dishes. If desired, add 5% blood to the medium without

mannitol to prepare a blood agar medium.

Appendix(8): Macconkey Agar

Formula in grams per liter

Bacteriological peptone.....20,00

Lactose..... 10,00

Sodium Chloride.....5,00

Bile Salts no 2 1,50

Neutral Red 0,05

Crystal Violet 0,001

Bacteriological Agar 13,50

Final pH 7,2 ± 0,2 at 25ºC

Preparation

Suspend 50 grams of the medium in one liter of distilled water. Mix well. Heat with frequent agitation and boil until
completely dissolved. Dispense into appropriate containers and sterilize at 121° C (15 lbs. sp.) for 15
minutes.

Appendix(9): Mannitol Salt Agar

Formula in grams per liter

Sodium Chloride.....75,00
Peptone Mixture..... 10,00
D-Mannitol 10,00
Beef Extract..... 1,00
Phenol Red.....0,025
Bacteriological Agar..... 15,00
Final pH 7,4 ± 0,2 at 25ºC

Preparation

Suspend 111 grams of the medium in one litre of distilled water. Mix well and heat with frequent agitation until
complete dissolution. Boil for one minute. Sterilize in autoclave at 121°C (15 lbs. of steam pressure)
for 15 minutes. Pour into Petri dishes.

Appendix(10) : Mueller Hinton Agar

Formula in grams per liter

Beef Infusion..... 2,00
Casein Peptone H 17,50
Starch..... 1,50
Bacteriological Agar 17,00

Preparation

Suspend 38 grams of medium in one liter of distilled water. Mix well. Heat agitating frequently and boil for about one minute. Dispense and sterilize in autoclave at 116 - 121°C (15 lbs.sp) for 15 minutes. Cool to 45° or 50° C and add defibrinated blood if desired. The blood mixture should be chocolated by heating to 80° C for 10 minutes if Neisseria development is desired. **DO NOT OVERHEAT**. To remelt the cold medium, heat as briefly as possible.

Appendix(11): Simmons Citrate Agar

Formula in grams per liter

Ammonium Dihydrogen Phosphate .....1,00 Dipotassium Phosphate..... 1,00

Sodium Chloride.....5,00

Sodium Citrate 2,00

Magnesium Sulfate 0,20

Bacteriological Agar..... 15,00

Bromthymol Blue.....0,08

Final pH 6,9 ± 0,2 at 25ºC

Preparation

Suspend 24,3 grams of the medium in one liter of distilled water. Mix well and heat with frequent agitation until completely dissolved. Dispense in tubes and sterilize in the autoclave at 121ºC (15 lbs sp.) for 15 minutes. Cool the tubes in a slanted position so that the base is short (1-1,5 cm. deep). Alternatively, the media can be poured into petri plates.

Appendix(12): Peptone Water

Formula in grams per liter

Bacteriological peptone.....10,00

Sodium Chloride 5,00

Final pH 7,2 ± 0,2 at 25ºC

Preparation

Suspend 15 grams of the medium in one liter of distilled water. Dissolve the medium completely. Distribute into
appropriate containers and sterilize in autoclave at 121ºC (15 lbs sp) for 15 minutes

Appendix(13): Kligler Iron Agar

Formula in grams per liter

Peptone mixture 20,00

Lactose 10,00

Sodium Chloride..... 5,00

Dextrose 1,00

Ferric Ammonium Citrate 0,50

Sodium Thiosulfate.....0,50

Phenol Red 0,025

Bacteriological Agar 15,00

Final pH 7,4 ± 0,2 at 25ºC

Preparation

Suspend 52 grams of the medium in one liter of distilled water. Mix well and heat with frequent agitation. Boil for
one minute. Dispense into tubes and sterilize at 121º C (15lbs. pressure) for 15 minutes. Allow to cool in a slanted position so as to obtain butts of 1’5-2 cm. Depth. For greater accuracy, Kligler Iron Agar should be used on the day of preparation or melted and solidified before use.

Appendix(14): Urea Agar (Christensen)

Formula in grams per liter

Gelatin Peptone..... 1,00

Dextrose 1,00

Sodium Chloride..... 5,00

Monopotassium Phosphate 2,00

Urea 20,00

Phenol Red.....0,012

Final pH 6,8 ± 0,2 at 25ºC

Preparation

Dissolve 29 grams of the medium in 100 ml. of distilled water. Sterilize by filtration. Separately dissolve 15 grams of agar in 900 ml. of distilled water by boiling. Sterilize in autoclave at 121°C (15 lbs.sp) for 15 minutes. Cool to 50°C and add to the 100 ml. of the sterile Urea Agar Base. Mix well and dispense aseptically in sterile tubes. Leave the medium to set in a slanted position so as to obtain deep butts. At a pH of 6.8 to 7.0 the solidified medium should have a light pinkish yellow colour. Do not remelt the slanted agar.

Appendix(15):Antiboitic Discs

Penicillin..... (10 units)

Methicilin..... 10 mcg)

Ciprofloxacin..... (5 mcg)

Imipenem..... (30 mcg)

Gentamicin ..... (30 mcg)

Tetracycline..... (30 mcg)

Erythromycin ..... (15 mcg)

Co-Trimoxazole ..... (25 mcg)

Appendix(16): Incubator (Trope PicenardiCCRI,Italy**)**

Appendix(17): Autoclave (Medical Instrumention MFG CO,Mumbia**)**

Appendix(18):Hot air oven (Leader Engineering Widness Cheshire, UK**)**

Appendix(19):Ethanol (Romile EU**)**