ANTIMICROBIAL EFFECT OF *DODONAEA VISCOSA* JACQ. EXTRACTS AGAINST SOME PATHOGENIC MICROORGANISMS

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Abstract

The present study has been conducted to evaluate the inhibitory effects of the aerial plant part (leaves and bark) extracts of *D. viscosa* before and during flowering against some pathogenic bacteria for human, plants, and against yeast (*Candida albicans*) by using different polarity organic solvents: Ethanol and Diethyl ether. The agar well diffusion method was used to evaluate the inhibitory actions of these extracts with eight concentrations: 0, 2.5, 5, 10, 20, 30, 40 or 50 mg/ml. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were also determined, in addition to compare the results of the plant extracts with the results of the susceptibility of pathogenic microorganisms for antibiotics, The completely randomized design (CRD) was used with three replications. Better effect was observed in the liquid dilution assay with all extracts showing a degree of effect. The results showed that ethanolic extracts of the bark and leaves, and diethyl ether extracts of the leaves demonstrated clear inhibitory effect against the tested microorganisms. ethanolic extracts of the bark was superior over leaf extracts in the inhibitory effects on the growth of *C. albicans*. In general, the results showed no significant differences between the concentrations of 30, 40 or 50 mg/ml. The microbial screening showed that the MIC of ethanolic extracts of the bark before and during flowering was at a concentration of 2.5 mg/ml, and for leaf extracts ranged between 2.5-10 mg/ml. As for MBC effects was ranged between 5-50 mg/ml, depending on the type of solvents, microorganism and the period of collection (before or during flowering). In comparison with antibiotics, the results showed high similitude between *D. viscosa* extracts at concentrations of 30, 40 or 50 mg/ml and antibiotics against the tested microorganisms. When our results compares with other studies that conducted in other parts of the world, we concluded that the type of solvent, method of extraction, period of collection (before or during flowering) and the geographical distribution of *D. viscosa* significantly affect the rate of the chemical components and its effect against microorganisms.

التأثير ضد الميكروبي لمستخلصات نبات الدودونيا *Dodonaea viscosa* Jacq. ضد بعض الأحياء المجهرية المرضية

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الخلاصة

أجريت هذه الدراسة بهدف تقدير فعالية المستخلصات الخام للأجزاء الهوائية للنبات (الأوراق والقلف) وفي فترتي ما قبل التزهير وخلال التزهير، كمضادات لبعض الأحياء المجهرية الممرضـة للإنسـان، و الممرضة للنبات والخميرة *Candida albicans* وباستعمال مذيبات عضوية مختلفة القطبيـة: الكحـول الأثيلي والداي أثيل أيثر.

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استعملت طريقة الانتشار في الحفر (The agar well diffusion method) في تقدير التأثير التثبيطـي لهذه المستخلصات وبثمانية تراكيز 0، 2.5، 5، 10، 20، 30، 40 و 50 ملغم/مللتر. كما حـددت قـيم التركيز المثبط الأدنـى(Minimum Inhibitory Concentration (MIC والتركيـز القاتـل الأدنـى Minimum Bactericidal Concentration (MBC) بالإضافة إلى مقارنة تأثير المستخلصات مع تأثير المضادات الحيوية. أُستعمل التصميم التام العشوائية في تنفيذ التجربة و بثلاث مكررات.

أشارت نتائج الدراسة إلى أن مستخلص قلف و أوراق نبات الدودونيا بالكحول الأثيلي والداي أثيل أيثر كان فعالاً في تثبيط نمو الأحياء المجهرية. وأن مستخلص القلف كان أكثر فعالية ضد الخميـرة *C. albicans* من مستخلص الأوراق. وعلى العموم فأن النتائج أظهرت عدم وجود فروق معنوية بين التراكيز 30 و 40 و 50 ملغم/مللتر في تأثيرها في أغلب الأحياء المجهرية المدروسة. وأشارت النتائج إلى أن التركيز المثبط الأدنى (MIC) لمستخلص القلف كان 2.5 ملغم/مللتـر، ولمسـتخلص الأوراق تـراوح مـابين 2.5-10 ملغم/مللتر. أما التركيز القاتل الأدنى (MBC) فقد تراوح مابين 5-50 ملغم/مللتر اعتمادا على نوع المذيب وفترة جمع النماذج (قبل أو خلال التزهير) ونوع الأحياء المجهرية. وعند القياس مع المضادات الحيويـة المستعملة في الدراسة، أظهرت النتائج وجود تقارب كبير بين مستخلصات قلف و أوراق نبات الـدودونيا عند التراكيز 30 و 40 و 50 ملغم/مللتر وبين المضادات الحيوية في فعاليتهـا التثبيطيـة ضـد الأحيـاء المجهرية.

يمكن الاستنتاج عند مقارنة نتائج هذه الدراسة مع نتائج دراسات أخرى أجريت في مناطق مختلفة من العالم يتضح بأن نوع المذيب المستعمل، وطريقة الاستخلاص، وفترة جمع النماذج والتوزيع الجغرافي يؤثر فـي محتوى النبات من المكونات الكيميائية وفي فعاليتها ضد الأحياء المجهرية.

Introduction

Human beings depend on plants for various obvious needs including food, clothing, shelter, timber, fuel, medicines, exchange of O_2 and CO_2 [1]. It is estimated that there are 250000 to 500000 species of plants on the earth, a relatively small percentage (1-10%) of these are used as food by both human and animals [2]. According to the World Health Organization (WHO), medicinal plants would be the best source for obtaining a variety of drugs [3]. Medicinal plants have been tested for biological, antimicrobial and hypoglycemic activity [4]. They have also tested for antiulcerogenic, antihelminthes, hepato protective, and insecticidal activities [5].

Due to the widespread and often indiscriminate use of antimicrobial drugs, many microorganisms have acquired resistance to specific antibiotic treatments and these strains are particularly evident in the hospital environment [6]. This has created immense clinical problems in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects on host, which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immune suppression and allergic reactions [7].

Therefore, the search for new drugs from novel sources, such as plants, is necessary. Because of the side effects and the resistance that pathogenic microorganisms build against the antibiotics, much recent attention has been paid to extract biological active compounds from plant species that used in herbal medicine. In many parts of the world, medicinal plants are used for their antibacterial, antifungal and antiviral activities. These plant extracts were used as a source of medicinal agents to cure urinary tract infections, cervicitis, vaginitis, gastrointestinal disorders [8] and skin infections such as herpes simplex virus type [9].World Health Organization (WHO) advocated traditional medicine as safe remedies for ailments of both microbial and non microbial origins [10]. These have been a revival of interest in herbal medicines. This is due to increase an awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plants Kingdome. Plants are the basic source of knowledge of modern medicine [11].

Dodonaea viscosa Jacq., which is belongs to the family Sapindaceae, used as a traditional medicine in different countries. Stem or leaf infusions were used to treat sore throats, root infusion to treat colds and seeds (in combination with other plants) used to treat malaria. The leaves are used to treat itching, digestive system disorders, including indigestion, ulcers and diarrhea; and the powdered leaves were given to expel round worms [12]. The plant is also used as antibacterial [13, 14] and has insecticidal activity [15].

The aims of this study were to determine the role of ethanol and diethyl ether extracts of leaves and bark of *D. viscosa* before and during flowering for potential antimicrobial effect, against human pathogenic bacteria, yeast (*Candida albicans*), and plant pathogenic bacteria , and also , study the Minimal Inhibitory Concentrations (MIC), and Minimal Bactericidal Concentrations (MBC) of the plant extracts, and to test the susceptibility of pathogenic microorg-anisms for antibiotics, and compared it with the effect of the plant extracts.

Materials and methods:

Plant material: Plant samples (leaves and bark) were collected from gardens of Baghdad University during flowering in March and April /2008 and before flowering in October, November and December /2008. In laboratory, plant samples were washed with distilled water and dried at room temperature, and then homogenized to fine powder by using electric mill, and then stored in airtight bottles.

Preparation of plant extracts: Two polar solvents were used in this study:

- Ethanol (80%): high polar solvent was used for extracted leaves and bark during and before flowering.
- Diethyl ether: low polar solvent was used for extracted leaves and bark during and before flowering.

The extraction for both solvents was done by Soxhlet extraction [16]. The air-dried and powdered plant materials (20 g) were kept in the Soxhlet′s apparatus, and 250ml of solvent was added and extracted for 8 hours at 70˚C for ethanol and at 40˚C for diethyl ether. Extracts were then filtered and evaporated using rotary evaporator to dry extracts. The extracts were stored at 4ºC until used.

Preparation of concentrations: Stock solutions were prepared by mixing 2 g from the dried extract with 20 ml of ethanol (80%), and then it was sterilized with Millipore membrane filter (0.22 µm). Then the concentrations (2.5, 5, 10, 20, 30, 40, or 50) mg/ml were prepared by mixing known volume from the stock solution with ethanol (80%) using the following equation:

C1 V1=C2 V2

C1= Stock solution concentration

C2= final concentration

V1= Volume that obtained from stock solution

V2= final volume

In addition to control treatment (only ethanol 80%).

Microorganisms: The microorganisms used were *Escherichia coli* , *Pseudomonas aeruginosa* , *Staphylococcus aureus* , *Streptococcus agalactiae* and *Candida albicans* were obtained from laboratory of Biology Dept., College of Science, University of Baghdad. .The *Agrobacterium tumefaciens* was obtained from Horticulture Dept., College of Agriculture, University of Baghdad . And the *Erwinia carotovora* was obtained from Biotechnology Dept., College of Science, University of Baghdad.

Determination of the antibacterial activity of plant extracts:

The agar well diffusion method:

The agar well diffusion method was used for the determination of antibacterial activity of the plant extracts [17,18]. Every 100ml of cultured media were inoculated with 1ml of bacterial inoculum (containing $1.5X10^8$ cell/ml). After proper homogenization it was poured into Petri dishes. Thereafter, wells were made by using sterilized cork borer (10mm). Then the extracts were introduced into the wells, and ethanol 80% was used as control. The plates were incubated for 24 hours at 37ºC (27ºC for *A. tumefaciens* and *E. carotovora*). The experiment was performed three times and the activity of plant extracts was determined by measuring the diameter of inhibition zone around each well by millimeter.

The Minimum inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the plant extracts:

a- Seven different concentrations of plant extracts were prepared which were (2.5, 5, 10, 20, 30, 40 or 50) by using nutrient broth and sabouraud dextrose broth.

b- Aliquot of 0.1ml of bacterial inoculum (containing$1.5X10^8$ cell/ml) was added to all test tubes, mixed well and incubated for 24 hours at 37ºC (27 ºC for *A. tumefaciens* and *E. carotovora*).

c- Results were recorded according to the turbidity appearance and compared with the control tubes. (Two control tubes: first tube was containing broth and bacterial inoculum, the second tube was containing broth and plant extracts).The minimum inhibitory concentration (MIC) was defined as the lowest concentration that prevents visible turbidity appeared clearly to the naked eye in the cultured broth [19], in which 0.1ml from test tubes that showed no turbidity, and spread on the culture media plates, by sterilized cotton swap stick, then the plates were incubated for 24 hours at 37ºC (27ºC for *A. tumefaciens* and *E. carotovora*). The results were recorded depending on the presence of colonies at less numbers. The minimum bactericidal concentration (MBC) was determined as a concentration where 99.9% or more of the initial inoculums is killed [20] by taking 0.1ml from prepared tubes and spread on the culture media plates by sterilized cotton swap stick then the plates were incubated for 24 hours at 37ºC (27ºC for *A. tumefaciens* and *E .carotovora*). The results were recorded by existing or not existing of bacterial growth [21].

Preparation of Antibiotics solutions:

A- Phosphate Buffer Solution:

This solution was prepared according to [23].

B- Sodium hydroxide Solution(1 M):

This solution was prepared by dissolving 0.4 g of Sodium hydroxide in 10 ml of distilled water in volume flask.

Statistical analysis : The experiments were conducted and analyzed as factorial experiments with three replications using a completely Randomized Design, and standard Error were calculated. The mean values were compared by using Duncan's multiple range tests at probability of 5% ($P \leq 0.05$) [24].

Results and Discussion:

Effect of crude extracts of *D. viscosa* on the growth of some pathogenic microorganisms:

In this study, agar well diffusion method was used for the determination of antibacterial effect of the crude extracts of the leaves and the bark. The results were recorded after 24 hours by measuring the diameter of inhibition zones. Kela and Kujefi [25] reported that antibiotics are not the only antimicrobial agents.

No reference for the inhibitory effect of bark extracts was found in the literature, however in this study, bark extracts with ethanol (80%) demonstrated a degree of inhibition at all tested microorganisms (Table ١). This may due to that ethanolic extracts contain terpenes, phenols, flavonoids and saponins which have a great effect as antimicrobial agents and have a good antimicrobial effect [26]. Both before and during flowering extracts were active, however, no significant

Table ١: Antimicrobial effect of ethanol extracts of *D. viscosa* bark before and during flowering.

Extract	Con. mg/ml	Diameters of inhibition zones (mm) #						
		E. coli	*P. aeruginosa*	*S. aureus*	. *agalactiae*	*C. albicans*	*E. carotovora*	*A. tumefaciens*
Before flowering	0	0.00±0.00 c	0.00±0.00 d	0.00±0.00 d	0.00±0.00 e	0.00±0.00 e	0.00±0.00 d	0.00±0.00 e
	2.5	12.00±0.57b D	14.00±1.00 c CD	15.00±1.00c BC	13.00±1.00d CD	15.00±0.57d BC	17.00±0.57c AB	19.00±0.57d A
	5	13.00±0.57ab D	18.00±0.57b AB	16.33±0.44bc BC	15.00±0.57cd CD	18.00±1.00c AB	19.00±0.57b A	20.00±1.00cd A
	10	13.00±0.57ab D	18.50±0.57ab BC	16.50±1.00bc C	16.50±0.57bc C	19.00±0.57bc AB	19.00±0.57b AB	21.00±0.57bc A
	20	14.00±1.00ab D	19.00±0.57ab BC	17.00±1.00bc C	17.00±0.57bc C	20.00±0.57abc AB	20.00±0.57b AB	22.00±0.57ab A
	30	15.00±0.57a E	19.00±1.00ab CD	17.50±0.57ab D	18.50±0.57ab CD	20.50±0.57ab BC	22.00±0.57a AB	23.00±0.57a A
	40	15.00±0.57a E	19.50±0.57ab CD	18.00±0.57ab D	20.00±1.00a CD	21.00±1.00ab BC	22.00±0.57a AB	23.00±0.57a A
	50	15.00±1.00a D	20.50±0.57a BC	19.50±0.57a C	20.00±1.00a C	21.50±1.00a ABC	23.00±0.57a AB	23.50±0.57a A
during flowering	0	0.00±0.00c	0.00±0.00c	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00c	0.00±0.00d
	2.5	13.00±0.57b BC	15.00±1.00b AB	16.00±0.57d A	12.00±057d C	13.00±1.00d BC	17.50±0.57b A	17.00±1.00c A
	5	13.00±0.57b C	18.50±0.57a A	16.50±0.57cd B	15.00±1.00c BC	15.00±0.57cd BC	20.00±0.57a A	20.00±0.57b A
	10	14.00±1.00ab C	18.50±0.57a AB	17.00±0.57bcd B	16.50±0.57bc B	17.00±0.57bc B	20.00±0.57a A	20.00±0.57b A
	20	14.50±0.57ab D	19.00±0.57a ABC	18.00±0.57abc BC	17.00±0.57abc C	18.00±1.00ab BC	20.00±0.57a AB	21.00±0.57b A
	30	15.00±0.57ab D	20.00±1.00a AB	18.50±0.57ab BC	17.00±0.57abc CD	18.00±1.00ab BC	21.00±0.57a A	22.00±0.57ab A
	40	15.00±0.57ab E	20.00±0.57a BC	19.00±0.57a CD	17.50±0.57ab D	20.00±0.57a BC	21.00±0.57a AB	22.00±0.57ab A
	50	16.00±0.57a C	20.00±0.57a B	19.00±0.57a B	19.00±1.00a B	20.00±0.57a B	21.00±0.57a B	23.50±0.57a A

 # Inhibition zones including cork borer (10 mm) diameter.

• Identical small letters refer to not significant differences between concentrations at probability of 5% (P≤0.05) by Duncan's multiple range test (Duncan, 1955).

• Identical capital letters refer to not significant differences between bacteria at probability of 5% (P≤0.05) by Duncan's multiple range test (Duncan, 1955).

•The star * refers to that the two extracts were significantly differs at this concentration.

differences were recorded between these two extracts. *E. carotovora* and *A. tumefaciens* showed the great sensitivity to these extracts and demonstrated the largest inhibition zones 23.00 and 23.50 mm, respectively at the concentration of 50 mg/ml (Figures 1 and 2). It was also an evident that concentrations of 30, 40 or 50 mg/ml have the same effect and there are no significant differences were recorded between them.

Escherichia coli was the less susceptible to the bark extracts and showed a diameter of inhibition zone reached to 15.00 and 16.00 mm before and during flowering, respectively at a concentration of 50 mg/ml. This less sensitivity of this bacterium may due to the presence of plasmid conferring resistance [27]. These extracts have a pronounced effect on the growth of *C. albicans* and the diameter of inhibition zones reached to 21.50 and 20.00 mm before and during flowering, respectively at a concentration of 50 mg/ml. The results also showed that no significant differences between the concentrations of 20, 30, 40 or 50 mg/ml. These results supported the work that conducted by Mehmet[28] who found that tannins exhibit antimicrobial effect against phytopathogenic fungi and bacteria.

The results of the antimicrobial effect of the investigated extracts were shown in table ٢. Leaf extracts of *D. viscosa* during flowering with ethanol (80%) inhibited the growth of all microorganisms at studied concentrations, except *C. albicans, P. aeruginosa* and *E. coli* which showed resistance to this extract at a concentration of 2.5 mg/ml. Maximum antibacterial effect was shown against *A. tumefaciens* followed by *E. carotovora, S. agalactiae* and *S. aureus* and the diameter of inhibition zones were 25.00, 23.00, 22.00 and 22.00 mm at 50 mg/ml respectively. This extract showed a clear effect against gram positive bacteria; *S. aureus* and *S. agalactiae* (Figure 2), while *E. coli* and *P. aeruginosa* were the less susceptible bacteria to this extract. Results differ from those of Getie *et al.* [29] who demonstrated that leaf extracts at concentrations of 25, 50 or 100 mg/ml showed weak antibacterial effect against *S. aureus.* In the present study, leaf extract were more effect against *S. aureus* and the inhibitory effect beginning with a concentration of 2.5 mg/ml. The antimicrobial effect of leaves extracts against *E. coli* and *P. aeruginosa* is in agreement with the results of [30]. These results supported the work that has been summarized by Rojas *et al.,* 1992; Mothana *et al.,* [13, 31] who reported that the methanolic extracts of *D. viscosa* have antimicrobial effects. Others described the isolation of diterpenoid and flavonoid derivatives from *D. viscosa* [32].

Candida. albicans showed less response to this extract started with the concentration of 5 mg/ml, and the diameter of inhibition zones reached to 15.00 and 14.00 mm before and during flowering at concentration of 50 mg/ml. Although the

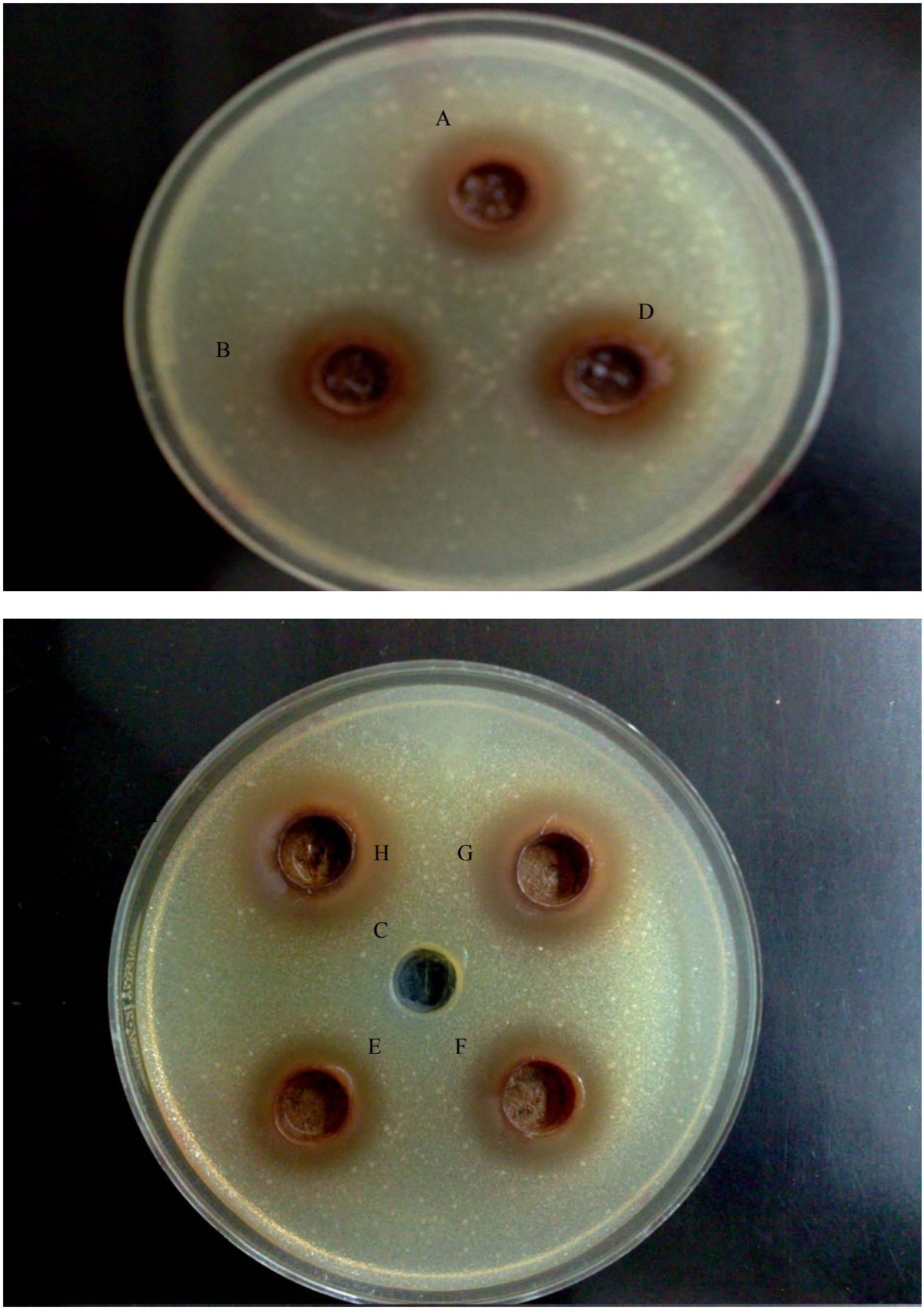

Figure 1. Effect of the ethanol extract of the bark before flowering on the growth of *A. tumefaciens*. A:2.5mg/ml, B:5mg/ml, C:control, D:10mg/ml, E:20mg/ml, F:30mg/ml, G:40mg/ml, H:50mg/ml

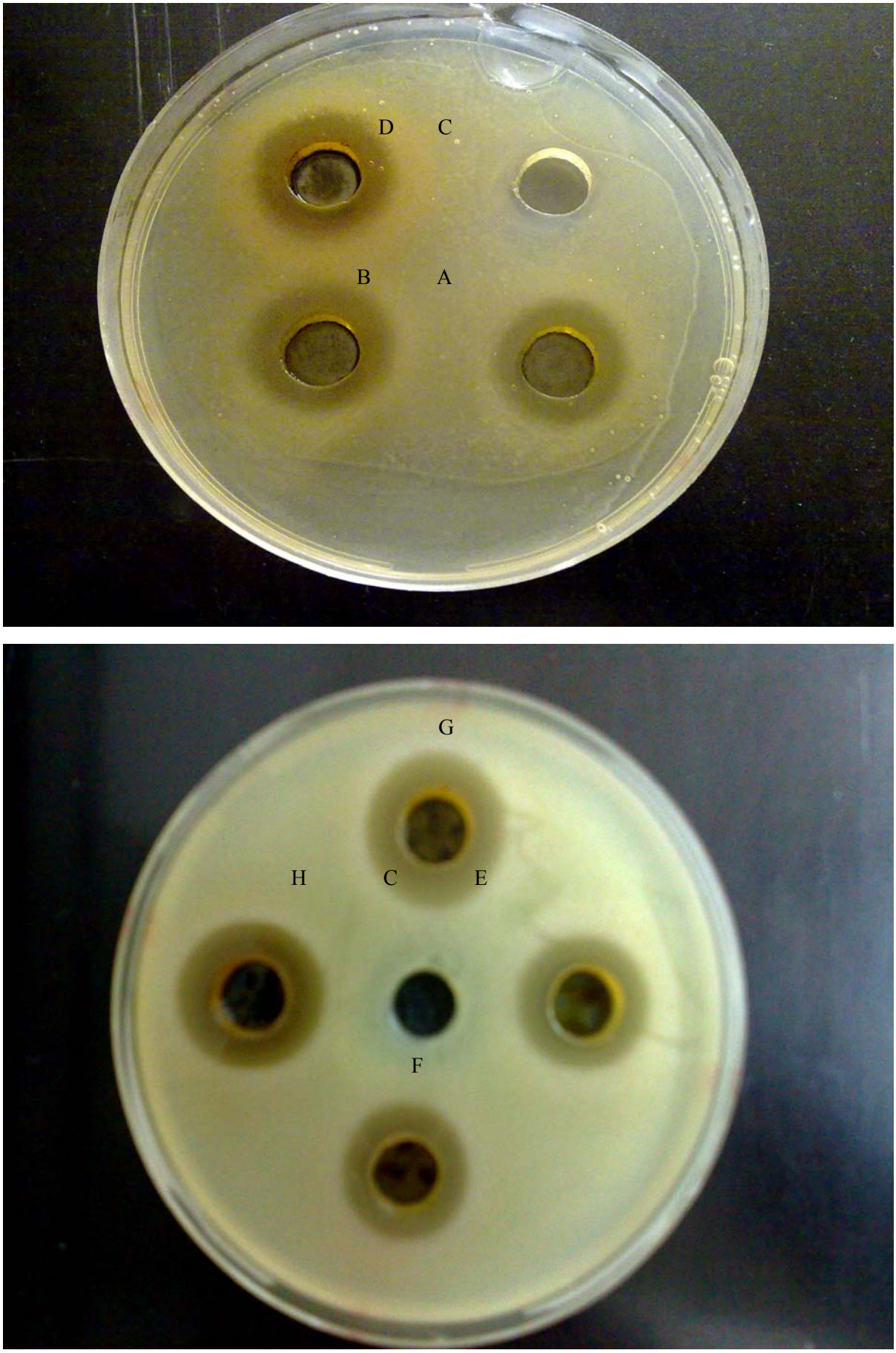

Figure 2: Effect of ethanol extract of leaves during flowering on the growth of *S.aureus* A:2.5mg/ml, B:5mg/ml, C:control, D:10mg/ml, E:20mg/ml, F:30mg/ml, G:40mg/ml, H:50mg/ml.

Table ٢: Antimicrobial effect of ethanol and diethyl ether extracts of *D. viscosa* leaves during flowering.

Extract	Con. mg/ml	Diameters of inhibition zones (mm) #						
		E. coli	*P. aeruginosa*	*S. aureus*	*S. agalactiae*	*C. albicans*	*E. carotovora*	*A. tumefaciens*
Ethanol	0	0.00±0.00d	0.00±0.00d	0.00±0.00e	0.00±0.00d	0.00±0.00c	0.00±0.00d	0.00±0.00f
	2.5	0.00±0.00d C	0.00±0.00d C	15.00±0.57d B	17.00±0.57c A*	0.00±0.00c C	18.00±0.57c A	15.00±0.57e B
	5	12.00±1.00c B	13.00±0.57c B	17.00±0.57cd A*	17.00±0.57c A*	12.00±1.00b B	18.00±0.57c A	18.00±1.00d A*
	10	13.00±0.57bc C	13.50±0.57bc C	18.00±1.00bc AB	17.00±0.57c B	12.50±0.57ab C	20.00±1.00bc A	20.00±0.57c A*
	20	14.50±0.57ab C	14.00±1.00bc C	20.00±0.57ab AB*	19.00±0.57b B	13.00±0.57ab C	22.00±0.57ab A	21.50±0.57bc A*
	30	15.00±0.57a C	15.00±0.57ab C	20.00±0.57ab B*	20.00±0.57b B	13.00±0.57ab D	22.00±0.57ab A	23.00±0.57b A*
	40	15.00±0.57a C	16.00±0.57a C	21.00±1.00a AB	20.00±0.57b B	14.00±0.57a C	23.00±1.00a A	23.00±0.57b A*
	50	15.50±0.57a CD	16.00±0.57a C	22.00±0.57a B	22.00±0.57a B	14.00±0.57a D	23.00±0.57a B	25.00±0.57a A*
Diethyl ether	0	0.00±0.00c	0.00±0.00d	0.00±0.00e	0.00±0.00e	0.00±0.00c	0.00±0.00d	0.00±0.00c
	2.5	11.00±1.00b B*	0.00±0.00d C	12.00±1.00d B	13.00±0.57d B	12.00±0.57b B*	18.00±1.00c A	13.00±0.57b B
	5	12.00±0.57b B	12.00±1.00c B	13.50±0.57cd B	14.00±0.57d B	12.50±0.57b B	21.00±0.57b A*	13.00±0.57b B
	10	12.00±0.57b C	13.00±0.57c BC	15.00±1.00bc B	21.00±1.00c A*	13.00±0.57b BC	21.00±0.57b A	13.50±0.57ab BC
	20	13.00±0.57b D	15.00±0.57b BC	16.00±0.57b B	23.00±0.57b A*	13.00±0.57b D	23.00±0.57ab A	14.00±0.57ab CD
	30	13.00±0.57b C	16.00±0.57b B	17.00±0.57b B	24.00±0.57ab A*	13.50±0.57ab C	23.00±0.57ab A	14.00±0.57ab C
	40	15.00±0.57a D	19.00±0.57a C*	22.00±0.57a B	25.00±0.57a A*	15.00±0.57a D	23.00±1.00ab B	14.50±0.57ab D
	50	15.00±0.57a C	20.00±0.57a B*	24.00±0.57a A	25.00±0.57a A*	15.00±0.57a C	25.00±0.57a A	15.00±0. 57a C

 # Inhibition zones including cork borer (10 mm) diameter.

• Identical small letters refer to not significant differences between concentrations at probability of 5% (P≤0.05) by Duncan's multiple range test (Duncan, 1955).

• Identical capital letters refer to not significant differences between bacteria at probability of 5% (P≤0.05) by Duncan's multiple range test (Duncan, 1955).

•The star * refers to that the two extracts were significantly differs at this concentration.

Concentration of 50 mg/ml was not significantly different from the concentration of 40 mg/ml against *E. carotovora, C. albicans, S. aureus* and *P. aeruginosa.*

The diethyl ether extract showed less effect against *A. tumefaciens* when compared with the effect of ethanolic extract and the great effect was shown at the concentration of 50 mg/ml with a diameter of inhibition zone reached to 15.00 mm. The greatest effect of the diethyl ether extract was against *E. carotovora, S. agalactiae, S. aureus* and *P. aeruginosa* achieving diameters of inhibition zones of 25.00, 25.00, 24.00 and 20.00 mm, respectively at the concentration of 50 mg/ml. Diethyl ether extract at the concentrations of 10, 20, 30, 40 or 50 mg/ml exhibited a great effect on *S. agalactiae* than ethanolic extract. Diethyl ether extract had no effect against *P. aeruginosa* at the concentration of 2.5 mg/ml, whereas this extract was more effective at the concentrations of 40 and 50 mg/ml and the diameter of inhibition zones were 19.00 and 20.00 mm, respectively. While *C. albicans* and *E. coli* were the less sensitive to this extract. The inhibition zone was 15.00 mm for both microbes. Although the concentration of 50 mg/ml was not significantly different from the concentration of 40 mg/ml.

The antibacterial effect of *D. viscosa* leaf extract (before flowering) of both solvents (ethanol and diethyl ether) against tested microorganisms were significant (Table ٣). *A. tumefaciens* and *E. carotovora* were the most sensitive bacteria to the ethanolic extract followed by *S. agalactia* then *S. aureus,* and the diameter of inhibition zones were 26.83, 24.00, 22.00 or 21.50 mm, respectively at a concentration of 50 mg/ml. Cowan [2] suggested that polyphenols act on the microbes by disrupting their membranes, depriving the substrate or inactivating the enzymes. While *P. aeruginosa, C. albicans* and *E. coli* were the less sensitive to this extract, the diameter of inhibition zones were 15.00, 16.00 and 13.00 mm at the concentration of 50 mg/ml, respectively. The concentrations of 5, 10, 20, 30, 40 or 50 mg/ml gave the same effect on *E. coli*. Thring *et al.* [14] working on *S. aureus, P. aeruginosa* and *C. albicans,* observed that *D. viscosa* extraction by ethyl acetate has no effect on these microorganisms.

Agrobacterium. tumefaciens showed less susceptibility to the diethyl ether extract of the leaves before flowering, while this extract has a great effect than ethanolic extract on *S. aureus, P. aeruginosa* and *E. coli* at the concentrations of 40 or 50 mg/ml with inhibition zones of 25.00, 17.33 and 16.00 mm at the concentration of 50 mg/ml, respectively. *E. carotovora* and *S. aureus* were the most susceptible among tested bacteria to the diethyl ether extract and the diameters reached to 25.00 mm followed by *S. agalactiae* and the diameter of inhibition zone was 20.33 mm. In general, the results showed no significant differences between the concentrations of 30, 40 and 50 mg/ml in their effects on the growth of most microorganisms.

Table ٣: Antimicrobial effect of ethanol and diethyl ether extracts of *D. viscosa* leaves before flowering.

Extract	Con. mg/ml	Diameters of inhibition zones (mm) #						
		E. coli	*P. aeruginosa*	*S. aureus*	*S. agalactiae*	*C. albicans*	*E. carotovora*	*A. tumefaciens*
Ethanol	0	0.00±0.00b	0.00±0.00c	0.00±0.00d	0.00±0.00e	0.00±0.00c	0.00±0.00d	0.00±0.00f
	2.5	0.00±0.00b D	13.00±0.57b C*	17.00±1.00c AB*	16.00±0.57d B	0.00±0.00c D	19.00±0.57c A	17.00±1.00e AB*
	5	11.00±0.57a E	14.00±0.57ab D	17.50±0.57c BC*	16.00±0.57d C	11.50±0.57b E	19.00±1.00c AB	20.00±0.57d A*
	10	12.00±0.57a	14.00±1.00ab	18.00±0.57bc	18.00±0.57c	12.00±0.57b	21.00±0.57bc	21.00±0.57cd

		C	C	B*	B	C	A	A*
	20	12.00±1.00a B	14.00±0.57ab B	20.00±1.00ab A	20.00±1.00b A	12.50±0.57b B	22.00±0.57ab A	22.50±0.57bc A*
	30	12.00±1.00a D	15.00±1.00ab C	20.00±0.57ab B	20.50±0.57ab AB	13.00±1.00b CD	23.00±0.57ab A	23.00±1.00bc A*
	40	13.00±0.57a D	16.00±0.57a C	20.00±0.57ab B	22.00±0.57a A	15.00±0.57a C	24.00±1.00a A	24.00±0.57b A*
	50	13.00±0.57a E	16.00±0.57a D	21.50±0.57a C	22.00±0.57a C	15.00±0.57a D	24.00±0.57a B	26.83±0.92a A*
Diethyl ether	0	0.00±0.00c	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00c	0.00±0.00e	0.00±0.00b
	2.5	12.00±0.57b C*	0.00±0.00e D	0.00±0.00e D	15.00±0.57d B	11.00±0.57b C*	18.00±1.00d A	0.00±0.00b D
	5	12.00±1.15b B	11.00±1.00d B	12.00±0.57d B	18.00±0.57c A	12.50±0.57ab B	20.00±1.00cd A	0.00±0.00b C
	10	14.00±0.57ab C	13.00±0.57c C	14.00±1.00c C	18.00±0.57c B	12.50±0.57ab C	22.00±0.57bc A	12.00±1.00a C
	20	14.00±0.57ab CD	15.00±0.57b C	17.00±1.00b B	18.50±0.57bc B	13.00±0.57a CD	23.00±0.57ab A	12.50±0.57a D
	30	15.00±0.57a CD	16.00±1.00ab C	24.00±0.57a A*	20.00±0.57ab B	13.50±0.57a D	23.00±1.00ab A	14.00±0.57a CD
	40	16.00±0.57a CD*	17.00±0.57a C	25.00±0.57a A*	20.00±0.57ab B	14.00±0.57a D	25.00±0.57a A	14.00±1.00a D
	50	16.00±0.57a C*	17.33±0.44a C	25.00±0.57a A*	20.33±0.44a B	14.00±0.57a D	25.00±1.00a A	14.00±0.57a D

 # Inhibition zones including cork borer (10 mm) diameter.
• Identical small letters refer to not significant differences between concentrations at probability of 5% (P≤0.05) by Duncan's multiple range test (Duncan, 1955).
• Identical capital letters refer to not significant differences between bacteria at probability of 5% (P≤0.05) by Duncan's multiple range test (Duncan, 1955).
•The star * refers to that the two extracts were significantly differs at this concentration.

Sensitivity test to antibiotics: This test has shown a development of resistance against antibiotics. The results from the bioassays are tabulated in table ٤, *E. coli* has shown a resistance against Nalidixic acid, while it was susceptible to other antibiotics; Ampicillin, Amoxycillin and Chloramphenicol. *S. aureus* showed a resistance against Erythromycin. Lincomycin had no effect on *S. agalactiae*. All tested bacteria were susceptible to Ampicillin and Amoxycillin, except *A. tumefaciens* which showed less susceptibility to Ampicillin and Amoxycillin with a diameter of inhibition zone reached to 12.00 and 15.00 mm, respectively. *Escherichia. coli, P. aeruginosa, E. carotovara* and *A. tumefaciens* were susceptible to chloramphenicol. Similar trends were observed with Nalidixic acid except *E. coli* which exhibited resistance against this antibiotic. *E. carotovora* was the most sensitive to the antibiotics that used in the study. The resistance against some antibiotics may occur due to the widespread and often indiscriminate used of commercial antimicrobial drugs commonly used for the treatment of infectious diseases [6, 33].

Determination of MIC of the plant extracts:

Results of MIC confirm that this bioassay is more sensitive since more effect was recorded (Table ٥). The antimicrobial screening showed that ethanol extract of the bark before and during flowering was effect against all microorganisms at a concentration of 2.5 mg/ml. Similar trends of ethanol extract of the leaves before and during flowering were observed with *S. aureus, S. agalactiae* and *E. carotovora.* Whereas it was effect against *E.*

coli and *C. albicans* at the concentration of 5 mg/ml.

The diethyl ether extracts of leaves before and during flowering showed a degree of effect at the three concentrations of 2.5, 5 or 10 mg/ml on the microorganisms. The lowest MIC value was 2.5 mg/ml which showed effect against all tested microbes during flowering, except *P. aeruginosa* which was affected at the concentration of 5 mg/ml, while before flowering reached to 10 mg/ml against *A. tumefaciens.* These results differ with some researches [34, 35] who reported high resistance of *C. albicans* to plant extracts and hence recommended antifungal testing at concentrations as high as 100 mg/ml.

This may suggest that the high concentration of the extracts administered to patients may be able to cure the disease.

Determination of MBC of the plant extracts:

Table ٦. Shows the killing effects of *D. viscosa* extracts on tested

Table ٤: Antibiotics sensitivity of some microorganisms.

Antibiotic	Diameter of Inhibition zone (mm) #						
	E. coli	*P. aeruginosa*	*S. agalactiae*	*S. aureus*	*E. carotovora*	*A. tumefaciens*	*C. albicans*
Erythromycin			**14.00±0.57 A c**	**0.00±0.00 B d**			
Ampicillin	**32.00±0.57 B a**	**18.00±0.57 C b**	**18.00±0.57 C b**	**16.00±0.57 D b**	**35.00±0.57 A b**	**12.00±0.57 E d**	
Amoxycillin	**32.00±1.00 B a**	**22.00±0.57 D a**	**25.00±0.57 Ca**	**19.00±0.57 E a**	**38.00±0.57 A a**	**15.00±0.57 E c**	
Lincomycin			**0.00±0.00 B d**	**13.00±0.57 A c**			
Chloramphenicol	**34.00±0.57 A a**	**18.50±0.57 D b**			**26.00±0.57 C c**	**30.00±0.57 B a**	
Nalidixic acid	**0.00±0.00 D b**	**15.00±0.57 C c**			**27.00±0.57 A c**	**20.00±1.00 B b**	
Nystatin							**17.00**

 # Inhibition zones including cork borer diameter (10mm).

• Identical small letters refer to not significant differences between antibiotics at probability of 5% (P≤0.05) by Duncan's multiple range test (Duncan, 1955).

Table ٥: Inhibition effects of ethanol and diethyl ether extracts of leaves and ethanolic extracts of *D. viscosa* bark before and during flowering on tested microorganisms.

Microorganisms	MIC mg/ml					
	Leaves before flowering		Leaves during flowering		Bark before flowering	Bark during flowering
	Ethanol 80%	Diethyl ether	Ethanol 80%	Diethyl ether	Ethanol 80%	Ethanol 80%
E. coli	5	2.5	5	2.5	2.5	2.5
P. aeruginosa	2.5	5	5	5	2.5	2.5
S. aureus	2.5	5	2.5	2.5	2.5	2.5
S. agalactiae	2.5	2.5	2.5	2.5	2.5	2.5
C. albicans	5	2.5	5	2.5	2.5	2.5
E. carotovora	2.5	2.5	2.5	2.5	2.5	2.5
A. tumefaciens	2.5	10	2.5	2.5	2.5	2.5

Table ٦. Killing effects of ethanol and diethyl ether extracts of leaves and ethanolic extracts of *D. viscosa* bark before and during flowering on tested microorganisms.

Microorganisms	MBC mg/ml					
	Leaves before flowering		Leaves during flowering		Bark before flowering	Bark during flowering
	Ethanol 80%	Diethyl ether	Ethanol 80%	Diethyl ether	Ethanol 80%	Ethanol 80%
E. coli	5	10	20	40	5	10
P. aeruginosa	5	30	30	40	10	5
S. aureus	20	30	20	40	30	20
S. agalactiae	40	30	20	30	30	20
C. albicans	40	5	10	30	20	20
E. carotovora	20	20	20	20	30	5
A. tumefaciens	50	10	20	10	20	30

microorganisms. The ethanol extracts of the bark before and during flowering showed a degree of killing effects. The lowest MBC value was at a concentration of 5 mg/ml with *E. coli, P. aeruginosa* and *E. carotovora.* While the highest value was at a concentration of 30 mg/ml. The ethanol and diethyl ether extracts of leaves before and during flowering exhibited a degree of killing effects ranged from 5-50 mg/ml. The susceptibility of microbes depends on the type of solvent and the period of collection (before or during flowering). For example, *E. coli* was killed at a concentration of 5 mg/ml by ethanolic extract before flowering, whereas it was killed at a concentration of 40 mg/ml by diethyl ether extract during flowering. *A. tumefaciens* was killed at the highest concentration 50 mg/ml by ethanol extract before flowering, while it was killed at concentration of 10 mg/ml by diethyl ether extract before and during flowering. Other microorganisms were killed between these ranges. It is concluded from this study that ethanolic extract (of bark and leaves) and diethyl ether extract of the leaves have clear effect on the tested microorganisms, and these results as compared with other studies [28,29,30,37], indicated that the type of solvents, method of extraction, period of collection and geographical distribution of *D. viscosa* significantly affects the rate of the chemical components, so some antimicrobial effects differ from other reported in the literature.

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