Egypt. J. Bot., **Vol. 57**, No, **3**. pp. 595 - 604 (2017)

Antibacterial Effect of Phytochemial Extracts from *Ziziphus-spina christi* against Some Pathogenic Bacteria

H.A. Temerk[#], W.M. Salem, W.F. Sayed and F.S. Hassan[*]

Department of Botany, Faculty of Science, South Valley University and [*]Department of Medical Analysis, El- Wafaa Clinic, General Authority for Health Insurance, Qena, Egypt.

THE ANTIBACTERIAL potential of *Ziziphus- spina christi* as methanol and ethanol extracts, of bark, fruit, roots, seeds and leaves, were evaluated against seven pathogenic bacterial strains using agar well diffusion technique: The used strains were *Pseudomonas aeruginosa* (ATCC 278223)*, Enterobacter cloaca*e (ATCC 13047)*, Enterobacter aerogenes* (ATCC 13084), *Escherichia coli* (ATCC 25922)*, Klebsiella pneumonia*e (ATCC 13888), *Enterococcus faecali*s (ATCC 29212) and *Methicillin-resistant Staphylococcus aureus (*MRSA, ATCC 43300). The antibiotic erythromycin was used as positive control. The presence of phytochemical compounds in the extracts was determined qualitatively, the functional bioactive groups were characterized by FTIR and the presence of bioactive elements was characterized by XRF. Antibacterial activity against the used bacterial strains was assessed by determining the minimal inhibitory and bactericidal concentrations (MIC and MBC) assays. All the used Gram negative and positive bacteria were sensitive to various plant extracts. Bark extract was the most active against all strains except for *Enterobacter aerogenes*. Ethanol bark extract showed great activity against *Enterococcus faecali*s with 16.2 mm clearing zone and MIC, MBC of 15 and 20 mg ml^{-1}, respectively. Methanol bark extract was also effective against *Klebsiella pneumonia*e with a clearing zone of 16.2 mm and 20 mg ml^{-1} for both MIC, MBC. Leaves extracts showed high antibacterial activity against all strains except *Escherichia coli*. Ethanol fruit extract also exhibited high activity against *Pseudomonas aeruginosa* with 20 mm clearing zone and 2.5, 10 mg ml^{-1} for both MIC and MBC. Finally, ethanol roots showed the largest clearing zone against *Enterococcus faecali*s (21mm) with 10 and 15 mg ml^{-1} for MIC and MBC, respectively.

Keywords: Antibacterial, FTIR, Pathogenic bacteria, Phytochemical constituents, XRF, *Ziziphus spina*.

Introduction

Some phytochemicals-plant including flavonoids, tannins, lipids, terpenes, alkaloids, steroids and carbohydrates were extracted from *Ziziphus-spina christi* (Shahat et al., 2001). These plant extracts and fractions of leaves, fruits and seeds has showed antiviral, antifungal and antibacterial activities and were used in the Egyptian folk medicine for the treatment of several diseases including gastrointestinal tract ailments, diabetes and diarrhea (Shahat et al., 2001)**.** Current antimicrobial therapy for the infectious diseases has certain limitations due to toxicity, side effects and multiple resistance of microorganisms. *Enterobacter* is usually a commensal bacterium, and is a common opportunistic pathogen responsible for urinary and respiratory tract infections and bacteremia (Talon et al., 2004). *Escherichia coli* is commonly found in the lower intestinal tract of healthy humans and animals but there are many types of *E. coli*, a few of which are pathogenic by a variety of infective and toxin-producing mechanisms (EFSA, 2011)**.** *S. aureus* causes human infections and gastrointestinal illness such as nausea, emesis, abdominal cramps and diarrhea (Scherrer et al., 2004). *K. pneumoniae* is an opportunistic pathogen that causes hospital- acquired urinary tract infections, pneumonia, septicemias and soft tissue infections, showing co-resistance to quinolones and aminoglycoside antibiotics (Jiang et al., 2008). Currently, there is a continuous search for new drugs with reduced levels of toxicity and side effects (AL-Haj et al., 2010 and Ali et al., 2001). This study was conducted to evaluate the effect of some *Ziziphus spina christi* extracts, as natural sources of antibacterial activity, on some pathogenic bacterial strains.

[#]Corresponding author email: temerk.hanan@gmail.com

DOI: 10.21608/ejbo.2017.665.1035

Materials and Methods

Bacterial strains and cultural conditions

Seven bacterial strains were provided by Luxor International Hospital at Luxor, Egypt. Including five Gram negative strains were *Pseudomonas aeruginosa* (ATCC 278223), *Enterobacter cloacae* (ATCC 13047), *Enterobacter aerogenes* (ATCC 13084), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13888), and two Gram positive strains were *Enterococcus faecalis* (ATCC 29212) and *Methicillin*-resistant *Staphylococcus aureus* (MRSA, ATCC 43300). All strains were streaked on Mueller- Hinton medium (Mueller & Hinton, 1941) ,a pure colony was transferred into agar slant and subculture on Mueller- Hinton medium broth and incubated at 37ºC for 24 h before carrying out the test.

Collection of plant material and preparation of extracts

Different healthy parts of *Ziziphus* plant were collected from Qena City, Egypt including bark, leaves, fruits, seeds and roots. Extracts were prepared according to Mann et al. (2008) as follows: Samples were washed under tap water followed by distilled water and air- dried at room temperature. Dried samples were ground into coarse powder using a blinder (Fresh-DL 717, Egypt) and packed in clean and dry containers for further use. Ten grams of each sample were dissolved in 100 ml of ethanol and methanol. The solutions were shaken for 72 h at room temperature using orbital shaker (Stuart scientific, United kingdom) and then filtered by using Whatman™ no. 1 filter paper. The remaining solvent traces were evaporated by leaving the filtrate at room temperature until completely dry.

Preliminary phytochemical screening of Ziziphus extracts

Phytochemical screening of the extracts was carried out according to the method described by Trease & Evans (1989) for the detection of active components as follows:

1- Alkaloids: 1 ml of 1% Hcl was added to 3 ml of the extract in a test tube. The mixture was then heated for 20 min, cooled and filtered, about 2 drops of Mayer's reagent were added to 1 ml of the extract. A creamy precipitate was an indication of the presence of alkaloids.

2- Tannins: 1 ml of freshly prepared 10% KOH was added to 1 ml of the extract. A dirty white precipitate showed the presence of tannins.

3- Glycosides

a) Fehling's method: 10 ml of 50% H_2SO_4 were added to 1ml of the extract and the mixture heated in boiling water for 15 min. then, 10 ml of Fehling's solution were then added and the mixture was boiled. Formation of a brick red precipitate was indicated the presence of reducing sugars.

b) Molish's method: Two ml of the prepared extract were mixed with 0.2 ml of alcoholic solution of α-naphthol in addition to 2 ml of 10% sulphuric acid, a bluish violet zone is formed indicating the presence of carbohydrates and/ or glycosides.

4- Saponins

a) Frothing test: 2 ml of the extract were vigorously shaken in a test tube for 2 min. A layer of foam was observed indicating the presence of saponins.

b) Emulsion test: 5 drops of olive oil were added to 3 ml of the extract in a test tube and vigorously shaken. Absence of stable emulsion formation indicated the absence of saponins.

5- Flavonoids: 1 ml of 10% NaOH was added to 3 ml of the extract. Yellow coloration is indicative for the presence of flavonoids.

6- Steroids: 5 drops of concentrated H_2SO_4 were added to 1 ml of the extract in a test tube. Appearance of red colour indicates for the presence of steroids.

7- Phlobatannins: 1ml of the extract was added to 1% HCl, a positive result indicated by formation of red precipitate.

8- Triterpenes: 1ml of the extract was added to 5 drops of acetic anhydride and a drop of concentrated H_2SO_4. The mixture was then steamed for 1 h and neutralized with NaOH followed by addition of chloroform. Development of blue-green color indicates the presence of triterpenes.

Fourier Transform Infrared spectrometer (FTIR)

The presence of bioactive functional groups in different parts of *Z. spina- christi* (bark, fruit, root, leaves and seeds) was determined by fourier transform infrared spectrophotometer (FTIR) in the plant powder. Analysis was carried out using a Magna-FTIR 560 (USA) instrument at a resolution of 2 cm^{-1} range from 4000 to 400 cm^{-1} in KBr pellet using diffuse reflectance

mode operated by Nicolet Omnic Software as instructed by the manufacturers.

X- Ray fluorescence analysis (XRF)

The presence of bioactive elements in the powder of different plant parts was also determined by Energy Dispersive X- ray Fluorescence system (EDXRF). Analysis was carried out using a JEOL JSX 3222 Element analyzer (JEOL, Japan).

Determination of antibacterial activity test of plant extracts

The antibacterial activity of each extract was tested against the bacterial strains by using agar-well diffusion method (Okeke et al., 2001), The dried extracts (50 mg each) were dissolved in 1 ml of 50% dimethyl sulphoxide (DMSO). Bacterial strains were inoculated into Mueller- Hinton broth and incubated in a shaking incubator at 150 rpm and 37ºC for 18 h. The bacterial suspension were spread on the surface of Mueller- Hinton agar by using a sterile cotton-swab, then wells were made in the agar plates (four well with"6 mm") for each plate with a sterile cork borer, The wells were then filled with 0.1 ml of each extract using a sterile micropipette and incubated at 37ºC for 24 h , A blank-well containing 50% DMSO was used as negative control, also 50 mg of the antibiotic erythromycin (powder were separately dissolved in 1 ml of sterile distilled water) which used as a positive control. The diameter of clearing zones around the well was measured in millimeters and compared to the tested-antibiotic.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Minimum inhibitory concentration (MIC) was determined by using the tube dilution technique according to Collins et al. (1995), varying concentration of the extracts were prepared (200, 100, 50, 25 mg/ml) and 1 ml introduced into 8 ml of Mueller- Hinton broth in test tube, 1 ml of 18 h of the bacterial culture diluted to 1×10^8 was added and incubated for 24 h at 37ºC, by observation the turbidity for each strain, the lowest concentration of the each plant extract that inhibit bacterial growth in the broth culture for each strain is consider as minimum inhibitory concentration (MIC), then the all test tubes were plated out onto Muller-Hinton agar plates. The plates were then incubated at 37°C for 24 h. The number of bacterial colonies developed on each agar plates was counted, and the lowest concentration of extracts that exhibited complete bactericidal effect considered as minimum bactericidal concentration (MBC).

Results

Phytochemical screening of plant extracts

The presence or absence of some phytochemical groups of compounds in plant ethanol and methanol extracts is presented in Table 1. All extracts had flavonoids, steroids, tannins and glycosides. Phlobatannins were present only in bark extracts. Other phytocomponents varied between different plant parts.

Determination of functional groups by FTIR

The recorded bands of the FTIR spectrum of the *Ziziphus* parts and functional groups are shown in Table 2.

X-Ray fluorescence analysis (XRF) for bioactive elements in Z. spina- christi

As shwon in Fig. 1, The presence of bioactive elements in the powder of *Z. spina- christi* parts was determined by using X- ray fluorescence analysis. All plant parts contained calcium, potassium and ferrous iones.The higher percentages of elements were found in different plant parts as follows: potassium in fruit (71.38 %), calcium in bark (94.23) and ferrous in roots (5.08 %). Other minerals were found such as titanium in roots (1.06 %), silicon and sulfur in seeds (0.92 and 1.98 %, respectively), and copper in leaves (0.641 %).

Antibacterial activity of plant extracts

Seven strains of pathogenic bacteria were tested against *Z. spina - christi* plant extracts, five strains were Gram negative rods and two strains were Gram positive cocci (Table 3), The strains of *Pseudomonas aeruginosa and Klebsiella pneumonia*e were highly sensitive to most extracts, showing MIC varying between (2.5 mg-20 mg/ml), for MBC range between (10 mg-20 mg /ml). Among Gram negative rods, *P. aeruginosa* was more sensitive to ethanolic extracts especially fruit extract with an inhibition zone of 20 mm and MIC of 2.5 mg ml^{-1}. The other ethanolic extracts showed antibacterial effects with inhibition zones between 10 mm to 15 mm. *K. pneumonia*e growth was inhibited by all *Ziziphus* extracts with the highest effect recorded for methanol bark extract (inhibition zone = 16.2 mm). *Enterococcus faecali*s was more sensitive to *Ziziphus* extracts than MRSA especially to methanol fruit extract (inhibition zone= 16.2 mm). MRSA and the other Gram negative rods (*Enterobacter cloaca*e, *Enterobacter aerogenes* and *Escherichia coli*) were moderately sensitive to most *Ziziphus* extracts.

TABLE 1. Qualitative phytochemical analysis of *Ziziphus spinac - christi* ethanol and methanol extracts.

Extract of	Solvent	Alkaloids	Tannins	Saponins	Flavonoids	Steroids	Phloba-tanins	Triterpenes	Glycosides	
									Fehling's test	molish's test
Bark extracts	Ethanol	-	+	+	+	+	+	-	-	+
	Methanol	+	+	+	+	+	+	-	-	+
Fruit extracts	Ethanol	+	+	-	+	+	-	+	+	+
	Methanol	+	+	-	+	+	-	+	+	+
Roots extracts	Ethanol	+	+	+	+	+	-	-	-	+
	Methanol	+	+	+	+	+	-	-	-	+
Seeds extracts	Ethanol	-	+	-	+	+	-	+	+	+
	Methanol	+	+	-	+	+	-	+	+	+
Leaves extracts	Ethanol	-	+	+	+	+	-	+	+	+
	Methanol	+	+	+	+	+	-	+	+	+

(+) present, (-) absent

TABLE 2. FTIR analysis for bioactive functional groups of *Ziziphus* plant parts.

Plant Part	Wavelength of peaks	Bioactive components
Bark	3421 cm-1	O–H bonds in water molecules, or to OH groups presented in cellulose, hemicelluloses and lignin
	1617 cm-1	Stretching vibrations of (C=N) Amide II of proteins
	1108 cm-1	The stretching vibrations of C-O and C-C, C-O-H and C-O-C deformation of carbohydrates
Fruits	3388 cm-1	N-H bonds of proteins or O–H bonds in water molecules, or to OH groups presented in cellulose, hemicelluloses and lignin
	2932 cm-1	Stretching of the aliphatic C–H bonds in –CH3 and -CH2 groups in lipids, cellulose, hemicelluloses and lignin.
	1618 cm-1	Related to the C=O stretching vibrations of the bonds in the aldehyde groups of hemicelluloses
	1618 cm-1	C=N Amide I of proteins stretching
	1068, 909 and 869 cm-1	C-H rocking of -CH2 bending.
Roots	3399 cm-1	Stretching vibrations of N-H bonds of proteins or O–H bonds in water molecules, or to OH groups presented in cellulose, hemicelluloses and lignin
	2928 cm-1	Aliphatic C-H -CH2- and -CH3 as from of lipids
	1618 cm-1	C=N Amide I vibration
	1054 cm-1	C-H rocking of -CH2
Seeds	3398 cm-1	N-H stretching of proteins, O-H stretching of hydroxyl groups as from water or to OH groups presented in cellulose, hemicelluloses and lignin
	2925 cm-1	C-H stretching of -CH2- and -CH3 as from lipids
	2854 cm-1	Stretching vibration of C-H , -CH2- and -CH3 from lipids
	1647 cm-1	(C=N) the vibration of Amide I of proteins
	1054 cm-1	C-H rocking of -CH2.
leaves	3385 cm-1	N-H stretching of proteins, O–H bonds in water molecules or to OH groups presented in cellulose, hemicelluloses and lignin
	1635 cm-1	(C=N) vibration of Amide I of proteins
	1058 cm-1	Amide II of proteins

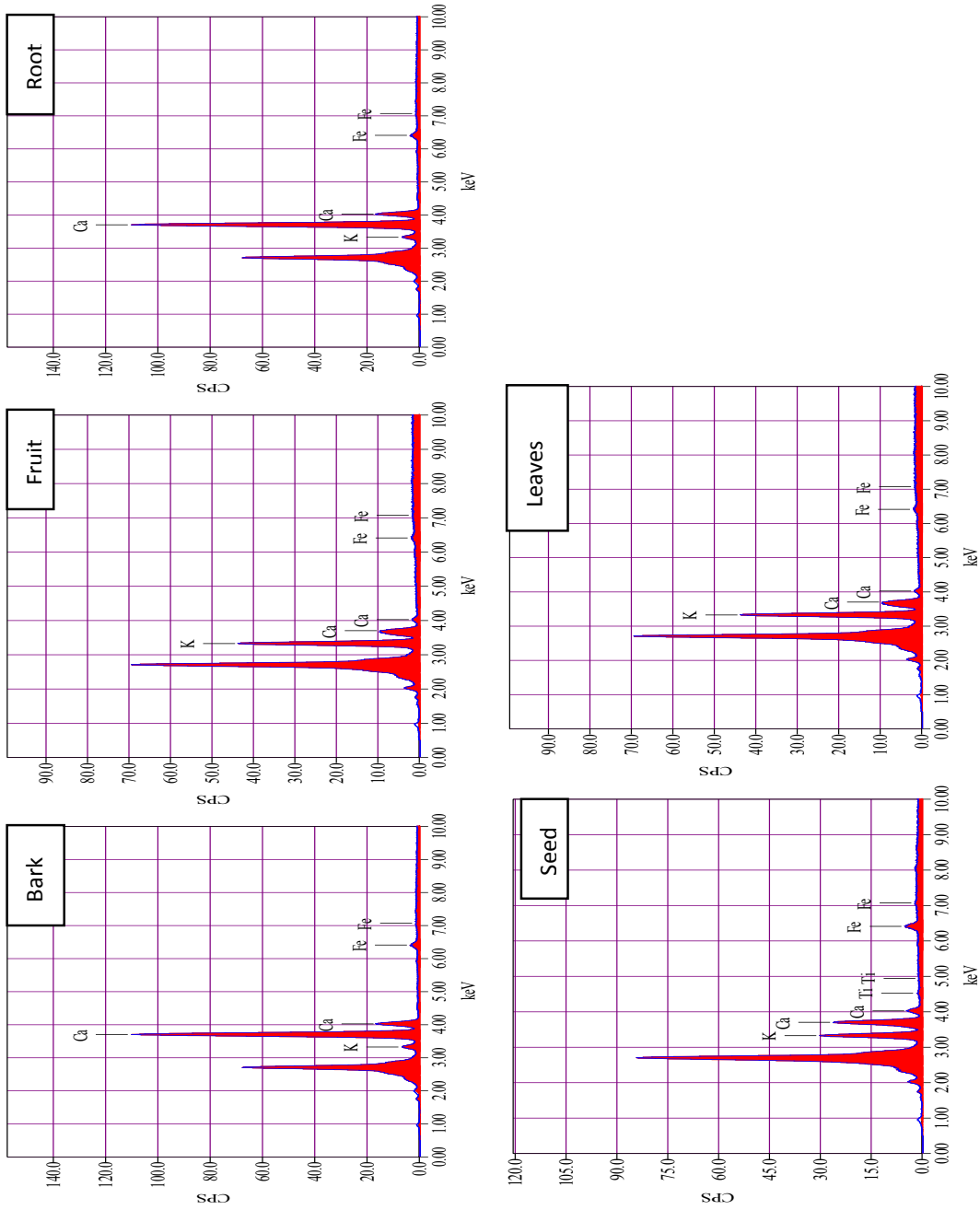

Fig. 1. X-Ray fluorescence analysis (XRF) spectrum of *Ziziphus spina- christi* parts showing bioactive elements.

TABLE 3. Antibacterial effects of *Ziziphus spina-christi* ethanol and methanol extracts.

Bacterial strains		*Ziziphus spina-christi* ethanol extracts					*Ziziphus spina-christi* methanol extracts					Antibiotic
		Bark	Fruit	Roots	Seeds	Leaves	Bark	Fruit	Roots	Seeds	Leaves	Erythromycin
***E. coli* (ATCC25922)**	Clear zone	15 ± 1*	- ve	- ve	- ve	- ve	8 ± 1	12 ± 1.5	15 ± 0.5*	- ve	- ve	- ve
	MIC	20	- ve	- ve	- ve	- ve	20	20	15	- ve	- ve	- ve
	MBC	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
***Enteroba-cter cloacae* (ATCC13047)**	Clear zone	12.5 ± 0.5	10.5 ± 0.5	- ve	- ve	12.4 ± 0.4	12 ± 1	- ve	- ve	13 ± 1	15 ± 2*	8 ± 2
	MIC	20	20	- ve	- ve	20	20	- ve	- ve	15	15	2.0
	MBC	20	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
***P. aeruginosa* (ATCC 278223)**	Clear zone	10 ± 1	20 ± 1*	15 ± 0.5	10 ± 0.5	14 ± 1	10 ± 0.6	- ve	- ve	- ve	12 ± 1	12 ± 0
	MIC	15	2.5	10	20	15	20	- ve	- ve	- ve	20	2.0
	MBC	20	10	15	- ve	20	- ve	- ve	- ve	- ve	20	2.0
***k. pneumoniae* (ATCC 13888)**	Clear zone	15 ± 1.5	12 ± 1	15 ± 0.5	16 ± 1.5	15 ± 1	16.2 ± 0.3*	14 ± 1.5	11.6 ± 0.5	11 ± 1	16 ± 1	10 ± 1.5
	MIC	20	20	15	15	15	20	15	20	20	15	2.0
	MBC	20	- ve	20	20	20	20	15	- ve	- ve	15	- ve
***Enterobacter aerogenes* (ATCC 13048)**	Clear zone	- ve	- ve	- ve	- ve	10 ± 0.5	- ve	13.3 ± 0.7*	12 ± 1	- ve	12.5 ± 0.5	10 ± 0.5
	MIC	- ve	- ve	- ve	- ve	20	- ve	20	20	- ve	20	1.5
	MBC	- ve	- ve	- ve	- ve	- ve	- ve	20	20	- ve	- ve	- ve
***Enterococcus faecalis* (ATCC 29212)**	Clear zone	16.2 ±0.5	10 ± 1	21 ± 1	19 ± 0.5	15 ± 1	13.7 ± 1.5	15 ±1	20 ± 2*	15 ± 1	15 ± 1	10 ± 0
	MIC	15	20	10	5	20	20	15	15	15	20	1.5
	MBC	20	- ve	15	10	20	20	20	15	20	20	- ve
***MRSA* (ATCC43300)**	Clear zone	11 ± 1	- ve	11 ± 1.5	- ve	10 ± 1	- ve	- ve	- ve	- ve	11 ± 1	- ve
	MIC	20	- ve	20	- ve	20	- ve	- ve	- ve	- ve	20	- ve
	MBC	- ve	- ve	20	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve

MIC = Minimum Inhibitory concentration (mg ml^{-1}), MBC = Minimum Bactericidal Concentration (mg ml^{-1}), mean ± SD, -ve=no inhibition

Discussion

The medicinal plant are important source for bioactive antibacterial phytochemical compound against majority of human pathogenic bacteria, according to our study ethanolic extracts of *Z. spina-christi* of all parts generally are more effective and act as a potential antibacterial agent than the methanolic extract against the tested pathogenic bacterial strains. Bark and leaves extracts were the most active extracts with small variation comparing with the other plant parts extracts, *P.aeruginosa* as a Gram negative bacteria and *E. faecalis* as a Gram positive bacteria were are more susceptible to the ethanolic extracts of *Z. spina-christi* of all parts. This activity could be attributed to the presence of tannins and leucocyanidin (Rizk et al., 1993), also due to the presence of other active components like saponins, flavonoids, steroids, Glycosides and Phlobatanins which are known to have antimicrobial properties (Lee, 2006; Lam, 2007 and Ogbunugafor et al., 2008). The antibacterial effect for tannins is due to its ability to react with protein to form stable water-insoluble components, since bacteria cell wall is made up of proteins, detoxifying agents by precipitating the protein components and inhibiting their growth (Dangoggo et al., 2012). In addition, it may bind to proline-rich proteins and interfere with the protein synthesis (Shimada, 2006) .Also, It has been reported to inhibit microbial enzymes (Chung et al., 1998), according to Jacob et al.(1991) and Zablotowicz et al. (1996) the complex with metal ions may due to its toxicity as tannic acid which chelate iron from the medium to be in unavailable form to aerobic microorganisms. Also antimicrobial property of saponins attributed to cause leakage of proteins and certain enzymes from the cell and had detergent-like properties that might increase the permeability of bacterial cell membranes without destroying them. The antibacterial effect of alkaloids due to its ability to interchelate with DNA of both Gram positive and negative bacteria and interfere with cell division (Bukar et al., 2015). While Flavonoids activity is likely to be due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Marjorie, 1999), inhibit bacterial DNA and RNA synthesis, protein and lipid synthesis (Mori et al, 1987), interfering with energy metabolism in a similar way to respiratory-inhibiting antibiotics, since energy is required for active uptake of various metabolites and for biosynthesis of macromolecules (Haraguchi et al., 1998). Antibacterial properties of steroids are due to association with membrane lipid and exerts its action by causing leakages. So bark extracts were effective against both Gram-positive and Gram-negative bacteria and this agreed with results recorded by El-Kamali & Mahjoub (2009). Each of the seed extracts was effective only against three bacterial strains, this could be attributed to the absence of saponins, phlobatanins and triterpenes as recorded in Epand et al (2007). Ethanol and methanol leaves extracts also were effective against most tested strains and this agrees with that obtained by Al-Mutairi et al. (2016) except for *Escherichia coli***,** also in contrast to their methanolic leaves extract which was more effective than ethanolic extract of leaves. That may be due to the presence of alkaloids in methanol leaves extracts and their absence in ethanol leaves extracts.

Conclusion

This study showed that ethanolic and methanolic extracts from different parts of *Z. spina-christi* inhibited the growth of various species of Gram positive and Gram negative bacteria. The ethanolic extracts showed slightly better killing action than the methanolic extracts. Ethanol bark extract had an inhibitory effect against all tested bacteria except for *Enterobacter aeruginosa*, also both ethanolic and methanolic extracts of leaves had an inhibitory effect against all tested strains except for *Escherichia coli* for both. *Pseudomonas aeruginosa* and *Klebsiella pneumonia*e which were highly sensitive to most *Ziziphus* extracts among Gram negative rods and *Enterococcus faecali*s among Gram positive strains. *Escherichia coli* and *MRSA* were moderately sensitive to most *Ziziphus* extracts while were resistance to erythromycin.

Refrences

AL-Haj, N.A., Mashan, M.N., Shamsudin, H., Vairappan, C.S. and Sekawi (2010) Antibacterial activity of marine source extracts against multidrug resistance organisms. *Am. J. Pharm. Toxicol*. **5**, 195-102.

Ali, N.A., Jülich, W.D., Kusnick, C. and Lindequist, U. (2001) Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *J. Hnopharmacol*.**74**,173-179.

Al-Mutairi, M.H., Ali, S., Aly, S.M. and Aldebasi,Y. (2016) Antibacterial activity of sider (*Ziziphus spina-christi*), leaves extract against selected pathogenic bacteria. *European Journal of Pharmaceutical and Medical Research*, **3**, 138 – 144.

Bukar, A. M., Kyari, M.Z., Gwaski, P. A., Gudusu, M., Kuburi, F.S. and Abadam, Y.I.(2015) Evaluation of phytochemical and potential antibacterial activity

of *Ziziphus spina-christi* against some medically important pathogenic bacteria obtained from University of Maiduguri Teaching Hospital, Maiduguri Borno State – Nigeria. *Journal of Pharmacognosy and Phytochemistry,* **3**(5), 98-101.

Chung, K.T., Wong, T.-Y., Wey, C.I., Huang, Y.-W. and Lin, Y. (1998) Tannins and human health, a review. *Crit. Rev. Food Sci. Nutr.* **38**, 421–464.

Collins, G.H., Lynes, P.M. and Grange, J.M. (1995) "*Microbiological Methods*" (7th ed.), pp.175-190. Butterwort-Heinemann Ltd, Britain.

Dangoggo, S.M., Hassan, L.G., Sadiq, I.S. and Manga S.B. (2012) Phytochemical analysis and antibacterial screening of leaves of diospyros espiliformis and *Ziziphus spina-christi*. *Journal of Chemical Engineering* , **1**(1), 31-37.

EFSA (2011) (European Food Safety Authority). Shiga toxin-producing *E. coli* (STEC) O104:H4 outbreaks in Europe: taking stock. *EFSA J*. **9**, 2390.

El-Kamali, H.H. and Mahjoub,S.A. (2009) Antibacterial Activity of *Francoeuria crispa, Pulicaria undulata, Ziziphus spina-christi* and *Cucurbita pepo* against seven standard pathogenic bacteria, *Ethnobotanical Leaflets*. **13**, 722-733.

Epand, R.F., Savage, P.B. and Epand, R.M. (2007) Bacterial lipid composition and the antimicrobial efficiency of cationic steroid compounds. *Biochimica et Biophysica Acta,* 2500–2509

Haraguchi, H., Tanimoto, K., Tamura, Y., Mizutani, K. and Kinoshita, T. (1998) Mode of antibacterial action of retrochalcones from Glycyrrhiza inflata. *Phytochemistry,* **48**,125–129.

Jacob, M.C., Favre, M. and Bensa, J.C. (1991) Membrane cell meabilisation with saponin and multiparametric analysis by flow cytometry. *Cytometry,* **12**(6), 550–558.

Jiang, Y.Z.Z., Qian, Y., Wei, Z., Yu, Y., Hu, S. and Li, L. (2008) Plasmid-mediated quinolone resistance determinants qnr and aac (6')-Ib-cr I extended spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in China. *J. Antimicrob. Chemother*. **61**, 1003-1006.

Lam, K.S. (2007) New aspects of natural products in drug discovery,*Trends Microbiology* ,**15** (6), 279-289.

Lee, C.P. (2006) Who's in the business of saving lives? *J. Med. Philos*. **31**, 465-482.

Marjorie, C. (1999) Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, **12**, 564-582.

Mann, A., Yahaya, A.Y., Banso, A. and Ajayi, G.O. (2008) Phytochemical and antibacterial screening of *Anogeissus leiocarpus* against some microorganisms associated with infectious wounds. *Afr. J. Microb. Res.* **2**, 60-62.

Mori, A., Nishino, C., Enoki, N. and Tawata, S. (1987) Antibacterial activity and mode of action of plant flavonoids against *Proteus vulgaris* and *Staphylococcus aureus*. *Phytochemistry*, **26**, 2231–2234.

Mueller, J.H. and Hinton, J. (1941) A protein –free medium for primary isolation of the *Gonococcus* and *Meningococcus*. *Proc. Soc. Exp. Biol. Med.* **48**, 330-333.

Ogbunugafor, H.A., Okochi,V.I., Okpuzor, J. and Emeka, P. (2008) Tolerance and antiplasmodial screening of *Ritchea longipedicellata* in *Plasmodium berghei. Biokemistri.* **20** (1), 23-27.

Okeke, M.L., Ireogbu,C.U., Eze, E.N., Okoli, A.S. and Esimone, C.O. (2001) Evaluation of extracts of the root of *Landolphia owerrience* for antimicrobial activity. *J. Ethnopharmacol*. **78**, 119-127.

Rizk, A.M., Hammouda, F.M., Ismail, S.I. and Hussiney, H.A. (1993) Constituents of plants growing in Qatar Xxiii. Flavonoids of F. Crispa. *Qater University. Sci J.* **13** (1), 51-52.

Scherrer, D., Corti, S., Muehlherr, J.E., Zweifel, C. and Stephan, R. (2004) Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from raw bulk-tank milk samples of goats and sheep. *Veterin. Microbiol*. **101,**101–107.

Shahat, A.A., Pieters, L., Apers, S., Nazeif, N.M., Abdel-Azim, N.S., Berghe, D.V. and Vlietinck, A.J. (2001) Chemical and biological investigations on *Zizyphus spina-christi L. Phytother Res*. **15** (7), 593- 597.

Shimada,T. (2006) Salivary proteins as a defense against dietary tannins. *J. Chem. Ecol.* **32** (6), 1149-1163.

Talon, D., Menget, P., Thouverez, M., Thiriez, G., Gbaguidi Haore, H., Fromentin, C., Muller, A. and Bertrand, X. (2004) Emergence of *Enterobacter cloacae* as a common pathogen in neonatal units: pulsed-field gel electrophoresis analysis. *J. Hosp. Infect*. **57,** 119–125. doi:10.1016/j.jhin.2004.01.023. PMID. : 15183241

Trease, G.E. and Evans, W.C. (1989) "*Pharmacognosy*" 13th ed, Bailere Traiadal, London p. 69.

Zablotowicz, R.M., Hoagland, R.E. and Wagner, S.C. (1996) Effect of saponins on the growth and activity of rhizosphere bacteria. Adv. Exp. Med. Biol. **405**, 83-95.

(Received 14/ 2/2017; *accepted* 26/ 9 /2017 *)*

التأثير المضاد للبكتريا للمستخلصات النباتية للسدر (النبق) على بعض انواع من البكتريا الممرضة

حنان عبد الفتاح تميرك، وسام محمد على سالم، وائل فرغلي سيد و فاطمة صلاح حسن*
قسم النبات- كلية العلوم- جامعة جنوب الوادي و *أخصائي تحاليل طبية - عيادة الوفاء - بالهيئة العامة للتامين الصحي - قنا- مصر.

في هذا البحث تم دراسة التأثير المضاد للبكتريا مستخلصي الايثانول والميثانول لخمس اجزاء منه هي : اللحاء والثمار والجذور والبذور والأوراق على نمو سبعة انواع من البكتريا الممرضة هى:
Pseudomonas aeruginosa (ATCC 278223), *Enterobacter cloacae* (ATCC 13047), *Enterobacter aerogenes* (ATCC 13084), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13888), *Enterococcus faecalis* (ATCC 29212) and *Methicillin-resistant Staphylococcus aureus* (MRSA, ATCC 43300).

وتم استخدام مضاد حيوي (اريثروميسين) على نفس السلالات البكتيرية ككونترول إيجابي . تم تقدير كيفي للمركبات الكيميائية النباتية لجميع المستخلصات. وتم تقدير المجموعات الكيميائية الحيوية بتقنية FTIR وايضا العناصر بتقنية XRF. تم تقدير نشاط المستخلصات المضاد للبكتريا عن طريق تحديد اقل تركيز يسبب تثبيط للنمو وتحديد اقل تركيز يسبب قتل تام للبكتريا. كل البكتريا الموجبة والسالبة لصبغة جرام كان لها حساسية لمستخلصات السدر المختلفة .مستخلصات اللحاء كانت الاكثر تأثيرا على جميع السلالات ماعدا *E. aerogenes* وجد ان المستخلص الإيثانولى للقلف له تأثير كبير على (16,2 مم) *E. faecalis*، وكان اقل تركيز للمستخلص له تاثير مثبط للبكتريا هو 15 مجم/مل واقل تركيز له سبب قتل تام للبكتريا هو 20 مجم/مل. لمستخلص الميثانولى للحاء كان الاكثر تأثيرا على (*K. pneumoniae* مم 2,16)، وكان اقل تركيز له سبب تثبيط للنمو وقتل للبكتريا هو20 مجم/ مل. مستخلصات الأوراق كان لها تأثير كبير على كل السلالات المستخدمة ما عدا *E. coli*.

المستخلص الإيثانولى للثمار كان له ايضا تأثير كبير على (20 مم *P. aeruginosa*)، اقل تركيز له مثبط للبكتريا هو 2,5 مجم/مل واقل تركيز له سبب قتل تام للبكتريا هو 10 مجم/مل. واخيرا المستخلص الإيثانولى للجذور كان اكثر تأثيرا على (21 مم *E. faecalis*)، اقل تركيز له مثبط للبكتريا هو 10 مجم/مل واقل تركيز سبب قتل تام للبكتريا هو 15 مجم/مل.