The genetic inhibition of some pathogenic bacterial isolates related to Enterobacteriaceae by using Different leaves extracts of Cider (Nabag) *Zizyphus spina-christa*

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Abstract:

The plant *Zizyphus spina-christa grows* wildly in the middle and southern of Iraq locally named Nabag. In this study the antibacterial activity of several different plant extract (alcoholic hot and cold extract 80%, aqueous hot and cold extract) was tested against some gram negative bacteria that related to Enterobacteriacea as follow; *Pseudomonas aeruginosa, Escherchia coli Proteus mirabilis, Serratia mercesence, . Aeromonas sp ، Klebsiella pneumoniae ,Shigella sp ،Salmonella enteritidis (134), S. typhi(97), S. typhimurium (300) , S. typhi, .* The results showed that efficient method of extract was alcoholic hot extract from other extract methods that are used in this study. The detection of active compound in crude extracts of the leaves showed positive reaction for alkaloids, flavonoides, saponin,
peptides, tannins and carbohydrates , while the aqueous hot and cold extract did not give any reaction against terpenes ,resins and coumarins . Minimum inhibitory concentration (MIC) of the ethanolic hot extracts of plants was determined and the results showed that MIC of S.typhi was 25 mg / ml and 250 mg/ml aganist *Klebsiella pneumoniae Serratia mercesence , Pseudomonas aeruginosa ,* while other isolates showed variety in their inhibitory action. the ethanolic hot extracts of plants did not show any bacteriocidal effect against all bacteria that included in this study within concentration that used except *S. typhi* in concentration 50 mg/ml . The effect of Sub – MIC of the ethanolic hot extract of plant on the production of some virulence factors from selective isolates *S. typhi* ,showed an inhibitory effect on production of H2S but no effect on others characteristic such, mannitol and glucose fermenter at 20 mg / ml . The electrophoresis of plasmid DNA isolated from bacterial cell treated with, alcoholic hot extract at sub-MIC concentrations had a curing effect on the plasmid of *S. typhi* .Using infrared spectroscopy spectrum indicates the possible effect of alcoholic hot extract on the conformation of the DNA molecules affecting some of its biological functions.

Key words:antimicrobial activity, *Zizyphus spina-christa,*chemical composition Enterobacteriaceae

Introduction

Infectious disease such as Gastroenteritis , Bacteremiae , and others diseases . Incidences of foodborne illnesses are still a major problem, even in developed countries. *Salmonella* spp., *Listeria monocytogenes* and other bacteria were the main pathogens incriminated in poisoning cases [1]. Several bacterial species, particularly enterobacterial members are involved in induction of these diseases .the treatment is still done with administration of antibacterial agents. However,

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bacterial resistance is the major problem during the course of treatment .Therefore replacement of antibacterial agents with a herbal medicine is partially useful for treatment of gastrointestinal infection [2].

In recent years there has been an increasing interest in the use of natural substances, and some questions concerning the safety of synthetic compounds have encouraged more detailed studies of plant resources. [3]. Zizyphus (Zizyphus spina-christi) is a plant that grows into a tree with thorny branches, grows wild in Asia and tropical Africa. The plant is originally of the Middle East South of the Euphrates and spread to Saharan Oases across Africa into the Sahel, in Iraq growing mainly in the Basra region.It is a member of 40 species of spiny shrubs and small trees in the buckthorn family Rhamnaceae which also included : Z. jujube, Z.mauritian, Z. joazeiro, Z._lotus, Z. rotundifolia, Z. mucronata [4].

Recently increasing attention to used Zizyphus (Rhamnaceae) species in folk medicine due to its antimicrobial, anti-inflammatory ,antidiarrhoeal , anticancer, antihypertensive, blood pressure reduction properties , antidiabetic activity and antioxidative constituents . Zizyphus contains a number of potentially biologically active compounds, including peptide and cyclopeptide alkaloids, flavonoids, sterols, tannins, betulinic and ceanothic acids and triterpenoidal saponin glycosides [4,5,6,7].

The widespread distribution of antibiotic resistant bacteria in hospitals specially the types of nosocomial infections caused major public problem which leads to many dead cases .Therefore , as part of our general interest in this study is the evaluation invitro antimicrobial activity of the leaves extracted from *Zizyphus spina-christi* against common Enterobacteriace pathogens isolated from patients suffered from different clinical cases, and study the inhibitory action of the plant extract on virulence factors and genetic material against selective isolates .

Materials and Methods:

Indicator Bacteria

The microorganisms used in this study were obtained from Biotechnology Department / college of Science / Baghdad University and Teaching Labs / Baghdad hospital, included: *Pseudomonas aeruginosa, Shigella sp,* and *Proteus sp ,Salmonella typhi(97), S.typhi* (isolated from blood), *Escherchia coli, Salmonella typhimurium* (300) and *S. entritidis*(134) , *KlebsielLa pneumoniae, Aeromonas sp, Serratia ,mercesence,* (isolated from stool) , these species isolated from different clinical specimens and were identified according to the method described by [8,9,10].

Methods used for detection of active compound

1- **Detection of alkaloids:** this test was done according to the method described by[11,12]

2- **Detection of carbohydrate:** this test was done according to the method described by[11].

3- **Detection of glycosides:** this test was done according to the method described by [13,14].

4- **Detection of flavones:** this test was done according to the method described by [11].

5- **Detection of phenolic:** this test was done according to the method described by [11].

6- **Detection of saponins :** this test was done according to the method described by [15] .

7- **Detection of resins :** this test was done according to the method described by [11] .

8- **Detection of coumarins:** this test was done according to the method described by [12].

9- **Detection of terpenes and steroids:** this test was done according to the method described by [14].

10- **Detection of tannins:** this test was done according to the method described by [11].

11- **Detection of peptide and free amine group:** this test was done according to the method described by [11].

12- **Detection of essential oil:** this test was done according to the method described by [12].

Plant collection and extraction

The plant *Zizyphus spina-christi* samples were collected during April 2008 from gardens of Al-jaderya in different regions .the leaves were washed with tap water to remove the dust and other related foreign materials, dried at room temperature, they were chopped into small pieces in a mortar, then a grinder finally ground it into final powder. Four extract were prepared from the dried leaves powder with tow types of solvents, as following:

1- Alcoholic extract: Two types of ethanol extract were done included:

a- Alcoholic hot extract : It was prepared according to the method described by[16] .

b- Alcoholic cold extract This extract was prepared according to the method described by[17] .

2-Aqueous extract: Two types of aqueous extract were done included:

a- Aqueous hot extract: It was prepared according to the method described by[18] .

b Aqueous cold extract It was prepared according to the method described by[12] .

McFarland tube standard (0.5)

A barium sulfate turbidity standard solution equivalent to a 0.5 McFarland standard was prepared as described by [15].

Determination of antibacterial activity of *Zizyphus* leaves extracts

The agar well diffusion method was used to evaluate the antibacterial activity of the four extracts of the plant. using cork borer . The final concentration (15.5 ,31,62.5,125,250,500) mg\ ml of the extracts were made from the stock solution (1000 mg\ml) , and methods was described by [19].

Determination of MICs and MBCs

The tube dilution method was used to determination of MICs of alcoholic hot extract that gave effective antibacterial activity against all indicator bacterial isolates were used in this assay. The final concentration (15,20,25,50,75,100,125,250,500) mg\ ml of the extracts were made from the stock solution (1000 mg\ml) and methods was described by [20].

MIC was defined as the lowest concentration of plant extract that completely suppressed colony growth (last tube lowest concentration in the series showing no growth compared with the growth of control).

The MBCs was determined and defined as the lowest concentration of plant extract that completely no colony growth were visible onto plate culture [20].

Detection of some virulence factors and biochemical test

Some virulence factors and biochemical test were Detected from selected isolated that exhibited wide susceptibility against alcoholic hot extract as follows:

Hemolysin production test: this test was done according to [8].

Urease production test: this test was done according to [8].

Gelatin liquefaction test: this test was done according to [9].

Production of H2S : this test was done according to[9]

Glugose , Mannitol and Lactose , fermentation . This test was done according to [8].

Study the effect of alcoholic hot extract in production of some virulence factors and biochemical test

After detection of MICs of alcoholic hot extract, we are study the effect of this extract in production of some virulence factors that give positive result from *S. typhi* as follows :

* put 100 µl from sub MIC (final concentration 20 µg/ml) from extract with 100 µl of bacterial culture (*S. typhi)* and subculturing in BHIB for 24 hr incubator at 37c ,then we removed 100 µl from it and cultured onto each of S-S agar ,.Mannitol salt agar, Phenol Red broth .
* incubate plates in 37c for (18-24) hr and then read the results.

Total DNA isolation (salting out method)

The method was used in this study described by Pospiech and Neumann [21] to isolate both plasmid and chromosomal DNA.

Agarose Gel Electrophoresis:

The method described by [22].

Absorbent spectrum of DNA in an infra red region

Add Drop of extraction from DNA (control and treated isolate) between cell discs (type Agcl), FTIR is used to measured the infra red radiation region at positive band ranging (500-4000) cm -1 .

Results and Discussion :

Antibacterial activity of different leaves extracts

The antibacterial activity of different Leaves extracts of *Zizyphus spina-christa* against ten gram negative bacteria species which considered as multi drug resistant bacteria(result not show),is summarized in table (1) and (2) .The results revealed that all extract showed antibacterial activity with varying magnitudes. The zone of inhibition above 7 mm in diameter was takes as positive results. The alcoholic hot extract exhibited maximum activity as showed in table (1) against all isolated bacterial species that are used in this study at concentration (250-500)mg/ml in diameter (15-40)(10-35)mm respectively .

Generally some bacterial isolates such as *P mirabilis, S typhimurium 300 ,S entritidis 134 S typhi 97 and S typhi* were sensitive at concentration 125 mg/ml in diameter(14,14,17,18,30)mm respectively, on the other hand *S. typhi* exhibit sensitivity at low concentration 15.6 mg/mlWhile *E.coli* showed sensitivity at concentration 31.25 mg/ml. The alcoholic cold extract and aqueous hot extract showed antagonistic activity at highest concentration 500 mg/ml against all bacteria in diameter (15- 25)(10-20)mm respectively (table 1) (table 2).

The bacterial isolates showed variable activity, when treated with the aqueous hot extract, 10 isolates (100%) appeared antibacterial activity at concentration (500) mg/ml, while 4 isolates did not show antibacterial activity when treated with cold extract at the same concentration.

Depending on the results above the alcoholic hot extract are most efficiency method than other extracted methods prepared according to wide inhibited spectrum against different isolates especially *S. typhi* which exhibit highest susceptibility at low concentration .and the efficiency of alcoholic hot extract may due to use alcoholic in percentage 80% in plant extraction to obtain components that dissolution in water beside that in alcoholic [13,23]. These results are in accordance with the results reported by Al- Bayatti *et al* .,[24] who mentioned

that the ethanolic and aqueous extract of *zizyphus spina christa* has inhibitory effects at various concentrations (25, 50, 100, 200, 400) mg/ml against five bacterial species *Staph.aureus, E.coli,P.aeruginosa,Enterococcus* sp and *Acinetobacter sp.*

Table (1): Antibacterial activity of Alcoholic hot and cold extract in different concentration against indicator bacterial isolated.

Indicator Bacteria	Alcoholic extract – (mg/ml)											
	hot						cold					
	500	250	125	62.5	31.25	15.6	500	250	125	62.5	31.25	15.6
E.coli	26	16	15	15	13	–	15	13	–	–	–	–
Pseudomonas aeruginosae	15	11	-	–	–	–	15	–	–	–	–	–
Klebsiella pneumoniae	15	10	–	–	–	–	15	–	–	–	–	–
Aeromonas sp.	20	15	-	–	–	–	15	–	–	–	–	–
Serratia marcescence	20	18	–	–	–	–	15	–	–	–	–	–
Proteus mirabilis	20	15	14	–	–	–	20	18	–	–	–	–
Salmonella typhi	40	35	30	25	22	21	25	20	15	–	–	–
S. typhi 97	25	20	18	–	–	–	20	18	–	–	–	–
S typhimurium 300	22	18	14	–	–	–	21	20	–	–	–	–
S . enteritidis. 134	22	20	17	–	–	–	22	20	17	–	–	–

***the diameter of inhibition zone assayed in mm**

Table (2): Antibacterial activity of Aqueous hot and cold extract in different concentration against indicator bacterial isolated.

Indicator Bacteria	Aqueous extract – (mg/ml)											
	hot						cold					
	500	250	125	62.5	31.25	15.6	500	250	125	62.5	31.25	15.6
E.coli	18	15	-	–	–	–	15	13	–	–	–	–
Pseudomonas aeruginosae	12		-	–	–	–	–	–	–	–	–	–
Klebsiella pneumoniae	10	-	–	–	–	–	–	–	–	–	–	–
Aeromonas sp.	12	–	-	–	–	–	–	–	–	–	–	–
Serratia marcescence	15	14	–	–	–	–	12	–	–	–	–	–
Proteus mirabilis	10	–	–	–	–	–	–	–	–	–	–	–
Salmonella typhi	20	18	15	–	–	–	20	15	–	–	–	–
S. typhi 97	14	12	–	–	–	–	14	–	–	–	–	–
S typhimurium 300	16	14	–	–	–	–	13	–	–	–	–	–
S . enteritidis. 134	14	13	–	–	–	–	13	–	–	–	–	–

***the diameter of inhibition zone assayed in mm**

Z. spina-christi has been shown activity against bacteria and fungi and also other pathogens that are normally quite resistant. [4].

Alanis and colleagues[13] tested the antibacterial activity of aqueous and methanolic extract of 26 medicinal plants including *Z. spina-Christi* against eight different species of enteropathogenic including *E.coli, Salmonella. spp. Shigella.soni ,S.flexanari* and the results showed that methanolic extract exhibited antibacterial activity against one or more bacterial isolates.

Chemical analysis of the plant extracts

As part of our general interest in the characterization of biological active compound from *Zizyphus spina-Christi*, several chemical reagents were used to detect the active compounds in four crude extracts of the leaves. The chemical detection revealed that four crude extracts showed positive reaction for alkaloids, phenolic , Tannins ,

flavonoides ,saponins , carbohydrates, essential oils, amine groups and peptides (table -3) . While alcoholic hot extract showed positive result to coumarins, on the other hand the alcoholic hot and cold extract give positive result to terpens, steroids.

Table (3): Chemical reagent to detection the anti medicinal components in different leaves extract of *Zizyphus spina-christa plant.*

Leave extract				
Active substance	Alcoholic hot extract	Alcoholic cold extract	Aqueous hot extract	Aqueous cold extract
Glycosides	-	-	-	-
Carbohydrates	+	+	+	+
Alkaloides.	+	+	+	+
Phenolic compound and tanines	+	+	+	+
Flevonoides	+	+	+	+
Saponins	+	+	+	+
Resins	+	+	+	+
Coumarins	+	–	–	–
Terpenes and Steroids	+	+	-	-
Amine groups and liplds	+	+	+	+
Essential oils	+	+	+	+

The phytochemical composition of *Z. spina-christi* reported indicates the presence of betulic and ceanothic acids from the different species of the genus Zizyphus, peptide and cyclopeptide alkaloids, flavonoids, sterols, tannins, lipids, terpenes, alkaloids, carbohydrate, betulinic acid and triterpenoidal saponin glycosides have been isolated and chemically identified [4].

Different extracts and fractions of the leaves of *Z. spina-christi*. grown in Egypt were investigated in vitro for their antiviral, antifungal and antibacterial activities. The flavonoids quercetin,hyperoside, rutin and quercetin-3-O-[_-xylosyl-(1–2)-_-rhamnoside] 4-O- rhamnoside) were isolated and showed activity against bacteria and fungi and also other pathogens that are normally quite resistant [4,25].

Determination of MIC and MBC

The MIC and MBC value of alcoholic hot extract was determined to all bacterial isolates. The MIC of alcoholic hot extract was (25-50) mg/ml for *S,typhi and Aeromonas sp* respectively , while *P.aeruginosae K. pneumoniae* and, *S. marcescence* showed a susceptibility at high concentration of MIC (250 mg /ml) (table - 4) , and this depends on the anti bacterial effect of this extract upon the different pathogenic bacteria and mode of action .

The result of MBC showed no bacteriocidal effect of alcoholic hot extract against all pathogenic bacteria that is included in this study at high concentration except *S. typhi* in concentration 50 mg/ml.

Table (4): The minimum inhibitory concentration(MICs) of alcoholic hot extract against indicator pathogenic bacterial isolated.

Indicator Bacteria	(MICs) mg/ml
E.coli	**75**
Pseudomonas aeruginosae	**250**
Klebsiella pneumoniae	**250**
Aeromonas sp.	**50**
Serratia marcescence	**250**
Proteus mirabilis	**125**
Salmonella typhi	**25**
S. typhi 97	**100**
S typhimurium 300	**125**
S . enteritidis. 134	**125**

Detection of some virulence factor

The results of detection of some virulence factors and biochemical tests from selected isolate *S,tyhi*, showed un ability of *S,tyhi* to produce hemolycin ,gelatinase ,urease and ferment lactose , but the capability to produce H2S , ferment glucose and mannitol. The effect of Sub – MIC of the ethanolic hot extract of plant was investigated on production of some virulence factor and biochemical tests that give positive reaction and the results indicate an inhibitory action on production of H2S, but did not effect on ferment glucose and mannitol at concentration 20 mg/ml.

The inhibitory action of alcoholic extract (80%) is consolation to the

subsistence soluble active compounds in alcoholic and aqueous which is detected in this study, included alkaloids, phenolic, flavonoides ,saponins , resins and tannins which act as inhibitors for enzymes and transport proteins in cell membrane [26], especially phenol compound interfere with proteins ,while flavonoides made complex compound with soluble proteins and extra cellular proteins [27]. In our study alcoholic hot extract exhibited strong activity against entero pathogenic bacterial isolates especially *S. typhi*, and offer an alternative method to treat drug resistant enteric infections and suggest that at least part of their action is due to their antibacterial propriety.

Isolation of total DNA

To investigate whether the antimicrobial action of *Zizyphus spina-Christi* extract was mediated by the effect on genetic material, an alcoholic hot extract was tested on the pathogenic bacterial DNA, as a curried agent instead of other chemical agents such as acridine orange, ethidium bromide, mitomycin-c and others [28]. Results illustrated in figure (1) shows that the local isolate *S .tyhi* (control without treated with extract) harbor two small plasmid bands (lane-1), on agarose gel , but the plasmid was lost when treated with plant extract and this indicate the extract had a curing effect on the plasmid at sub-MIC concentration (20 mg/ml) (lane-3) These results in agreement with result of inhibit product of H2S from this isolate when treated with plant extract , so this leads to conclude the extract of *Zizyphus spina-Christi* may act as a cured agent to plasmid isolated from *S.typhi* at concentrations 20mg\ml. and this result may be confirmed by studies in future against others pathogeic bacteria .

.Shriram *et al* [29] reported a novel plasmid-curing compound was identified as 8-epidiosbulbin E acetate (EEA) from *Dioscorea bulbifera* L which exhibited broad-spectrum plasmid-curing activity against multidrug-resistant (MDR) bacteria, including vancomycin-resistant *Enterococcus faecalis*, *Escherichia coli*, *Shigella sonnei* and *Pseudomonas aeruginosa* with 12–48% curing efficiency ,and 16 - 64% from *Bacillus subtilis* (pUB110), *E. coli* (RP4), *P. aeruginosa* (RIP64). and *Salmonella typhi* (R136).

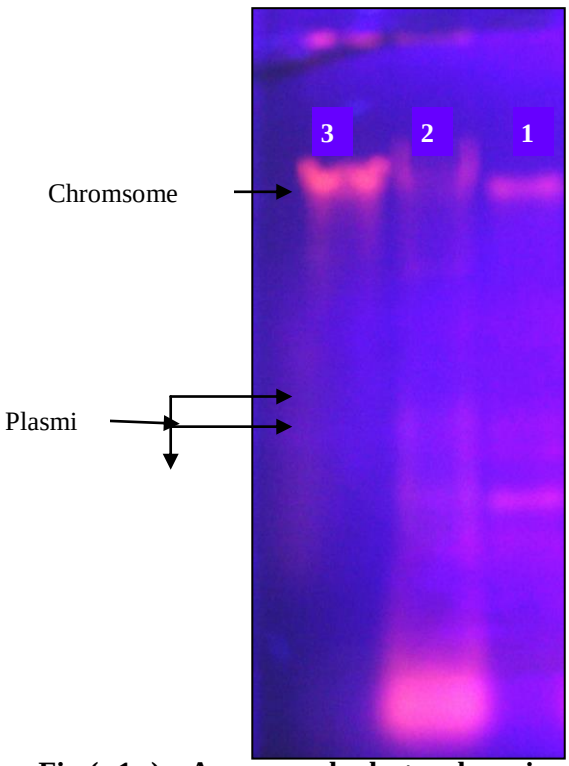

Fig (1) : Agarose gel electrophoresis of plasmid DNA extracted from *S.typhi Treated with* alcoholic hot extract *of Z. spina-christi* (agarose concentration (0.7%),voltage 5volt/cm,during 2hr).
Lane 1 : plasmid profile of *S.typhi without treated* (control).
Lane 2 : plasmid profile of *S.typhi aftert treated With plant extract* at concentration 15 mg\ml
Lane 3 : plasmid profile of *S.typhi after treated with plant extract* at concentration 20 mg\ml

Absorbent spectrum of DNA in an infra red region

Vising infrared spectroscopy spectrum showed in figure (2) and (3)

that the sub MICs of alcoholic hot extracts of. *Zizyphus spina-christa* (20 mg/ml) cause an alteration in NH^{+}_{3} groups at wave band (1500-3200) cm^{-1} to (2098.41 , 2545.86) , and C=O group (back bone for DNA) at wave band (1500-2800) cm^{-1} to (1535.23 , 1488.94) cm^{-1} . The PO_2^- groups (2500-2900) altered to 2545.86 cm^{-1} and 2862.12 cm^{-1}. Two band appeared (1488.94, 1535.23) cm^{-1} after being treated with plant extract. These results indicate the possible effect of plant extracts on the conformation of the DNA molecules affecting some of its biological functions.

This result agrees with that of Passat [30] who found that the sub MICs of ethanolic and aqueous extracts of other plant such as. *Withenia. somnifera* (4 mg / ml , 150 mg / ml) made an alteration in NH+3 groups at wave band (2000-3100) cm-1 and C=O group at 1649 cm-1 , while the Sub-MICs of the ethanolic and aqueous extracts of U. urens (20 mg / ml , 175 mg / ml) made changes in NH+3 , C=O and PO2- groups at the wave bands (2000-3100) , 1649 , 690-810 cm-1 respectively.

In recent study used plant extracts in causing damage in DNA, such as in De-Oliveira *et al* [31] reported the ethanolic extract of *Casearia sylvestris* and its *Clerodane diterpan* can protect cell against DNA damage induced by cyclophosphamide at low concentrations, but at high concentrations these compounds also induce DNA damage. From all above, this study indicate clear result of variation in DNA positions (hydrogenated) which sharing in obtaining variation values in biotic function and also whole cell level [32].

The results in last experiments in our study showed, the inhibitory action of the leaves extracted from *Zizyphus spina-christi* on some virulence factors and genetic material against selective isolate *S.tyhi*.

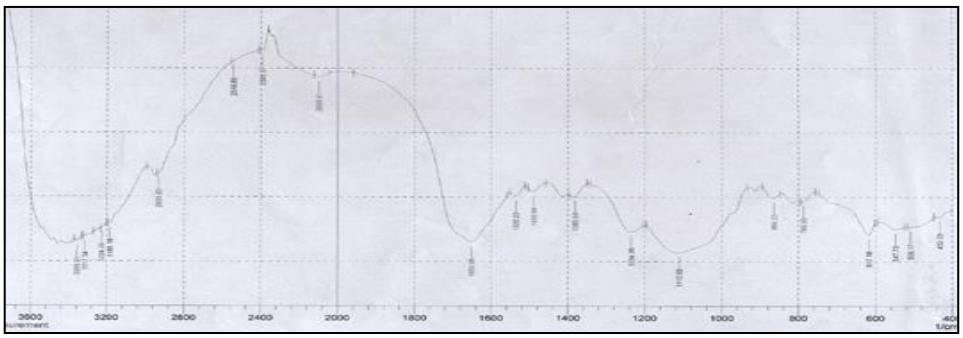

Fig (2): Vising infrared spectroscopy (400-5000) cm^{-1} showed the absorbency values of plasmid DNA (control) not treated with alcoholic hot extract of *Z. spina-christi* .

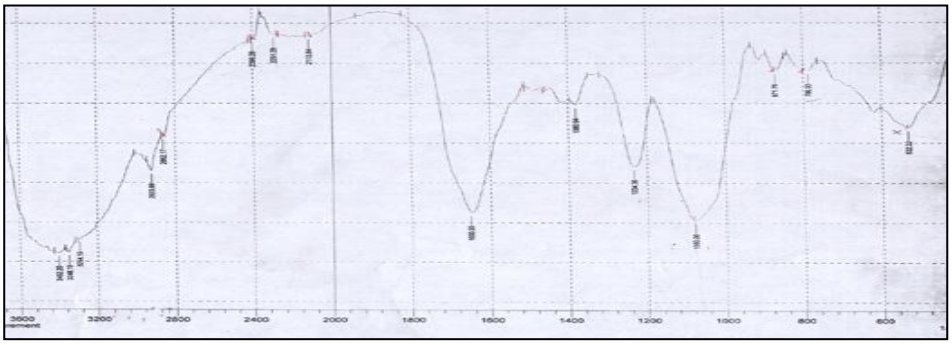

Fig (3): Vising infrared spectroscopy (400-5000) cm^{-1} showed the absorbency values of plasmid DNA after treated with alcoholic hot extract of *Z. spina-christi* in concentration 20 mg/ml.

References :

1- Alzoreky ,N,S. and Nakahara ,K.. 2003 . Antibacterial activity of extracts from some edible plantscommonly consumed in Asia . Int.J. Food Microbiol., 80 : 223–230.

2- Dorman,H.I.D. and Deans,S.D. 2000 . Antimicrobial agents from plants : antimicrobial activity of plant volatile oils.J.Appl.Microbiol.88:308-316.

3- Burt**,**S. 2004. Essential oils: their antibacterial properties and potential applications in foods. Int.J. Food Microbiol.,94:223 – 253 .

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

5- Abdel-Zaher ,A.O, Safa ,Y. S.; Assaf, M. H. , and Abdel-Hady, R. H. 2005. Antidiabetic activity and toxicity of *Zizyphus spina-christi* leaves. J. Ethnopharmacol.101 : 129–138.

6- Michel,G.C.; Demiana I.N.and. Manal,F.I. 2010. Anti-diabetic activity and stability study of the formulated leaf extract of *Zizyphus spina-christi (L.)* Willd with the influence of seasonal variation. J. Ethnopharmacol .5(5): 6320-6330.

7- Hussein, M.H.; Eman, M.E.and Aataa, A.S. 2006. Antihyperglycemic, Antihyperlipidemic and Antioxidant effect of Zizyphus spina christa and Zizyphus jujube in Alloxan Diabetic Rats .Int. J. Pharmacol.2(5):563-570

8- Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolke, R.H., 1995 . Manual of Clinical Microbiology, vol. 6th ed. ASM, Washington, DC.

9- Macfaddin,J.F. 2000 .Biochemical tests for identification of medical bacteria.3ed Lippincott Williams and Wilkins.

10- Holt, J.G., Krieg,N.R., Sneath, H.A. Staley,J.T. and Williams,S.T. 1994 . Berge's Manual of Determinative Bacteriology. 9th (ed) Williams and Wilkins. U.S.A.

11- AL-Kazaraji,S.M. 1999 .Biopharmacological study of *Artemisia herbaalba*.M.Sc.thesis, College of Pharmacy – University of Baghdad

12- Harborne,J.B.(1973).Phytochemical methods.A guide to modern teqniques of plant analysis. Chapman and Hall,London.

13- Alanis,A.D.;Calzada,F.;Cervantes,J .A.;Torres. J.; and Ceballons,G.M. 2005. Antibacterial properties of some plants used in Maxican traditional medicine for the treatment of Gastrointestinal disorders .J.Ethnopharmacol ,100.: 153-157.

14- El-Falla,A.A.;and El-Kattan.M.H. 1997 .Effect of plant extracts on the mycelial growth of some cultivated mushrooms. Egypt. J. Microbial,32(1):41-48.

15- National Committee for Clinical Laboratory Standards(NCCLS). 2003. Performance standards for disk susceptibility tests, 8th ed. Approved standard M2-A8. National Committee for Clinical Laboratory Standards, Wayne, Pa.

16- Geisman,T.A. 1962 .Chemistry of Flavonoid compound .Macmillan Co.,New York.

17- Indian herbal pharmacopeia, (vol.1). 1998 .Ajoint publication of Regional Research Laboratory. Council of Scientific and Industrial Research.Jammutawi.p:1-10.

18- AL- abid,M.R. 1985 .Aurzu sammen strungder Abschla B membrane in phenix dactilyfra.Wurzhurg University.

Wurzhurg,F.R. Germany.Cited by Asmaa,O. 2002. .Effect of *Teucrium polium* extracts on the growth of some pathogenic microorganisms.M.sc. thesis submitted to the college of science,Al-Nahrain University.

19- Vignolo G.M, Suriani F., de Ruiz Holgado A.A.P., and Oliver G.1993. Antibacterial activity of Lactobacillus strains isolated from dry fermented sausages. J. Appl. Bacteriol., 75; 344-349.

20- Miles , R.S. and Amyes S.G.B. 1996. Laboratory control of antimicrobial therapy. In : Collee, J.G. Fraser, A.G., Marmiom, B.P., and Simmon, A. (eds.) Mackie & McCarteny Practical Medical Microbiology. 4th ed. Churchill Livingstone Inc., USA.

21- Pospiech T. and Neumann . 1995. Preparation and analysis of genomic and plasmid DNA. (ed. Kieser, T.). Norwich, U.K.

22- Sambrook J, and Rusell DW. 2001. Molecular cloning. A laboratory manual. Third ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press, N.Y.

23- Al-Shamma,A.;Kasal,N,;andAl-Hiti, M. 1989. Screening of indigenous Iraqi plant for alkaloids and antimicrobial activity . Iraq .J.Pharm.Sci.,2(2):81-89.

24- Al-Bayatti,K.K;Aziz,F.M.and Issam,M.2010. Astudy of antibacterial activity of cider *Z.spina christa* on bacterial pathogens isolated from skin infection . Acceptance letter for published in A[-Mustansiriyah Journal of Pharmaceutical Sciences.

25- Nazif,N.M. 2002. Pharmacognosy and Chemistry of MedicinalPlants. Egypt. *FoodChemistry*76(1) :77-81

26- Greuloch , V.A. 1973. Plant function and structure .The Macmillan .Co ., New York

27- Cowan, M. M. 1999. Plants products as antimicrobial agents. C. M. R. 12 (4) : 564 – 582.

28- Trevorse**,** J.T. 1986 . Plasmid curing in bacteria . FEMS. Microbiol . Rev . 32 : 149–157.

29- Shriram,V.;, Sheetal.J. C.;Lathac, V. K.; Vedavati ,P.; Supada, R.; Prashant ,K. D.and Shitole, M.G. 2008 . A potential plasmid-curing agent, 8-epidiosbulbin E acetate,from *Dioscorea bulbifera* L. against multidrug-resistant bacteria . Int. J..Antimicrob Agents.32 : 405–410.

30- Passat ,D.N.2006.The Effect of aqueous and alcoholic extracts of *Withania somnifera* (L.) Dun. and *Urtica urens* (L.) in some virulence factors and plasmid DNA in local isolates of *Pseudomonas aeruginosa* . M. Sc thesis .College of Science, University of Baghdad.

31- De-Oliveira,A.M.; Dos-Santos, A.G.; Dos-Santos, R.;Csipak, A.R.; Olivate, C.;Silva,I.C.; Freitas,M.B.; Bassi, C.L.; Cavalheiro, A.J.; Bolzani, V.S.; Silva, D.H.S.; Sakamoto-Hojo,E.T.; Takahashi,C. and Soares,C.P. 2009 . Ethanolic extract of *Casearia sylvestria* and its *clerodane diterpen* (caseargrewiin F) protect against DNA damage at high concentrations and cause DNA damage at high concentrations in mice's Blood cells. Mutagenesis 24(6):501-506.

32- Mourant, J. R. ; Gibson, R. R. ; Johnson, T. M. ; Carpenter, S. ; Short, K. W. ; Yamada, Y. R. and Freyer, J. 2003 . Methods for measuring the infrared spectra of biological cells. Phys. Med. Biol. 48 : 243 – 257.

التثبيط الوراثي لبعض الممرضات البكترية التابعة للعائلة المعوية Enterobacteriaceae باستخدام مستخلصات اوراق نبات السدر (النبق) *Zizyphus spina-christa*

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الخلاصة:

ينمو نبات السدر *Zizyphus spina-christa* بشكل واسع في جنوب ووسط العراق والذي يعرف محلياً النبق او النبك Nabag . في هذه الدراسة اختبرت الفعالية ضد البكترية لمستخلصات مختلفة لاوراق هذا النبات والتي شملت (الاستخلاص بالكحول الحار والبارد 80% ، ،الاستخلاص المائي الحار و البارد) ضد العزلات البكتيرية السالبة لصبغة كرام التي تعود للعائلة المعوية والتي *شملت Pseudomonas aeruginosa* و*Escherchia coli* ، *Serratia. mercesence* و. *Aeromonas sp* و *KlebsielLa pneumoniae , Shigella sp* و. *S. entritidis (134), Salmonella typhi(97), Salmonella. Typhimurium (300)*،.. *S. typhi* و *Proteus mirabilis*. وأظهرت النتائج كفاءة طريقة الاستخلاص بالكحول الحار لأوراق نبات السدر عن باقي طرق الاستخلاص المستخدمة

تم في هذه الدراسة التحري عن محتوى المركبات الفعالة للمستخلصات المختلفة لاوراق نبات السدر وقد لوحظ من الكشوف النوعية للاوراق احتوائها على الفينولات والتانينات والقلويدات والفلافونات والبيتيدات والكاربوهيدرات والصابونيات، في حين لم يظهر كل من المستخلص المائي الحار والمستخلص المائي البارد اختبارا ايجابيا تجاه الراتنجات والتربينات والكومارينات . وشملت الدراسة أيضاً تحديد التركيز المثبط الادنى والقاتل للمستخلص الكحولي الحار لاوراق نبات السدرتجاه جميع عزلات بكتريا الاختبار، اذ بلغ التركيز المثبط الادنى 25 ملغم/مل تجاه العزلة البكترية *S. typh*i في حين بلغ اعلى تركيز مثبط ادنى 250 ملغم/ مل تجاه العزلات*Klebsiella pneumonia* و *Serratia mercesence* و*Pseudomonas aeruginosa* في حين تباينت قابلية باقي العزلات في تثبيطها. ولم يبدي المستخلص الكحولي الحار لاوراق هذا النبات تاثير قاتل ضد جميع العزلات البكترية المشمولة في هذه الدراسة ضمن التراكيز المستخدمة باستثناء بكتريا *S. typh*i عند تركيز 50 ملغم/ مل .كما درس تاثير المستخلص الكحولي الحار في التركيز تحت التركيز المثبط الادنى (20) ملغم تجاه بعض عوامل الضراوة لبكتريا *S. typhi*. . وكان مثبطاً لانتاج الـ H2S ولم يكن له تأثير في تخمير المانتول والكلكوز، كما اظهر تاثيرا محيد لبلازميد بكتريا *S.typhi*. وعند تحليل الدنا باستخدام الاشعة تحت الحمراء وجد ان المستخلص الكحولي الحار عند التركيزاعلاه قد احدثا تغييراً في الوضعية العامة لجزيئة الـ DNA من ناحية التركيب الثانوي وبالتالي على بعض ادواره الوظيفية الحيوية.