CHAPTER ONE

INTRODUCTION

1. Introduction

Microbial infection is major public health problem in the developing countries. Antibiotics are used to treat these infections. Due to the miss use of antibiotics, the incidence of multiple antibiotic resistances among human pathogens is increasing; this has forced the scientists to search for new antimicrobial substances from natural sources (Deshpande, 2013).

Plants possess active ingredients for defense against plant pathogens many of these antimicrobial substances were found to produce the same affects against human pathogens. Hence; researchers started screening plenty of plant extracts and essential oils against various human pathogens. Screening medicinal plants for antimicrobial sensitivity has led to encouraging results However, it is essential to investigate the toxicity of the plant (Kavitha and Salish, 2013). Antibiotics have undesirable side effects while the emergence of previously uncommon infections is also a serious medical problem. Over 75% of the antimicrobial in clinical use are of natural origin and most of them are obtained from fungal source (Kavitha and Salish, 2013).

Ambrosia maritime L is a plant 5 to 20 mıter high with a thick spherical crown, stems and branches usually black colored to grey-pinkish slash, the plant has straight, light thin, grey spines in axillaries pairs, the leaves are bipinnate, with 3 to 6 pairs of pinnulae and 10 to 30 pairs of leaflets each, rachis with a gland at the bottom of the last pair of pinnulae flower in globules head 1.2 to 1.5 cenmiter in diameter of bright golden-yellow color(Kavitha and Salish,2013).

Ambrosia maritime L has been reported to be very useful in treating urinary tract infection in diabetic patients (Amin,1990). Ali *et al.,* reported the usefulness of *Ambrosia maritime L* extract against various bacteria claiming

that *Ambrosia maritime L* is more effective against Gram negative bacteria than Gram positive (Ali *et al*., 2015).

1.2Rationale

The continuous emergence of multidrug resistance bacteria is a major threat to the live of human around the globe. Antibiotics and synthetic chemo therapeutic agents were thought of as miracle tablets that cure all infectious diseases. This fact was shortly found to be incorrect as Methicillin resistance strains emerged since the 1960s (pakleung, 2011).

Plants produces antimicrobial substances that protect the plants against plants pathogens. The research has proved that antimicrobial substances produced by plants could produce the same effects on human pathogens. *Ambrosia maritime L* is one of the medicinal plants that are used in Sudan and other countries in order to treat bacterial infections like urinary tract infection (Pakleung, 2011).

1.3.1General Objective

To determine the antimicrobial activity of *Ambrosia maritime L* extract against selected clinical isolates from diabetic patients with recurrent urinary tract infection attending Al-Rıbat University Hospıtal .

1.3.2Specific Objective

To isolate, identify and determine the antimicrobial sensitivity of clinical isolates against selected antibiotics.

To determine the antimicrobial potential of methanolic extract of *Ambrosia maritime L* extract against selected urinary tract pathogens.

CHAPTER TWO
LITERATURE REVIEW

2.1. Urinary Tract Infections (UTIs)

UTI is the most common bacterial infection encountered in the care setting in the United States (Rechtsteiner, 2011).

Recurrent UTI is defined as uncomplicated UTIs in 6 months or, more traditionally, as ≥3 positive cultures within the preceding 12 months. Most recurrences occur within the first months after the primary infection, and there can often be clustering of infections. When the initial infection is caused by *Escherichia. coli*(*E. coli*), there is a higher risk of reinfection within the first 6 months. When there is recurrent infection with the same organism despite adequate therapy, it is considered a relapse (Annette *et al.,* 2010).

2.1.1.Pathogenesis of aerobic bacteria cause urinary tract infection

Symptomatic UTI infection in a healthy human is a complex event. It is initiated when potential urinary pathogens from the bowel or in some cases from the vagina (as a result of direct inoculation during sexual activity), Colonize the pre urethral mucosa and second through the urethra to the bladder and in the some case through the ureter to the kidney uro pathogenic *E.coli* is the predominate pathogen in the UTI infection, are a specific subset of *E.coli* that have the potential for enhanced virulence.

Virulence and fitness factor include flagella, toxin and polysaccharide coating and the other properties that assist the bacteria in avoiding the human response (Johnson, 2003).

2.1.2 Symptoms of UTI

Aburring feeling when you urinated, Dysuria, frequency, urgency,urinary in continence, suprapubic discomfort, sensation of in complete bladder

emptying post void, fever, back pain, abdominal palpation, vaginal discharge, hematuria, nocturia,offensive smelling with /out purulent (Andrew,2010).

2.1.3. Sterile pyuria:

Is defined as the presence of 10 or more white cells per cubic millimeter in a urine sample, 3 or more white cells pre high-power field of urine, positive result on Gram's staining of urine specimen.

Sterile pyuria is the present finding of white cells in the urine in the absence of bacteria as determined by wet preparation techniques (Horan *et al*., 2008).

2.1.5. Causes of Sterile pyuria

causes related to infection: Current use of antibiotic, recently treated UTI within past 2 weeks, gynecologic infection, urethritis due to Chlamydia, Mycoplasma, prostatitis,balanitis, appendicitis, viral infection of the lower genitourinary tract, genitourinary tuberculosis, fungal infection and parasitic disease such as trichomoniasis (Horan *et al*., 2008).

causes not related to infection: Presence used of urinary catheter, recent cystoscopy, urinary tract stone, foraged body such us surgical partials in the urethra, pelvic irradiation, urinary fistula, rejection of a renal transplant and inflammatory disease such as systematic lupus erythematous (Horan *etal*., 2008).

2.2. Diabetes mellitus

More commonly referred to as "diabetes" a chronic disease associated with abnormally high levels of the sugar glucose in the blood (WHO, 2006).

2.2.1. Diabetes mechanisms

Inadequate production of insulin (which is made by the pancreas and lower Blood glucose), or inadequate sensitivity of cells to the action of insulin (WHO, 2006).The two main types of diabetes correspond to these two mechanisms and are called insulin dependent (type 1) and non-insulin dependent (type 2) diabetes. In type 1 diabetes there is no insulin or not

enough of it. In type 2 diabetes, there is generally enough insulin but the cell supon it should act are not normally sensitive to its action (WHO, 2006).

The major complications of diabetes include dangerously elevated blood sugar, abnormally low blood sugar due to diabetes medications, and disease of the blood vessels which can damage the eyes, kidneys, nerves and heart (WHO, 2006).

2.2.2. Urinary tract infection in Diabetics patients

Patients with diabetes mellitus (DM) are more susceptible to urinary tract infection (UTI) than non-diabetics (WHO, 2006), due to the emergence of multidrug resistant (MDR) uropathogenic strains; the choice of antimicrobial agent is restricted (WHO, 2006). The etiology and the antibiotic resistance of uropathogens have been changing over the past years. Predisposition to UTIs in diabetes mellitus results from several factors. Susceptibility increases with longer duration and greater severity of diabetes (Chen *et al*., 2009).

The epidemiology of urinary tract infections (UTIs) among men and women with diabetes is similar to the epidemiology of those without.Women have greater risk than men and the frequency of sexual activity is a risk factor. The bacteriology and antibiotic susceptibility patterns also do not in general differ from those without diabetes. Although persons with diabetes are more likely to have asymptomatic bacteriuria, asymptomatic bacteriuria does not lead to increased risk of symptomatic infection, except during pregnancy or prior to genital-urinary or gastrointestinal surgery and should not be treated otherwise. However, diabetes doubles the risk of UTI. The source of this increase is not well understood, although bladder dysfunction, which increases with duration of diabetes, and glycosuria are hypothesized mechanisms. As treatment using sodium glucose co transporter 2 inhibitors could lead to glycosuria, there is a potential for the frequency of UTI to increase among those with diabetes (Lindsay and Nicolls, 2002).

2.3. *Ambrosia maritime L*

Ambrosia is sometimes the food and drink of the Gods, The word has been derived from Greek a (not) and (mortal); hence the food or drink of the immortals.

However, the word may be derived from the Semiticambar(Ambergris) to which Eastern nations attribute miraculous properties (Täckholm and Viv, 1974) both nectar and ambrosia were kinds of honey, in which case their power of conferring immortality would be due to the supposed healing and cleansing power of honey (Amin,1990).

The use of medicinal plants to treat human diseases has its roots in pre historical times. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries. In Sudan, the percentage of people depends on medicinal plants for health care is estimated as over 90%. These plants and derived products play an important role in the primary health care of Sudan (Unido, 1996).

2.3.1. Phytochemical of *Ambrosia maritime L*

The pseudoguaianolide sesqutierpenes of *Ambrosia maritime L* ,which contain neoambrosin ,damsinic acid ,damsin, volatile oil and hymenin were isolated chloroform extract of *Ambrosia maritime L* as previously described by (Abdelgaleil *et al*., 2011).The chemical substances was confirmed on the basis of their ultra violet, infrared,nuclear magnetic resonance and mass spectrometry.(Abdelgaleil *et al*.,2011).

2.3.2. Uses of *Ambrosia maritime L*

Ambrosia maritime L. is a widely distributed plant in African countries and is locally known as Damsissa. Traditionally, it is used to cure gastrointestinal disturbance, abdominal pain, kidney inflammation and renal colic. In addition its therapeutic properties extend to include anti- molluscicidal, anti-malarial and anti-tumor activities (Abdelgaleil *et al.,* 2011).

2.3.3. Effect of *Ambrosia maritime L*on Gram positive and Gram negative bacteria

The extraction of this plant by methanol of *Ambrosia maritime L,* Showed equal or nearly equal antibacterial activity both against Gram positive and Gram negative bacteria (EL-Kamali, 2010).

The phytochemistry of *Cassia spp* e.g. *Ambrosia maritime L* (volatile oil) and other plants of same species have been received considerable interest (Modawi *et al.*, 1984).

2.3.4Pharmacological action and Toxicity of *Ambrosia maritime L*

Ambrosia maritime L relaxes pain muscles of the intestine, uterus and blood vessels, where it inhibits the force and frequency of pain muscle contractionit increases the urine output/day and the level of sodium inurine. It decreases body weight. It has antimicrobial activity against *Streptococcus pyogenes* and *Aspergillus niger*. A guide to Medicinal Plants in North Africa It has an excellent molluscicidal activity due to its content of volatile oil and sesquiterpenes (El-sawy*et al*., 1986).

CHAPTER THREE
MATERIALS & METHODS

3.1 Study design

This was prospective cross sectional and hospital based study.

3.2 Study area

The study was conducted in AL-Ribat University Hospital.

3.3 Study duration

This study was conducted during April to July 2015.

3.4 Study population

diabetic patients male &female with recurrent urinary tract infections were in whom approved to participated in this study were enrolled .

3.5 Inclusion criteria

Diabetic patients with recurrent urinary tract infection.

3.6 Exclusion criteria

Diabetic patients without recurrent urinary tract infection.

3.7 Sample size

One hundred urine samples (n=100) were collected randomly from diabetic patients. After explaining the study and its goal, a verbal consent was taken from the study recruits before proceeding with the study and collecting the samples.

3.8. Collection and processing of urine samples

Mid stream urine samples were collected in universal wide mouth sterile urine containers. The samples were carried in ice bag and transported to the microbiology lab.Macro examination was done todetect the color, smell & turbidity ofsamples. Then was done microscopic examination was done by wet preparation method, started with immersing the strip of urine general in the urine samples to detect the presenceof glucose, protein, ketones.

The urine samples were centrifuged &the deposite was tested for the presence of puscells, red blood cellsand yeast cells.Then the samples were inoculatedunder aseptic condition on Cysteine lysine Electrolyte Deficient (CLED) for the isolation and identification of the pathogens. The inoculated culture media were incubated aerobically at 37C˚for 18-24 hours and examined for significant growth.

3.9. Identification of the clinical isolates

The clinical isolates were identified in the lab by standard microbiology procedures including the following steps.

3.9.1 Colonial morphology

Colonial morphology used as first identification depending on size, color, edges and fermentation of lactose in CLED.

3.9.2 Preservation and storage of isolated organism

Isolated organisms were kept in nutrient agar slope at 4C˚for further identification and susceptibility tests. Nutrient glycerol broth used for long stage preservation for isolates at -20 C˚.

3.9.3 Microscopic examination

Fixed and dried smears were prepared from growth. The smear was` stain with Gram stain was applied crystal violet stain for 30- 60 sec, washed with water followed by log`s iodine for 30-60 sec washed again then decolorized rapidly by alcohol, washed immediately with water and covered with safranin for 2 min then washed and dried to examined microscopically by oil immersion lens (×100) tdetected the Gram reaction and formed of bacteria.

3.9.4 Biochemical test

Biochemical tests including Kliglar ion agar test(KIA),indole, urease, citrate, motility test,esculin hydrolysis and catalase test as well as inoculation on differential selected media such as Mannitol Salt Agar (MSA) was used to distinguish between the clinical isolates. The principles of tests started with indole test ,the test organism is cultured in a medium which contain

tryptophan,indole production is detected by Kovs reagent which contain 4-dimethylaminobenzaldehyde.This react with indole to give red color compound . Then urease test,the test organism culture in medium contain urea and indicator phenol red. The enzyme will break down the urea to give ammonia and carbon dioxide which release the ammonium showing change the color from yellow to pink color. then citrate test, the test organism cultured in medium contain sodium citrate, ammonia salt and indicator bromo-thymol blue showing turbidity and change in color of the indicator from green to blue due to the alkaline reaction, the motility test depend on the properties of bacteria structure which contain flagella or no. also esculin test, the test organism culture in esculin agar yellow in slope, the H2S produce when the sulphur-containing amino acids are decomposed to give black color. While the principle of Catalase test depend on the enzyme act as break down of hydrogen peroxide to give oxygen and water indicated produced by air bubble, then kliglar ion agar test using for detected the H2S and gases production sugar fermentation (lactose), help identification of enterobacteriasae. Finally used special media like mannitol salt agar to help differeinated the Staphylococcus species, to detect the mannitol fermentation to change color from yellow to pink color or no mannitol ferment,and DNA test ,the test organism cultured in medium contain DNA ,after inoculation and incubation at overnight used HCL acid ,which precipitates the unhydrolyzed DNA to give clear zone around the colonies.

3.10. Modified Kirby–Bauer Method

Isolated pathogen were tested against antibiotics by using Kirby-Bauer disk-diffusion method in which 3-5colonies were touched by sterile standard wire loop then emulsified into sterile normal saline and adjusted to 0.5 McFarland standard (Cheesbrough, 2006).

Antibiotic discs (Hi Media laboratories PV+Ltd, India) were applied on inoculated plates: Ciprofloxacin (30 mcg), Gentamicin (10mcg), and

Imipenem (10 mcg) Ceftazidime (30 mcg), Vancomycin (30 mcg) and AmiKacin (20 mcg), Aztreonam, Tetracycline, Piperacillin, Cotrimoxaz and Nitrofurantoin.

Plates were left at room temperature and incubated at 37° C for 18-24 hrs. Zone of inhibition were measured in mm and results interpreted according to standardized chart.

3.11.Collection and identification of plant material

*Ambrosia maritime L*was collected from Khartoum local market Sudan. The plants were taxonomically identified by Medicinal &Aromatic Plants& Traditional Medicine Research Institute National Center for Khartoum, Sudan.

3.12. Preparation of extract

Extraction was carried out according to the method described by (Sukhdev *et al.*, 2008). 65grams of *Ambrosia maritime L* was grinded using mortar and pestle and extracted with 80 % methanol using sox hlet extractor apparatus. Extraction carried out for about eight hours till the solvent returned colorless at the last siphoning time. Solvent was evaporated under reduced pressure using rotary evaporator apparatus. Finally the extract was allowed to dry in Petri dish and the yield percentage was calculated as followed:

Weight of extract obtained / weight of plant sample * 100

3.13. Cup plate method

The agar well diffusion using Mueller Hinton Agar medium for the assay was done to screen the antimicrobial activity of *Ambrosia maritime L* extracts against selected pathogens. The microorganism was activated by inoculating the strain in nutrient agar incubated at 37C° for 18-24 hrs, each strain was suspended in sterile normal saline. Then 0.1 ml of inoculums (adjusted according to McFarland standard) were inoculated into the molten Mueller Hinton agar media and after proper homogenization it was poured into sterile petri dishes. Wells were made into the seeded plates by using crock-borer size

(10mm).Various concentrations of *Ambrosia maritime L* (100%, 50%, 25 %)(w/v) were introduced into the wells subsequently and all the plates were incubated at 37 C° for 24 hrs (Abdullah,1991).

Positive control was Chloramphenicol and negative control was methanol, each step was under strict aseptic conditions. Bacterial growth was determined by measuring the diameter of the zone of inhibition (in mm) and the mean were calculated.

3.14 Statistical Analysis the data analysis was done by SPSS version 11.5

3.15 Ethical consideration

Permission to carry out the study was taken from the collage of Graduated Studies, Sudan of Science &Technology .All subject examined were informed for the purpose of the study before collection of the specimen and consent was taken from them.

CHAPTER FOUR

RESULTS

4. Results

Antimicrobial activity of methanolic extract of *Ambrosia Maritime L* in various concentrations 100%(w/v),50%(w/v) and 25%(w/v) was determined against selected Gram positive and Gram negative organisms including. *Escherichia. coli* (*E. coli 41%), Klebsiella. pneumoniae,(K. pneumonia* 20%), *Staphylococcus. aureus* (*S. aureus12%), Proteus. mirabilis* (*P. mirabilis7%*),*Enterococcus.faecalis* (*E.faecalis*12%)and *Staphylococcus. saprophyticus ,(S.saprophyticus8%).*

Table (4.1) Distribution and frequency of isolated bacteria in percentage

Microorganism	**Frequency**	**Percent**
E. coli	41	41%
K. pneumonia	20	20%
S. aureus	12	12%
E. faecalis	12	12%
S.saprophyticus	8	8%
P. mirabilis	7	7%
Total	100	100%

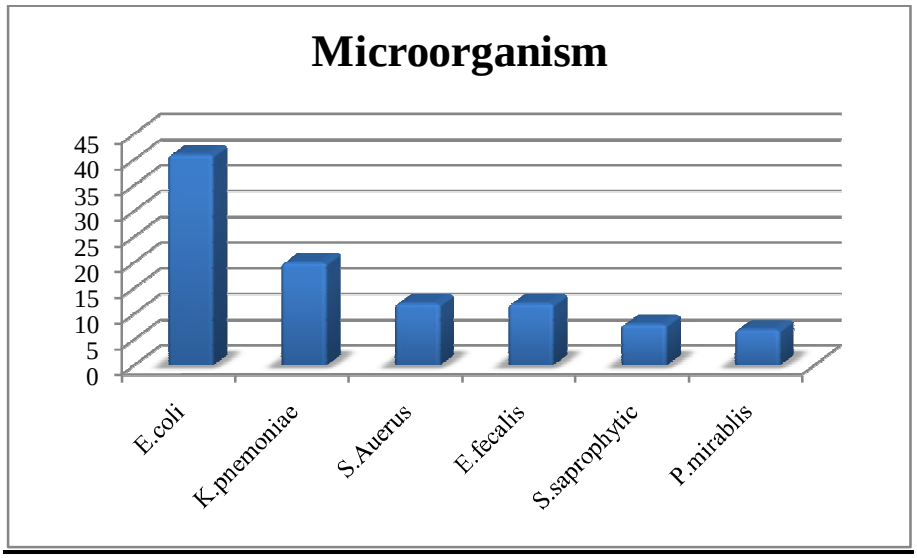


Figure (4.1) Distribution and frequency of isolated bacteria in percentage

Table (4.2) Biochemical results of Gram negative bacteria isolated from patients with recurrent urinary tract infection

Isolated bacteria	Biochemical tests							Motility test
	Indole	Urease	Citrate	KIA				
				Slope	Butt	Gas	H2S	
E. coli	+ve	- ve	- ve	Y	Y	+ve	- ve	+ve
K. pneumoniae	- ve	+ve	+ve	Y	Y	- ve	- ve	-ve
P. mirabilis	+ve	+ve	+ve	R	Y	+ve	+ve	+ve

Table (4.3)Biochemical results of Gram positive bacteria isolated from patients with recurrent urinary tract infections

Isolates	**Catalase**	**Mannitol fermentation**	**DNase test**	**Esculin hydrolysis**
S. aureus	+ve	+ve	+ve	-ve
E. faecalis	-ve	-ve	-ve	+ve
S.saprophyticus	+ve	-ve	-ve	-ve

Table (4.4) Results of antimicrobial sensitivity test of Gram positive bacteria

	Microorganism			
Antibiotics		*S. auerus*	*E. faecalis*	*S. saprophyticus*
Gen	S	12(37.5%)	12(37.5%)	8(25%)
Aug	S	12(37.5%)	12(37.5%)	8(25%)
Ery	S	12(37.5%)	12(37.5%)	8(25%)
Cip	S	12(37.5%)	12(37.5%)	8(25%)
Fus	S	12(60%)	12(37.5%)	8(40%)
Cho	S	12(57.90%)	12(37.5%)	8(40%)

Key words

Cip=Ciprofloxacin Gen=Gentamycin Aug =Augmentin

Ery= ErythromycinCho=ChloramphenicolFus=Fuidic acid

Table (4.5) Results of antimicrobial sensitivity tests of Gram negative bacteria

Antibiotics		Microorganism		
		E .coli	*K.pneumoniae*	*P.mirabilis*
Ami	S	41(60.30%)	20(29.40%)	7(10.30%)
Cef	S	41(60.30%)	20(29.40%)	7(10.30%)
Azt	S	41(60.30%)	20(29.40%)	7(10.30%)
Tet	S	41(60.30%)	20(29.40%)	7(10.30%)
Pip	S	41(60.30%)	20(29.40%)	7(10.30%)
Imi	S	41(60.30%)	20(29.40%)	7(10.30%)

Key word

Ami= Amikacin

Cef=ceftazidime

Azt=Aztreonam

Pip=Piperacillin

Tet=Tetracycline

Imi=Imipenem

Table (4.6) Sensitivity test of bacterial isolates

		E.c	*K.p*	*S.a*	*E.f*	*S.s*	*P.m*
Cot	S	41 (40%)	20 (20%)	12 (12%)	0	8 (8%)	7 (7%)
	R	0	0	0	12 (14.30%)	0	
Nit	S	41 (56.9%)	20 (27.80%)	12 (15.30%)	12 (14.30%)	8 (8%)	7 (7%)
Van	S	41 (56.90 %)	20 (27.80%)	12 (15.30%)	12 (14.30%)	8 (8%)	
	R	0	0	0	0	0	7 (7%)
Mixed = 100 (100%)							

Table (4.7) Yield percent of extracts

Sample	Weight of samples (g)	Weight of extract (g)	Yield %
AML(Ambrosia maritime L)	100 g	35.5	35.5%

**g= gram.*

Table (4.8): Effect of *Ambrosia maritime L* against *E.coli* with different concentrations

Concentration of *Ambrosia maritime L*	Clinical isolate *E.coli*	Inhibition zones (mm)	STD.deviation
100 w/v (%)	3	19	1
50 w/v (%)	3	16	1
25 w/v (%)	3	12	1

One way ANOVA *P-value =0.000108*

*Note One way Anova P value less than 0.05 is considered statistically significant

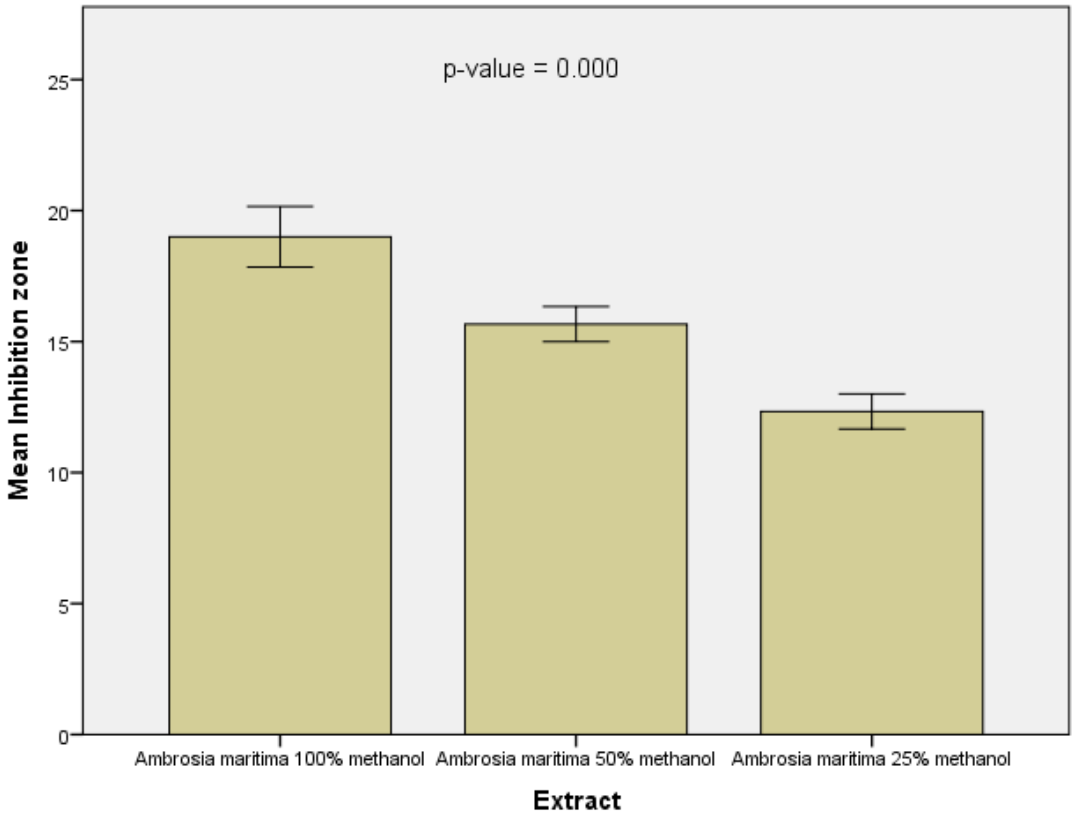


Figure (4.8): Effect of *Ambrosia maritime L* against *E.coli* with different concentrations

Table (4.9): Effect of *Ambrosia maritime L* against K . *pneumonia* with different concentrations

Concentration of *Ambrosia maritime L*	Clinical isolate *K .pneumonia*	Inhibition zones (mm)	STD.deviation
100 w/v (%)	3	20	1
50 w/v (%)	3	16	1
25 w/v (%)	3	13	1

One way ANOVA *P-value = 0.000107*

*Note One way Anova P value less than 0.05 is considered statistically significant

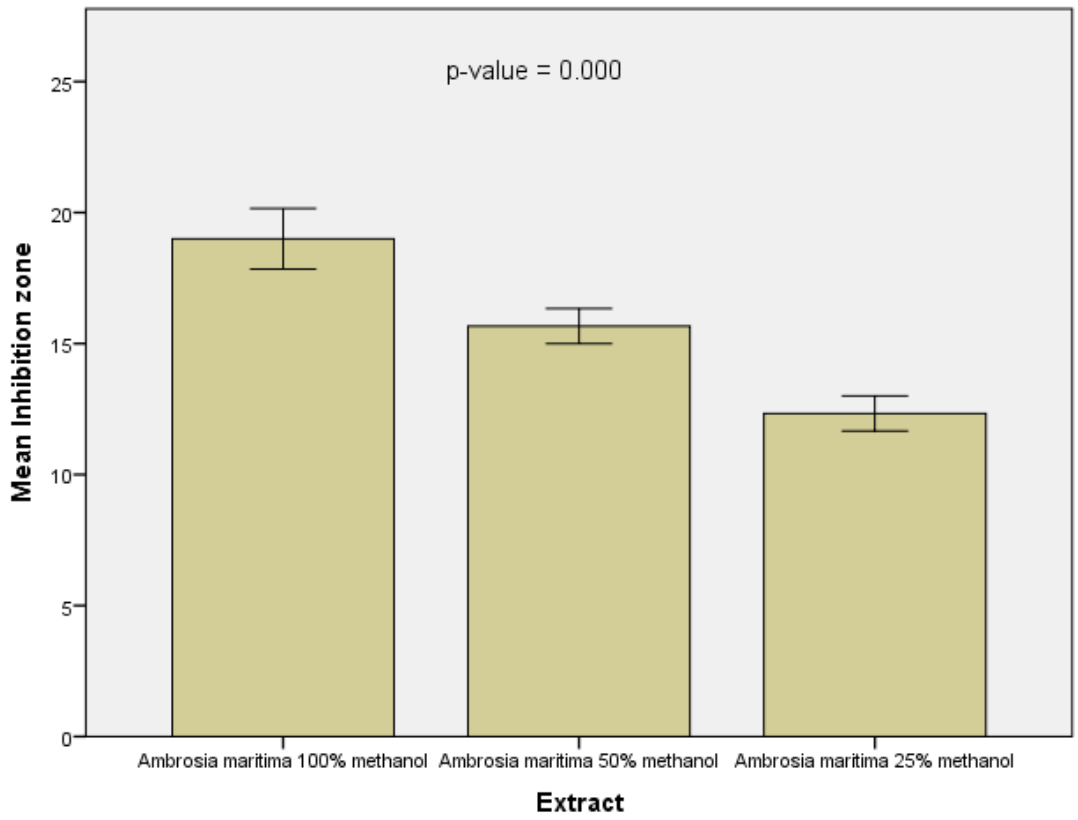


Table (4.9): Effect of *Ambrosia maritime L* against K . *pneumonia* with different concentration

Table (4.10): Effect of *Ambrosia maritime L* against *S .aureus* with different concentrations

Concentration of *Ambrosia maritime L*	Clinical isolate *S .aureus*	Inhibition zones (mm)	STD.deviation
100 w/v (%)	3	19	1
50 w/v (%)	3	15	1
25 w/v (%)	3	12	1

One way ANOVA *P-value = 0.000242*

*Note One way Anova P value less than 0.05 is considered statistically significant

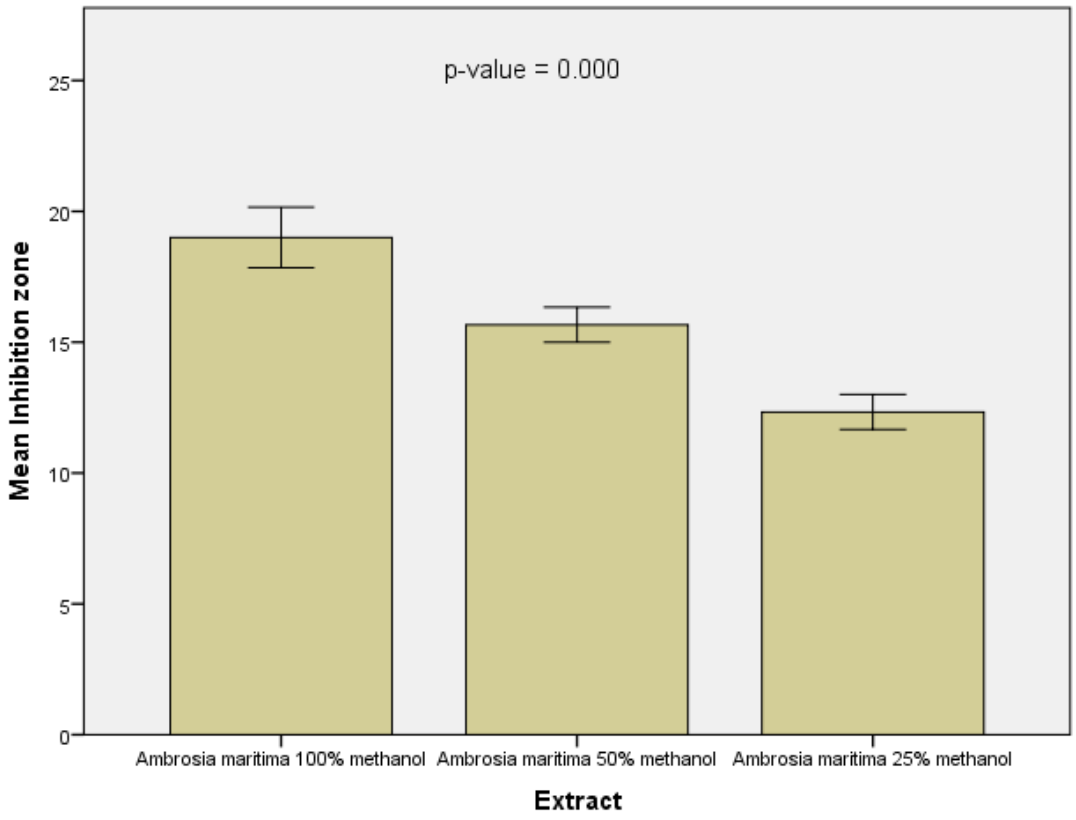


Figure (4.10): Effect of *Ambrosia maritime L* against *S .aureus* with different concentrations

Table (4.11): Effect of *Ambrosia maritime L* against *P .mirabilis* strains with different concentrations

Concentration of *Ambrosia maritime L*	Clinical isolate *P .mirabilis*	Inhibition zones (mm)	STD.deviation
100 w/v (%)	3	19	1
50 w/v (%)	3	15	1
25 w/v (%)	3	12	0

One way ANOVA *P-value = 0.000034*

*Note One way Anova P value less than 0.05 is considered statistically significant

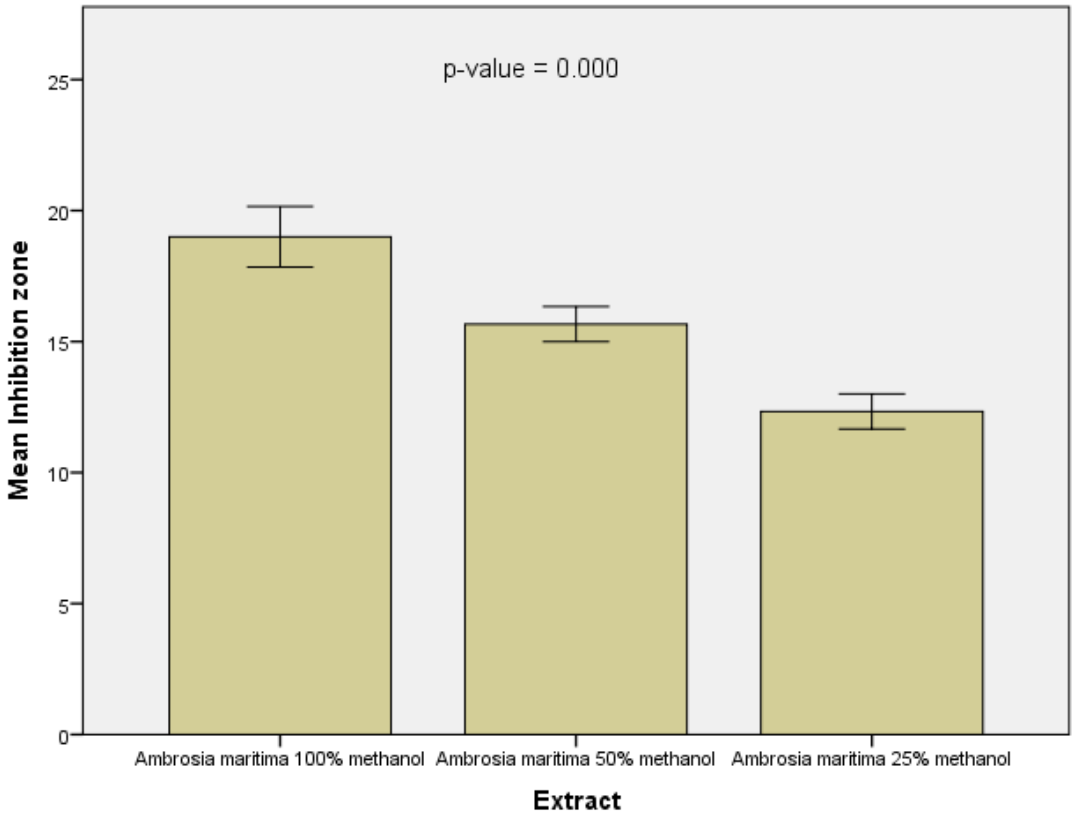


Figure (4.11): Effect of *Ambrosia maritime L* against *P .mirabilis* strains with different concentration

Table (4.12): Effect of *Ambrosia maritime L* against *E .fecalis* strains with different concentrations

Concentration of *Ambrosia maritime L*	Clinical isolate *E .fecalis*	Inhibition zones (mm)	STD.deviation
100 w/v (%)	3	20	1
50 w/v (%)	3	16	1
25 w/v (%)	3	14	1

One way ANOVA *P-value = 0.000595*

*Note One way Anova P value less than 0.05 is considered statistically significant

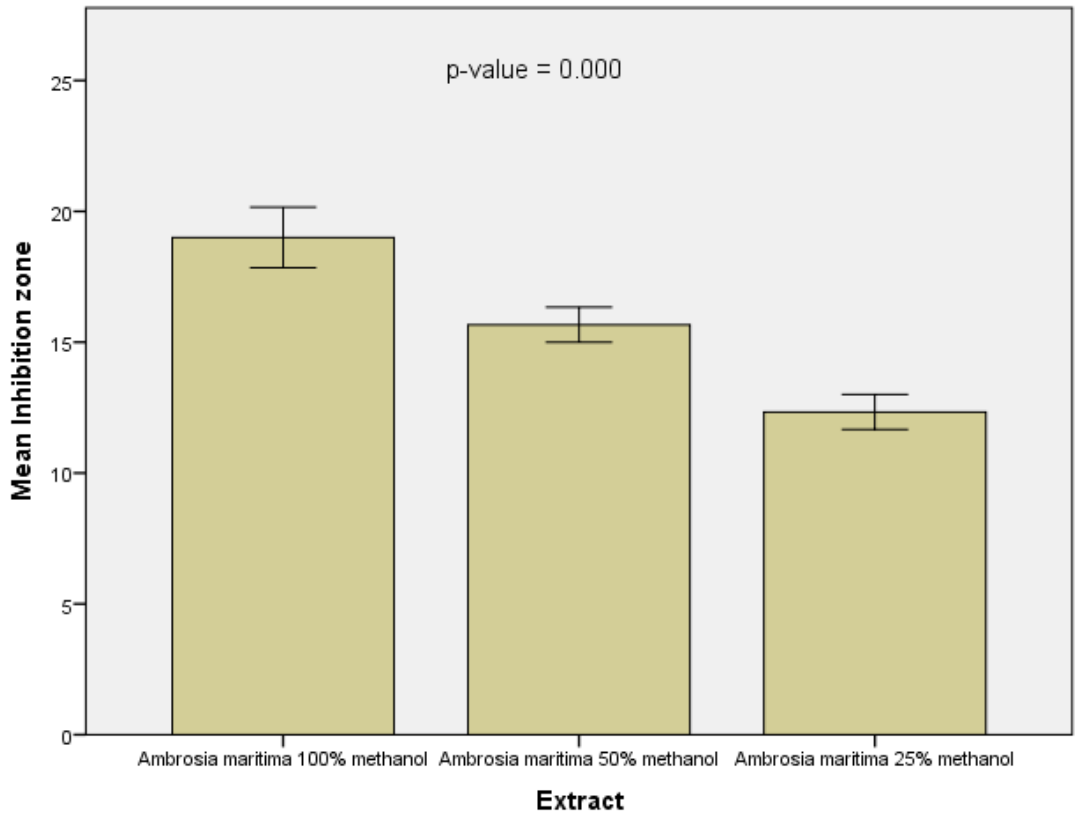


Figure (4.12): Effect of *Ambrosia maritime L* against *E .fecalis* strains with different concentrations

Table (4.13): Effect of *Ambrosia maritime L* against S .saprophytic us strains with different concentrations

Concentration of *Ambrosia maritime L*	Clinical isolate S.saprophyticus	Inhibition zones (mm)	STD.deviation
100 w/v (%)	3	20	1
50 w/v (%)	3	16	1
25 w/v (%)	3	14	1

One way ANOVA *P-value = 0.000595*

*Note One way Anova P value less than 0.05 is considered statistically significant

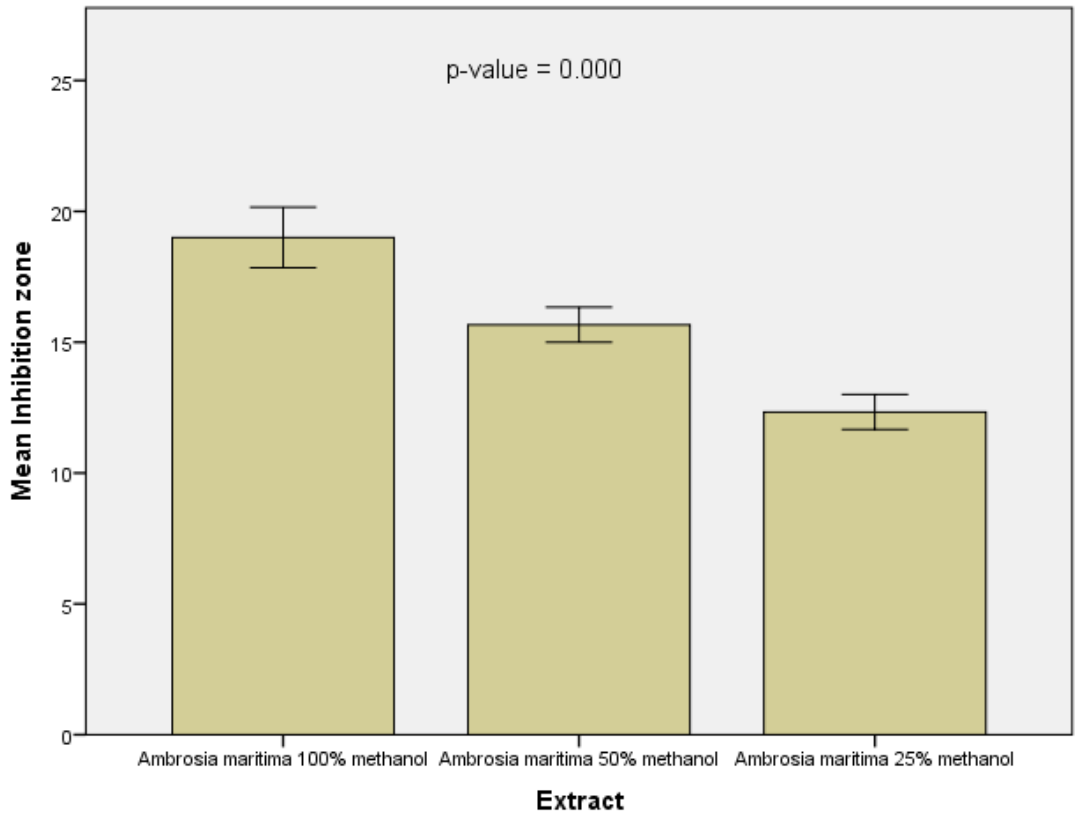


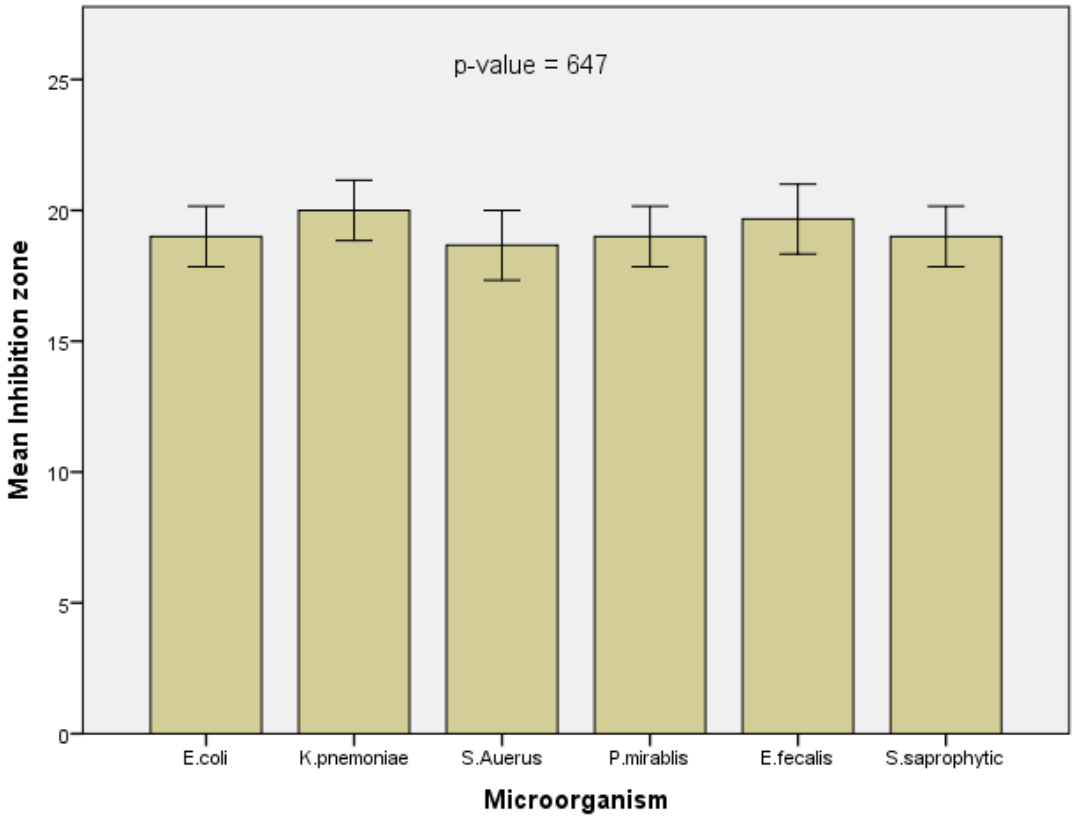
Figure (4.13): Effect of *Ambrosia maritime L* against S .saprophytic us strains with different concentration

Table (4.14): The inhibition zone (mm) of 100 % of *Ambrosia maritime L* against bacterial isolates

Isolated bacteria	Inhibition zones	Std. Deviation
E .coli	19	1
K .pneumonia	20	1
S .aureus	19	1
P .mirabilis	19	1
E .faecalis	20	1
S.saprophyticus	19	1

One way ANOVA *P value =0.647*

*Note One way Anova P value less than 0.05 is considered statistically insignificant

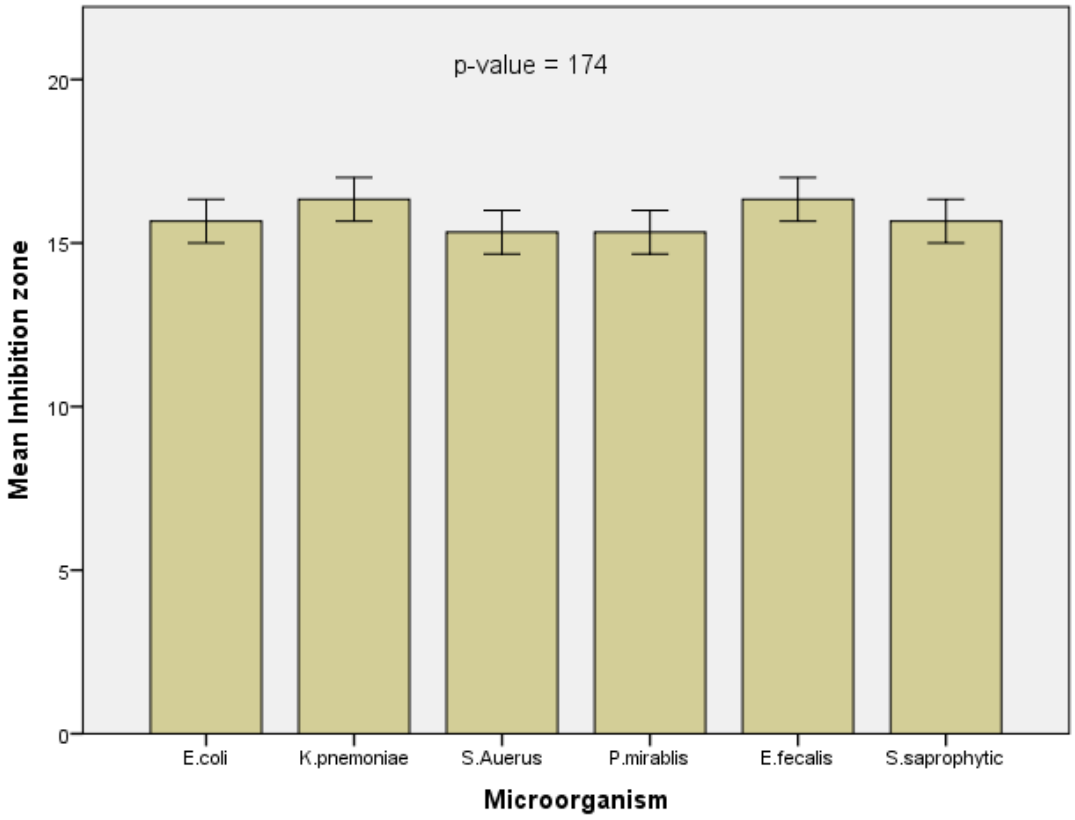


Figure(4.14): The inhibition zone (mm) of 100 % of *Ambrosia maritime L* against bacterial isolates

Table (4.15): The inhibition zone (mm) of 50 % of *Ambrosia maritime L* against bacterial isolates

Isolated bacteria	Inhibition zones	Std. Deviation
E .coli	16	1
K .pneumonia	16	1
S .aureus	15	1
P .mirabilis	15	1
E .faecalis	16	1
S.saprophyticus	16	1

One way ANOVA *P value = 0.174*



Figure(4.15): The inhibition zone (mm) of 50 % of *Ambrosia maritime L* against bacterial isolates

Table (4.16): The inhibition zone (mm) of 25 % of *Ambrosia maritime L* against bacterial isolates

Isolated bacteria	Inhibition zones	Std. Deviation
E .coli	12	1
K .pneumonia	13	1
S .aureus	12	1
P .mirabilis	12	1
E .faecalis	14	1
S.saprophyticus	12	1

One way ANOVA *P value = 0.008*

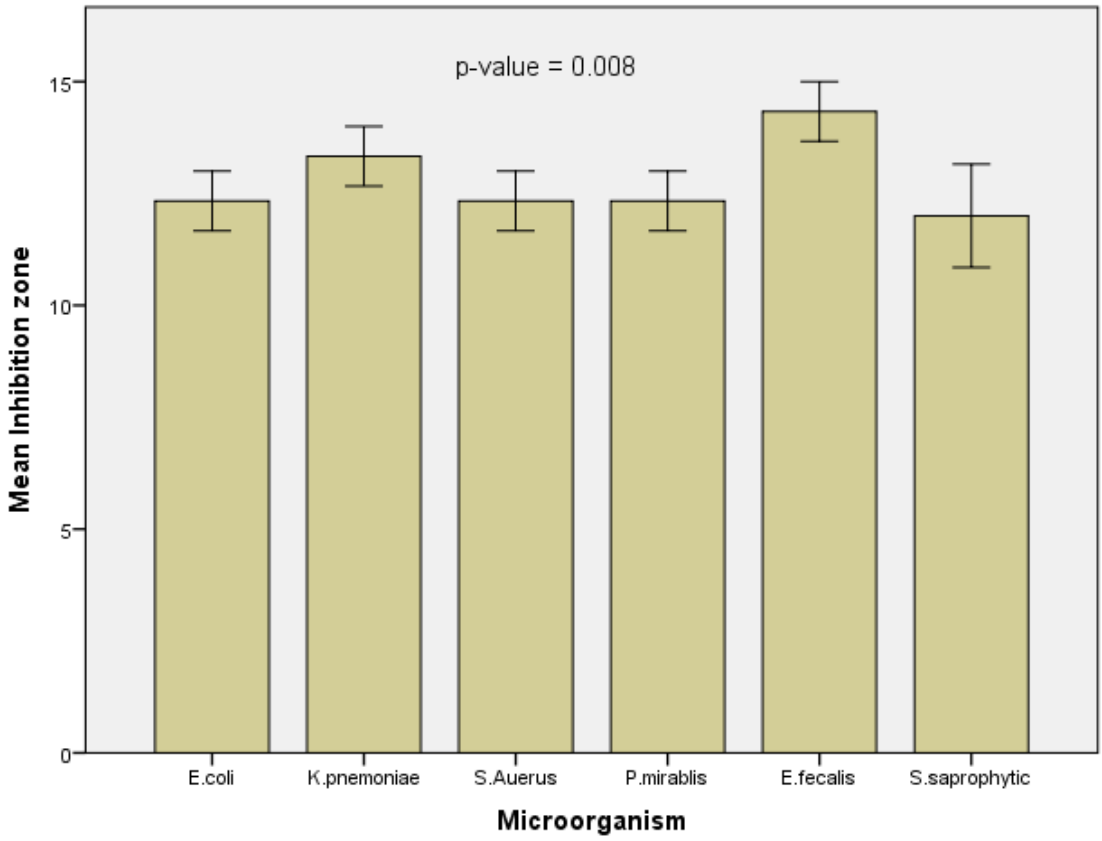


Figure (4.16): The inhibition zone (mm) of 25 % of *Ambrosia maritime L* against bacterial isolated

CHAPTER FIVE

Discussion, Conclusions and Recommendations

5.1. Discussion

The misuse of antimicrobial drugs has led to emergence of 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 for possible antibacterial properties(Khan *et al*.,2009).

According to the results, the effect of *Ambrosia maritime L on* isolated bacteria *E.coli, K.pnemoniae, S.auerus, E.faecalis, S.saprophyticus* and *P.mirabli*s,showed antimicrobial activity on the isolated bacteria showing higher effect on *E.coli*, which is similar to the result obtained by (Hamadan *et al*., 2015), which showed the high effect of *Ambrosia maritime L* on *E.coli* (54%). This study also agree with the report of (Ali *et al.*, 2015)who mentioned that concentration 83.3%of petroleum ether extract of *Ambrosia maritime L on E. coli*, *K. pnemoinae* and *S. auerus* was inhibited and 33.3% with methanols, also similar to(peng *et al*.,2006) whom detect to chemical composition of *Ambrosia maritime L* like oil ,in this study showing the inhibition zone of *E.coli* 20.0mm,*S.auerus* 25.0mm, *E. faecalis*20.0mm and *K.pneumoniae* 23.5mm.

The different between studies maybe artiputted to use of solvent, sample size, climate and location collection of samples, and preparation of patients, different extracted methods and finally isolated bacteria(Khan *et al*.,2009).

5.2Conclusion

In conclusion the results of this study showed that the methanolic extract of *Ambrosia maritime L* has antimicrobial potential against urinary pathogens. Further investigations with reference strains are essential before it is possible to draw any final conclusions since**,** high concentrations were required for the inhibition of urinary pathogens.

5.3 Recommendations

1-Futures studies should verify the effect of water extract as well as other extracts of *Ambrosia maritime L* using different solvents such as ether & chloroforom is necessary since other solvents might extract other active ingredients that were not extracted with methanol .

2-It is essential to run cytotoxicity tests for this plant since the study showed that high concentrations of methanolic extract are required to inhibit the bacterial growth.

3-Further research into the antimicrobial potential of *Ambrosia maritime L* using reference strains is required in order to confirm the effectiveness of this medicinal plant.

References

1-Abdallah,O, Ali,A and Itokawa,H, (1991). Cytotoxic activity of sesquiterpone Lactones isolated from *Ambrosia maritime L.Journal of Medicine Plant*, 46:60-472.

2-Abdelgaleil,S, Badawy,**M**, **Suganuma,T and Kitahara, k** (2011) Antifungal and Biochemical effects of Pseudo guanolides sesquiterpenes isolated from *Ambrosia maritime L. African Journal of Microbiology Research*,**5**(21): 3385-3392.

3-Amin, W, (1990). A pharmacolognostical and structure study of *Ambrosia maritime L. Journal of MedicinePlant,*1:30-39.

4-Annette,E,Annicklarochelle, A, and Dannylovatsis, D,(2010).Recurrent urinary tract infection. *Journal of Obstetrics and Gnaecology*,**32** (11)1082-1096.

5-Andrew, l, (2010).Symptoms of urinary tract infection .*Journal of Health scope Medical Center*. (1)2-11.

6-Ali,j, MaKKwi,k, Eman,M, Kesh,K, **Maha,M, ELshamy**,**E and Mamdou Abdel-Mogib** (2015).Phytochemistry and biological evaluation of *Ambrosia maritime L. Journal of Pharmaceutical Biological and Chemical Science* **6**(4):16-78.

7-Cheesbrough, **M,** (2006).District Laboratory Practice in Tropical Countries2:157-162.

8-Chen , S , Jackson , S, and Boyko , E ,(2009) .Diabetes mellitus and urinary tract infection epidemiology, pathogenesis and proposed studies in animal models. *Journal of Urology* ,**182**(6):51-65.

9-Chowdhury, M, Nayem,M ,Ali,M,Lutfun, N, and Kazi , M, (2013) Antimicrobial activity of some medicinal plants against multi drug resistance human pathogens *Advance In Bioscience and Bioengineering*. **(**1)1-24.

10-Deshpande, S, (2013). Microbial infection .*The Cobrane Library*.**2** (3-5).

11-EL-amir,(2010).Effect of *Ambrosia maritime L* against bacteria. *Current Resarch Journal of Biological Sciences* .2(**2**) 150-159.

12-EL-Kamali, H, (2010).Antimicrobial activity of *Ambrosia maritime L* against bacteria infection. *Journal of Medicine Plant* .**1**(1):45-49.

13-El-Sawy,M,El-Hamd,Z,Loutfy,**N,El- Masry,S and AbdelGaulil,M,** (1986).Pharmacological action of *Ambrosia maritime L. Journal of Pharmacognocy and Photochemistry of Plant* 2:57-64.

14-Farnsworth, **N**, (1995).The phytochemistry of *Ambrosia maritime L.Current Research Journal of Biological Sciences* (2):143-146.

15-Franz,**G,**(1993).The phytochemistry of *Ambrosia maritime L. Journal of Medicine Plant.* (1):2-6.

16-Hamdan, Z, Eman, K, Amar,M,Onab, S, Sarah, O, and Ishag, A, (2015). Urinary tract infections treatment and antimicrobial sensitivity among diabetic patients at Khartoum, Sudan. *Annals of Clinical Microbiology and Antimicrobials*,**14** (1),p1.

17-Hamdorf,M, (2010).Test and treatment of urinary tract infection. *Journal of Health Scope Center* 4: 6-8.

18-Horan, **T,Andrwus**, **M ,DudecK,M**, (2008). Definition and cause of sterile bacteruria associated with urinary tract infection . *The New E ngland journal of M edicine (* **36)**:309-32.

19-Johnson, **J**. (2003). Pathogenesis and virulence of the Urinary tract infection infection. *The Journal of Clinical Microbial Research* (**17**):261-278.

20-Junel,**A**,**Brusch, M** ,(2015) .Relationship between Urinary tract infection and diabetes mellitus .*Website of urinary tract infection in diabetes mellitus* 4:1-3.

21-Kavitha, K,Satish, S, (2013).Antimicrobial activity of *Ambrosia maritime L* & structure. *Journal of Analytical Science Technology.4:23*-26.

22- **Khan**, **R**,**Islam**, **B**, **Akram**, **M**,(2009).Antimicrobial activity of herbal extract against multi- durg resistance strains of bacteria .*Journal of Clinical Biology, Agriculture and Health Care*.(14)**:**586-597.

23-Khalid,H, Abdalla,W, Abdelgadir,H, Opatz,T and Efferth,T, (2012).Uses of *Ambrosia martima L*in sudan . *Journal of Medicine Plant*.2: 92-103.

24-Modawi, **B, Sattiand,R,and Dupery**,**D**,(1984). Chemistry *of Ambrosia maritime L .Current Research of Biological Sciences* .**47**(1):167-169.

25-NieLubowicz, **R**, **Mobley**, **H**, (2010). Host pathogen interaction in urinary tract infection .*The New England Journal of Medicine*.7:430-41.

26-Lindsay,E, **Nicolle,M ,**(2002) Epidemiology of urinary tract infection. *Journal of Clinical Microbiology* **24(**18):135-140.

27-Rechtsteine, E,(2011). Uncomplicated urinary tract infection. *Journal of* the *Microbial Research* .**1(**169**):**1-38.

28-Rizk, **A,** (1986). The phytochemistry of the *Ambrosia martima L .Current Resarch Journal of Biological Sciences*.47:50-66.

29-Ross,**S, EL-Saved**,**k, Hamann, M, Abdel-Halim**,**O,Ahmed,M,** (1997). Phytochemical analysis of Geigeria a lata and Francocuriacrispa essential oils.*Current Research Journal of Biological Sciences*. 63(5):479-482.

30-Pak, **leung**, **H**,(2011) Multi drug resistance of bacteria .*Current of Microbiology Research***16**(**4**)1-15.

31-Peng, W, ChuiHua,K,andChaoXian, Z,(2006).Chemical composition and antimicrobial activity of the essential oil from *Ambrosia maritime L. Journal of Medicine Plants*. (11) p549-555.

32-Sukhdev, S ,Suman, P,Gennaro, L, and Dev, D, (2008).Extraction technologies for medicinal and aromatic plants.*The Journal of Medicine Plant* 2:250-255.

33-Tackholm, v,(1974).Characteristic of *Ambrosia maritime L. Journal of Medicine Plant***.3** (1)25-28.

34-Unido, (1996).Project for the Government of Sudan prepared by unido industrial utilization of medicinal and aromatic plants in Sudan. *Journal of Medicine Plant*.**80** (502):26-32.

35-WHO (2006). Definition, classification, complication of Diabetes mellutis, *World Health Organization*.1:11-15.

Appendix (1) Mueller Hinton II Agar

Ingredients	g/L
beef infusion	300.0
Casein acid Hydrolysate	17.50
Starch	1.50
Agar	17.00

preparation:

Suspend 38g of the powder in 1 litre of Distilled water Mix thoroughly.

Heat, to completely dissolve the powder.Autoclave at 121^0C for 15 minutes.

Appendix (2) : CLED Agar (Cystine-Lactose-Electrolyte-Deficient Agar)

Ingredients g/L	
Enzymatic Digest of Gelatin	4 g
Enzymatic Digest of Casein	4 g
Beef Extract	3 g
Lactose	10 g
L-Cystine	0.128 g
Bromthymol Blue	0.02 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

preparation:

Suspend 36g of the powder in 1 litre of Distilled water, Mix thoroughly.

Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121^0C for 15 minutes.

Appendix (3): Nutrient agar

Ingredients	g/L
Peptic digest of animal tissue	5.00
Beef extract/yeast extract	3.00
Agar	15.00
NaCl	5.00

pH is adjusted to neutral (7.4) ± at 25 °C.

preparation

Suspend 23 g of the powder in 1 litre of Distilledwater . Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121˚C for 15 minutes.

Appendix (4) :Christensen's Urea Agar

Ingredients	g/L
Sodium Chloride	5.00
Monopotassium Phosphate	0.8
Dipotassium Phosphate	1.2
Peptone	1.00
Dextrose	1.00
Phenol Red	0.012
Agar	15.0

Final pH 7.4 ± 0.2 at 25 ° C.

Preparation

Suspend 24 grams in 950 ml of distilled water heat boil to dissolve completelyAutoclave at 121 ° C for 15 minutes.then cool to 50° C add Aseptically 40% urea solution and mix well , dispense in strile tube and allow to set at slant position

Appendix (5) Simmon's Citrate Agar

Ingredients	g/L
Sodium Chloride	5.0
Sodium Citrate	2.0
Ammonium Dihydrogen Phosphate	1.0
Dipotassium Phosphate	1.0
Magnesium Sulfate	0.2
Bromothymol Blue	0.08
Agar	15.0

Final pH 7.4 ± 0.2 at 25 ° C

Preparation

Suspend 24.28 grams in 100 ml of distilled water heat boil to dissolve completelyAutoclave at 15 Ibs pressure (121° C) for 15 minutes. then cool to 50° C aseptically dispense in sterile tube and allow to set

Appendix (6) Peptone Water

Ingredients	g/L
Sodium Chloride	5.0
Peptic digest of animal tissue	10.0

Final pH 7.4 ± 0.2 at 25 ° C

Preparation

Suspend 15 grams in 100 ml of distilled water heat boil to dissolve completelyAutoclave at 15 Ibs pressure (121° C)for 15 minutes. then cool aseptically, dispense in sterile tube and allow to set

Appendix (7) :Kligler Iron Agar (KIA)

Ingredients	g/L
Peptic digest of animal tissue	15
Lactose	10.0
Proteose Peptone	10.0
Sodium Chloride	5.0
Beef Extract	3.0
Yeast Extract	3.0
Dextrose	1.0
Sodium Trisulphate	0.3
Ferrous Sulfate	0.2
Phenol Red	0.024
Agar	15.0

Preparation

Suspend 57.52 grams in 1000 ml of distilled water heat boil to dissolve completelyAutoclave at 15 Ibs pressure (121° C)for 15 minutes. Then cool aseptically, dispense in sterile tube and allow to set slant position

Appendix (8) :Mannitol Salt Agar (MSA)

Ingredients	g/L
Proteose Peptone	10.0
Sodium Chloride	10.0
Beef Extract	1.0
D-mannitol	10.0
Phenol Red	0.025
Agar	15.0

Preparation

Suspend 111.02 grams in 1000 ml of distilled water heat boil to dissolve completelyAutoclave at 15 Ibs pressure (121° C) for 15 minutes. Then cool aseptically, dispense in sterile tube and allow to set slant position

Appendix (9):DNase Test Agar

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Animal Tissue	5 g
Sodium Chloride	5 g
Deoxyribonucleic Acid	2 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Preparation

Suspend 24 g of the medium in 100ml of Distilled water .Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121°C for 15 minutes

Appendix (10) :Antibiotic Disc

CIP	Ciprofloxacin	30 mcg/disc
Gen	Gentamicin	10 mcg/disc
CXR	Ceftriaxone	30mcg/disc
Cefo	Cefoxitin	30 mcg /disc
VA	Vancomycin	30 mcg/disc
CAZ	Ceftazidime	30 mcg /disc
Imp	Imipenem	10 mcg/disc
AMC	Amoxicillin	20mcg/dis

Appendix (11):Autoclave (Medical Instrumention MFG CO,Mumbia)

Appendix(12):Hot air oven (Leader Engineering Widness Cheshire , UK)

Appendix(13): Incubator (TorpePicenardiCCRI, Italy)

Appendix(14): Soxhlet(Duran UK)

Appendix(15): Rotary evaporator (Buchiswitzerland)

Appendix(16):Methanol (Romile EU)