

## INTRODUCTION

Plants have been used for thousands years for many purposes ranging from medicinal to religious (in rituals) and from production against spirits to culinary delights, perfumery and cosmetics (Agra *et al.*, 2007). Plants have been considered an important source of indispensable drugs. The relationship between human and plants has always been a very close one throughout the development of human culture, and no doubt, the herbalist is probably one of the first professional in the evolution of human culture. The uses of the plants as drugs lead researchers to study more about these plants to determine their mode of active principles i.e. pharmaceutical value(Albsher, 2005). Traditional medicine in Sudan is practiced in rural as well as urban areas of Sudan for many years ago. Sudanese people used many traditional and folklore as the uses of medicinal plants for curing the disease and relief pain(Ebnaaouf,2015). The plants selected for this investigation are used in folk medicine for their effects are known effects as antimicrobials but this has not been confirmed by experiments. Historically , medicinal plants have provide the basic building blocks for a number of highly effective drugs and they remain as an attractive option for discovery of new molecular entities, due to their largely untapped chemical diversity(Malherbs, *et al.*, 2012). Medicinal plant, in general, has been as issue of great controversy through the history of mankind, For early peoples, they came easily to hand, and were intricately connected to diet and healing. Through observation and experimentation; they learned which plants promoted health and well-being .Actually , the use of plants and herbs as medicine has almost become a differentiating aspect between first world and third world countries and cultures. This seems ironic as many of the modern medicinal products are actually derived or extracted from plants(Cowan,1999). Clinical microbiologists have two reasons to be interested in topic of antimicrobial plants extracts. First it is very likely that these phytochemicals will find their way in to the arsenal of antimicrobial drugs prescribed by the physicians;

several are already being tested in human . Scientists realize that the effective life span of any antibiotic is limited , so new sources especially plant sources are also being investigated. Second the public is becoming increasingly aware of the problems with the over prescription and misuse traditional antibiotics. In addition many people are interested in having more autonomy over their medical care. A multitude of plants compounds(often of unreliable purity) is readily available over the counter from herbal supplier and national food stores and self medication with these substances is a common practice to certain extent (Cowan, 1999).

### **Objective of study**

#### **General objective**

The present study investigates the antimicrobial activities of different solvent extracts from *Nicotiana tabacum* against few selected species of Gram +ve and Gram –ve bacteria.

#### **Specific objective**

To compare the activity of *Nicotaina tabaccum* extract with some Antibiotics

## CHAPTER ONE

### LITERATURE REVIEW

Tobacco nicotine inhibits the growth of pathogens which is dose dependent (Agra *et al.*, 2007),( Wang *et al.*, 2008) and (Suresh *et al.*, 2008).The extracts of leaves also applied for muscle relaxation and relieving pain. Antibacterial activity of different extracts of the leaves of *Nicotiana tabacum* was studied Yildirim *et al.*, (2001) reported that the ether extracts of both the leaves and seeds and ethanol extract of leaves had shown antimicrobial activities on *Staphylococcus* spp. Wang *et al.*, (2008 ) found inhibition of the activities of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* by crude polyphenols extracted from tobacco leaf by 80% ethanol solution. Strong antimicrobial activities against *Klebsiella pneumoniae*, *Escherichia coli*,*Streptococcus faecalis*, *Mycobacterium phlei*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and the human pathogenic yeast, *Candida albicans* were detected in methanolic extracts of 24 plants used medicinally in the treatment of skin infections in four different regions of Colombia. Twenty-two extracts displayed activity against Gram-positive bacteria whereas none was active against the Gram-negative species (Lopez *et al.*, 2001).

## **1.1 Bacteria and disease they caused**

### **1.1.1 The genus *Staphylococcus***

*Staphylococci* are Gram-positive, spherical cells occurring singly in pairs or clusters. They are natural habitat from birth to death. Man lives in an environment that is rarely free of *S.aureus*. The human nose is a natural reservoir for the organism (Ahmed 2006). The skin and large intestine represent additional source for the contamination of the atmosphere with *S.aureus* (Buchanan and Gibbons, 1974; Melville and Russel, 1975; Talaro and Talaro, 1993). Traditionally, *staphylococci* have been divided into two groups according to their ability to clot blood plasma (coagulase reaction). Coagulase-positive *staphylococci* strains have been subdivided according to animal host in to serotype and phagotypes. In general, names for new subspecies of coagulase-positive *Staphylococcus* have not been proposed (Cohen, 1986; Thomas, 1988). In contrast, Coagulase-negative *staphylococci* have been differentiated according to their biochemical characteristic and cell wall chemistry.

### **1.1.2 *Staphylococcus* infection**

*Staphylococci* are perhaps the best example that have great pathogenic potential, yet are able to live in symbiotic balance with their host. In spite of their ability to produce serious, life threatening disease, pathogenic *staphylococci* are present on skin or mucous membrane of all human (Jensen and Warright, 1998). *Staphylococci* are part of the normal microflora of upper respiratory tract (Kumar and Clark, 1996). *Staphylococci* cause a wide range of infection both in man and animals, which are summarized in two groups Suppurative (Skin) and Systemic (Davis *et al* 1973; Melville and Russell, 1975; Jawets *et al* 1980; Talaro and Talaro, 1993).

### **1.1.3 Pathogenicity of *Staphylococcus aureus***

*Saphylococcus aureus* is usually a secondary infection in patient with chronic lung disease (MacSween and Whaly, 1992). *S.aureus* causes boils,

pustules , impetigo, infection of wounds, ulcers and burns, osteomyelitis, mastitis , septicemia , meningitis, pneumonia and pleural empyema (Cheesburgh, 2000).

#### **1.1.4 The genus *Escherichia***

*Echerichia* is a genus of the family *Enterobacteriaceae* and types *Eschericheae*. Like many other *eterobacteria* contains numerous serotypes some of which are associated with infections in human and animals, some are particulary associated with diarrhoeal disease while other causes a variety of extra intestinal infection(Orskov and Orskov, 1982).

##### **1.1.4.1 General characteristic of *Escherichia coli***

*E.coli* is Garm negative rod-shaped, oxidase negative, catalase positive . *E.coli* has a worldwide distribution . Many *E.coli* are part of normal flora of the intestinal tract of human and animal .Some species are free living occurring in soil, water and vegetation(Jawetz *et al.*,2001).

##### **1.1.4.2 *E.coli* infections**

*Escherichia coli* causes various diseases in human and animals including several types of diarrhea(watery diarrhea, cholera like diarrhea, watery to dysentery like diarrhea and mucoid diarrhea ), urinary tract infections sepsis , heamorrhagic uraemic syndrome and meningitis in human ( Nataro and Kaper, 1998). In animal *E.coli* causes profused watery diarrhea in most animal species, odema, hemorrhagic colitis, septicemia and mastitis (Abdalgader 2003).

##### **1.1.5 *Pseudomonas aeruginosa***

*Pseudomonas aeruginosa* is a gram- negative rod, non capsulated. It is an obligate aerobe and can grow in anaerobic condition as found in biofilms if nitrate is available. It can grow in wide range temperature(37-42°C) and awide variety of culture media . It produces diffusible pigment, typically the colony and surrounded medium is greenish blue or yellowish green .It is catalase and oxidase-positive. It causes mild ear infection (otitis media), wound infection in

skin after operation, urinary tract infection and endocarditis. Also it causes eye infection and sepsis progressed to skin lesions which called ecthymagangreosum (Jawetz *et al.*, 2001).

### **1.1.6 *Bacillus subtilis***

*Bacillus subtilis* is a gram-positive bacterium, rod-shaped, spore forming often occurs in long threads and the endospores are terminal and not bulging. It forms rhizoid colonies, usually grows both aerobic and anaerobically. It is a common environmental organism and is frequently isolated in laboratory as a contaminant of media or specimens. It is saprophytic organism prevalent in soil, water, air and on vegetation. It is motile by peritrichous flagella, catalase and oxidase-positive (Jawetz *et al.*, 2001).

## **1.2 Chemotherapy of bacterial infection**

### **1.2.1 Anti microbial agents:**

Antimicrobial drugs are divided in two classes, based upon their general effect on bacterial population. These are bacteriocidal and bacteriostatic. Ehrlich found that the arsenical compound, arsphenamine, was selectively toxic for *Treponema pallidum*, this was the first along series of drugs to be synthesized in the laboratory. A number of years later. Showed that the red dye prontosil was effective in the treatment of streptococcal infections. Later it has been discovered that it was due to sulfonamide derived from prontosil. The success of this drug stimulated a research for related compounds and resulted in the synthesis of effective compound which is the sulfonamide group (Carter *et al.*, 1986)

### **1.2.2 Antibiotics**

The antibiotics are a group of complex organic chemicals which are produced initially by microorganism during their growth and which in minute quantities have detrimental effects on other organisms (Brander *et al.*, 1970). Pasteur and Joubert (1877) first reported that air borne contaminants had lethal

effect on culture of *B.anthraxis* . In 1929, Fleming observed that a fungus *Penicillim notatum*, was strong inhibitor to the growth of *Staphylococci*, when present on cultcure plate. In 1940, chain, Florery, and associated team succeeded in obtaining a preparation from *penicillium* . After the discovery of penicillin an extensive search of antibiotics was began (Cart *et al.*, 1986). In 1949, Waksman and Lechevalier isolated a soil organism, *Streptomyces fradiae* which produced an antibiotics that in crude contained an antifungal compound (Fradine) and a groups of antibacterial substances that were labeled neomycin (Rolinson and Steven, 1961).

### **1.2.3 Problems of antibiotics**

Firstly broad spectrum antibiotics cause disturbance in digestion when used orally due to their effect on microflora. Secondly the development of resistance among bacterial population exposed to antibiotics received great deal of attention both in human and veterinary medicine( Brander and Bugh 1977). Resistance can be caused by a variety of mechanisms such as the presence of enzymes that inactivate the antimicrobial agent; a mutation in the antimicrobial agents target, which reduces the binding of antimicrobial agent; the presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent; reduce uptake of the antimicrobial agent; active efflux of the antimicrobial agent; and over production of target of the antimicrobial agent. In addition , resistance may be caused by unrecognized mechanisms(Tenover and Unger, 1993). Thirdly antibiotics have been used in therapy of animal it may be harmful to human, and it may cause problems in the control of human disease. Finally some antibiotic are very expensive so in recent years, medicinal plant represented a primary health source for the pharmaceutical industry (Brander and Bugh 1977).

### **1.3 Medicinal plants**

Many cultures around the world have strong believes on the use of different plants as medicine which has rendered some culture, up to date almost

completely dependent on the use of plants in medicine rather than common commercial medicinal products (Stockwell, 1988). Medicinal plant in general have been an issue of great controversy through the history of mankind for early peoples they come easily to hand, and were intricately connected to diet and healing through observation and experimentation they learned which plants promoted health and well being( Jawetz *et al.*,2001).

### **1.3.1 Use of plants in medicine**

The use of plants for treatment of various disease is universal and has been practiced by many people since ancient times. Hippocrates in the late fifth century B.C. mentioned 300 to 400 medicinal plants (Schultes, 1978). In the first century A.D. Discorides wrote *De Materia Medica*, a medicinal plant catalog which became the prototype for modern pharmacology. The use of medicinal plants varies from disease to another. The Neem tree (*Malia azadirach*), for example, has been known in Asia for long time. The sun –dried seeds of the plant are used by Indians to control pests in house as well as stored cereal grains, and as a detergent resembling shampoo, for the removal of lice from the hair (Jotwani and Srivastova, 1984; Ibrahim, 1990). The Neem tree has also been found to have an antimicrobial effect (Khalid *et al.*, 1989). In African countries, the ripe fruit of *Balanites egyptiaca* , (Laloub) is a popular medicinal plant and is used as a purgative and anthelmintic (Oliver, 1986 ;Nakhla , 1990). *Cassia senna* and *C.italica*, both of the family *Leguminasceae* were the first *cassia* species reported in literature for their therapeutic value as purgative . The chemical constituents in the ripe pods of these plants are emodin, aloe and chrysophanol (Friedrich and Steftem, 1973).

*Cucurbita maxima* and *C.peppo* seeds karnel (Kousa) are used in many African countries including the Sudan as well as Europe and Asia as anthelmintic for tape worms and as diuretic and the scraped fruit pulp is applied as a poultice to burns, boils and swelling or as cooling application for headache (Oliver, 1986; Mohamed, 1992). Plant constituents possessing antibacterial activity include



flavonoids in *Polygonum senegalense* (Lewis and Elvin Lewis, 1977). phenol chlorophenol in *Clorophora excelsa* thymol and euganol in (Jain and Jain, 1972). The volatile oils of black peppers, clove, geranium, nutmeg, oregano and thyme were assessed for antibacterial activity against 25 different genera of bacteria. These include animal and plant pathogens, food poisoning and spoilage bacteria. The volatile oils exhibited considerable inhibitory effects against all the organisms under test while their major components demonstrated various degrees of growth inhibition (Dorman and Deans, 2000).

#### **1.4 The plant *Nicotiana tabacum***

Is a green plant that harvests most of its energy from sunlight via photosynthesis and belongs to the family *Solanaceae* which also includes some other important crop species such as tomatoes, potatoes and peppers etc. Genus: *Solanus* Species: *Nicotiana tabacum* Scientific/ medical name: *Nicotiana tabacum* Common name: Tobacco. (Wang *et al.*, 2008).

## CHAPTER TWO

### MATERIAL AND METHOD

#### **2.1 Plant collection**

Plants of *Nicotiana tabacum* were collected from Alfasher .leaves were separated out from stems washed with distilled water to remove dirt and soil particles leaves were cut in to small pieces and then leaves were dried in shaded area at room temperature for a period of one week .The dried leaves were grinded and stored dried in a plastic container until the time of use ,The method was Adopted by Bakht *et al.*,(2012).

**2.2 Source of bacteria strain :** from the Ministry of Enviroment Department of Microbiology (Khartoum).fresh bacteria in petri dish.

#### **2.3 Plant Extracts**

**2.3.1 Preparation extract :** In the Ministry of Enviroment-Department of medicinal and aromatic plants – Department of plant Chemistry(Khartoum).

##### **2.3.2 Water extract**

100 grams of *Nicotiana tabaccum* were Soaked in 200 ml of distilled water in a sterile flask for 24 hour. The contents of flask were then filtered The filtrate was kept at 4°C for later use.

##### **2.3.3 Ethanol extraction**

Hundred grams of dried powdered plant material of tobacco (leaves ) were taken into separate round bottom flasks and filled with 95% ethanol until dipped and then fixed with the condenser. This assembly were adjusted in heating mental and connected to the a tape water supply. The material were boiled at 50°C for 24 hrs and then filtered with the help of vacuum pump using Buchner funnel. Ethanol was isolated from the mixture of the extract through rotary evaporator at 60°C under reduced pressure. Then Ethanol extract was collected

from the flask and dried through water bath at 60°C. After drying, the extract was weighed and stored into a vial.( Bakht *et al.*,2012).

#### **2.3.4 Acetone extraction**

Hundred grams of dried powdered plant material of tobacco (leaves ) were taken into separate round bottom flasks and filled with 95% Acetone until dipped and then fixed with the condenser. This assembly were adjusted in heating mental and connected to the tape water supply. The material were boiled at 50°C for 24 hrs and then filtered with the help of vacuum pump using Buchner funnel. acetone was isolated from the mixture of the extract through rotary evaporator at 60°C under reduce pressure. Then Acetone extract was collected from the flask and dried through water bath at 60°C. After drying, the extract was weighed and stored into a vial. Bakht *et al.* (2012).

#### **2.4 Preparation of different concentration of *nicotina tabacum* extract**

Four concentration of the extract ( 100 %, 50 %, 25 %, 12.5 %). were made by dissolving 1gram of extract in 5ml of sterile distilled water to prepare the first concentration and double fold dilution was made to obtain 100 %, 50%, 25%, 12.5% concentration( Kavanagh 1972).

#### **2.5 Sterilization**

All instrument and media used in different procedure for bacteriological techniques were sterilized by method adopted by Cowan and Steels (1993).

#### **2.6 Testing of the extract for antibacterial activity**

##### **2.6.1 Well-agar diffusion method (cup plate method)**

The cup- plate agar diffusion method (Kavanagh, 1972) was adopted to assess the antimicrobial activity of the prepared extract. Antibacterial activities of *Nictiana tabacum* was evaluated using well diffusion method on nutrient agar media adopted by (Murray *et al.*,1995 and Jahangirian *et al.*, 2013). The suspension culture for bacterial cells growth was done by preparing nutrient Broth1colony of bacteria in 1ml nutrient Broth .The bacteria used for

antibacterial assay of *Nictiana tabacum* leaves *S.aureus*, *E.coli*, *Bacillus subtilis*, *Pseudomonas earoginosa* Nutrient agar media plates were inoculated with 50 µl bacterial strain suspension under aseptic conditions and wells is a piece of sterile iron 6mm in diameter and about 2cm in apart was filled by sterile syringe with 50 µl of different concentrations of different plant solvent extracts and then test samples incubated at 37°C for 24hour Normal saline will be used as negative control and Penicillin, Gantamicin and Ciprofloxacin as positive control. After the incubation period Antibacterial activity was determined by measuring the diameter of zones of inhibition (mm). This experiment was carried out in triplicate for confirmation(Sharma *et al.*, 2015).

## CHAPTER THREE

### RESULTS

#### 3.1 Antibacterial test

Inhibition zone was calculated in order to reveal its inhibitory effect against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and its antibacterial activity compared with standard antibiotics such as penicillin, ciprofloxacin and Gantamicin **Table(3 and 4)**.

#### 3.2 Water extract

Water extract of *Nicotina tabaccum* showed no inhibitory effect against *S.aureus*, *E.coli*, *Pseudomonas.aeruginosa* and *Bacillus.subtilis* (zero inhibition zone).

#### 3.3 Acetone extract

The inhibitory effect was also found to be present in the acetone extract 12.5% against *S.aureus* and *E.coli* with inhibition zone  $3.67 \pm 0.58$  mm and  $2.33 \pm 0.58$  mm respectively but no inhibition was observed against *Pseudomonus. aeruginosa* and *Bacillus.subtilis*

Acetone extract 25% also has showed inhibitory effect against *S.aureus* and *E.coli* with inhibition zone  $4.00 \pm 1.00$  bmm,  $2.50 \pm 0.50$  mm respectively and not seen effect against *Pseudomonas.aeruginosa* and *Bacillus.subtilis*. Acetone extract 50% showed inhibitory effect against *S.aureus* and *E.coli* with inhibition zone  $5.17 \pm 1.15$  b mm and  $4.67 \pm 1.53$  mm respectively and no inhibition effect against *Pseudomonas.aeruginosa* and *Bacillus.subtilis*. Growth of *S.aureus*, *E.coli* and *Bacillus subtilis* found to be inhibited by the 100% acetone extract with inhibition zone  $8.83 \pm 2.02$  mm,  $7.33 \pm 1.53$  mm and  $3.67 \pm 0.58$  mm respectively **Table (1 and 5)**

### 3.4 Ethanolic extract

Ethanolic extract 12.5% showed inhibitory effect against *S.aureus*, *E.coli* with inhibition zone  $1.33 \pm 0.58$  mm and  $1.17 \pm 0.29$  mm respectively inhibitory effect against *Pseudomonas.aeruginosa* and *Bacillus.subtillis* was no noticed zero inhibition.

Ethanolic Extract 25% also showed inhibitory effect against *S.aureus* and *E.coli* with inhibition zone  $2.33 \pm 0.58$  mm and  $2.17 \pm 1.26$  mm respectively whereas inhibitory effect against *Pseudomonas.aeruginosa* and *Bacillus.subtillis* was zero inhibition . Ethanolic extract 50% showed inhibitory effect against *E.coli* and *S.aureus* with inhibition zone  $4.33 \pm 1.16$  mm and  $4.67 \pm 0.58$  mm and it,s has showed a less inhibitory effect against *Bacillus.subtilis* with inhibition zone of  $1.33 \pm 0.58$  mm and while inhibitory effect against *Pseudomonas.aeruginosa* was zero inhibition. Ethanolic extract 100% showed also inhibitory effect against *S.aureus*, *E coli*. and *Bacillus.subtilis*, with inhibition zone  $6.33 \pm 0.58$  mm,  $6.33 \pm 1.53$  mm and  $5.00 \pm 2.00$ mm **Table respectively (2 and 6 )**.

**Table1. Inhibition zone of four concentration of acetone extract against tested strains.**

Concentration Inhibition Zone	100%			50%			25%			12.5		
	<i>Staph.aureus</i>	6.5	10	10	3.5	5.5	6.5	3	4	5	4	3
<i>E.coli</i>	12	6	5	6	5	3	2.5	3	2	2	2	3
<i>Pseudomonas aeruginosa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bacillu ssubtilis</i>	4	4	3	0	0	0	0	0	0	0	0	0

**Table 2. Inhibition zone of four concentration of ethanolic extract against tested strains.**

Concentration Inhibition Zone	100%			50%			25%			12.5		
	<i>Staph.aureus</i>	6	6	7	5	3	5	2	3	2	2	1
<i>E.coli</i>	5	8	6	5	4	5	2	1	3.5	1.5	1	1
<i>Pseudomonas aeroginosa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bacillus subtilis</i>	7	5	3	2	1	1	0	0	0	0	0	0

**Table 3. Antibiotic inhibition zone aganist bacterial strains**

Antibiotics Inhibition Zone	Penicillin			Ciprofloxacin			Gentamycin		
<i>Staph.aureus</i>	9	7	9	7	7.5	6	5	3	4
<i>E.coli</i>	0	0	0	12	11	10	5	7	7
<i>Pseudomonas aeroginosa</i>	0	0	0	0	0	0	0	0	0
<i>Bacillus subtilis</i>	8	7.3	7	9	5	7	10	5	6

### 3.5 Sensitivity of tested organisms to some known antimicrobial agents

#### Antibacterial agents

*Pseudomonas earuginosa* were found to be resistant to penicillin, ciprofloxacin and gentamicin. But *E.coli* resistant to penicillin and gentamicin, *S.aureus* and *B.sbtilis* were found to be sensitive to all antibiotics.

**Table4. Sensitivity of tested organisms to some known antimicrobial agents.**

Antibiotic	<i>Staph. Aureus</i>	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>
Penicillin	8.67±0.58 <sup>a</sup>	0.00 <sup>b</sup>	0.00	7.43±0.51 <sup>a</sup>
Ciprofloxacin	6.83±0.76 <sup>b</sup>	11.00±1.00 <sup>a</sup>	0.00	7.00±2.00 <sup>a</sup>
Gentamycin	4.00±1.00 <sup>c</sup>	0.00 <sup>b</sup>	0.00	7.00±2.65 <sup>a</sup>
Water	0.00 <sup>d</sup>	0.00 <sup>c</sup>	0.00	0.00 <sup>b</sup>
Significant	**	**	NS	**

For this table and subsequent tables

N=12

NS= No significant difference

\*\*=significant at P<0.01

Different superscript letters within the same column are significant different at P<0.05



**Table 5. Antibacterial activity of acetone extracts of *Nicotiana tabaccum* leaves against bacterial pathogens**

Acetone %	<i>Staph.aureus</i>	<i>E.coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>
12.5	3.67± 0.58c	2.33±0.58c	0.00	0.00b
25	4.00±1.00bc	2.50± 0.50c	0.00	0.00b
50	5.17±1.15b	4.67±1.53b	0.00	0.00b
100	8.83±2.02a	7.67±3.79a	0.00	3.67±0.58a
Water	0.00d	0.00d	0.00	0.00b
Significant	**	**	NS	**

**Table 6. Antibacterial activity Ethanolic extracts of *Nicotiana tabaccum* leaves against bacterial pathogens .**

Ethanol %	<i>Staph.aureus</i>	<i>E.coli</i>	<i>pseudomona aeroginosa</i>	<i>Bacillus subtilis</i>
12.5	1.33±.58c	1.17±.29b	0.00	0.00b
25	2.33±.58c	2.17±1.26b	0.00	0.00b
50	4.33±1.16b	4.67±0.58a	0.00	1.33±.58b
100	6.33±.58a	6.33±1.53a	0.00	5.00±2.00a
Water	0.00d	0.00c	0.00	0.00b
Significant	**	**	NS	**

## CHAPTER FOUR

### DISCUSSION

Some plants have been used for centuries as a treatment of infections and other illness in humans and animals. Some of them are believed having antimicrobial activities (Stockwell, 1988). In present study , the plant of *Nicotaina tabaccum* ,which is believed amongst herbal therapist as antimicrobial agent , was examined . the possible in agar well effect of *Nicotaina tabaccum* on growth of isolated Gram positive and Gram negative bacteria were examined. Three different solvents , water, acetone and ethanol were used for extraction . the extracts were tested by agar well method. In contrast to previous studies water extract did not inhibit the growth of *S.aureus* , *B.subtilis* .*E.coli* (0mm inhibition) at any concentration and were more effective to control *pseudomonas aeruginosa* (35.22mm) at highest concentration when compared with other concentrations of the same extract. Reported by (Yildirim *et al.*, 2001) .

In case of *S.aureus*, acetone extract maximum inhibition (45.66mm)being achieved at highest concentrations when compared with other concentration in the same extract. And in case of *pseudomonas aeruginosa* acetone extract controlled the growth of this organism by an inhibition zone of (29.51mm) only at highest concentration, *E.coli* revealed the largest inhibition zone ( 77mm) at highest concentration (Wang *et al* 2008).

In study ethanol extract did not inhibit the activity of *Bacillus subtilis* and *E.coli* (zero inhibition zone) at any concentration of the same extract. In case of *S.aureus* ethanol extract inhibited the growth by 0.85mm at highest concentration when compared with other extract in the same solvent. In previous study, ethanolic extract of the leaves of *Nicotaina tabaccum* has showed effect against *pseudomonas aeruginosa* with inhibition zone of 10.33mm.(Sharma *et al.*, 2015). When comparing antimicrobial activity of the

extracts and some antibiotics it was observed that water plant extract activity had no activity as compared with antibiotics.

Actone and ethanolic extract have the same activity as compared with antibiotics

In contrast, previous studies which used of extracts of *Nicotaina tabaccum* and antibiotics , it was observed that plant extracts were in most cases of higher antibacterial activity than the antibiotics (Abdalla 2008).

## CONCLUSIONS AND RCOMMENDATION

### Conclusions

From the present study it can be concluded that *Nicotaina tabaccum* is plant possessing antibacterial effects against Gram positive and Gram negative bacteria.

Acetone and ethanolic extracts of *Nicotaina tabaccum* are more effective in their action than water extract(water extract is not effective).

The agar- well diffusion method is a simple and it give clear results.

### Recommendation

1-Further testing of water extract on Gram positive and Gram negative bacteria should be carried out.

2-Field trial on larger scales should be conducted to ensure the effectiveness of the different extracts of *Nicotaina tabaccum*.

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