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Preliminary Biochemical Study for Three Extracts of Lycium sp. Plant

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Abstract

An aquous hot , cold and hot ethanolic extracts fraction for *Lycium species* have been prepared. Qualitative tests have been carried out for detection of their general chemical composition ,and UV-Visible spectra were also obtained .The antibacterial activity of the extracts was tested by the well diffusion method .Human blood cells were used to determine cytotoxicity for the extracts in concentration 500 mg/ml . No cytotoxic effects were observed by this concentration,and this is considered the first step in the work positively and in the continuity of testing the extracts on human by pharmacy and medicine colleges.

Key word : *Lycium sp*. plant, aqueous and ethanolic extracts , antibacterial activity .

1:Introduction

In the recent years , researches have been directed towards curing a lot of various diseases by using the popular medicine known as (Folkloric medicine) for activity , safety ,and economic factors [1].

Recently , there are an interest in planting and investing medical plants through describing them as a natural source to make the remedy instead of some synthesized drugs of bad side effects [2&3].

Furthermore , the study of the activity of many medical plant extracts as anti- microbes has led to an important results which include their influence on an target ,other than that affected by manufactured antibiotics [4].

The cases of the infecting with many pathogenic microorganism such as gram positive , gram negative bacteria or fungi which exhibited recently resistance for many antibiotics. Also, increasing of treatment cost ,decreasing of averages of antibiotics production ,and appearing of the isolates resisting the treatment had led many researchers to search for new sources for treatment instead of chemical drugs such as plants [5] .For a long time , excavating of scientists continued in the equatorial forests, the farms, and the environmental systems of earth about unusual materials for saving human needs ,so the nature was the classical source for organic chemical compounds which used in medicine. since more than 3000 years, the first societies have known that their environments rich with plants which saved methods of treatment of many camelish diseases and many bacterial and fungal infections[6].

Along every the centuries ,the focusing was on drugs which extracted from plants and to ensure continuation in obtaining new chemical compounds and to discover useful drugs for humanity ,the biological techniques was merged with chemistry of natural products to facilitate the obtaining the new and rare natural products ,also in the present time the increased focusing on techniques of genetic geometry and genetic mutations may be useful in generating changes in chemical content for plant which may gives positive restricken back in changing type of chemical compounds in plant[7].

The discovery of many new antibiotics from the plant and their use was one of great scientific achievements because of the controlling some bacterial and fungal infections and human became indeed capable to treat killing diseases ,while other diseases was obliterated utterly[8].

In Iraq , there are many plants and herbs that we have to do our best efforts to discover these fortunes enrich the science . The object of the present work is to investigate some of the chemical compositions of the plant *Lycium sp*. extracts and their activity towards some clinical bacterial isolates

2 :Materials and Methods

2-1: **Preparing plants for study**

The plant (stem and leaves) was collected from Abul - Kasseb region in Basrah city of Iraq at April 2005, and dried at room temperature in dark condition and milled electronically to a coarse powder and stored till use at April 2007 .

2-2: **Preparation of the extracts**

Both hot aqueous or ethanolic extracts were prepared by extracting 20 gm of plant powder with 250ml of distilled water or ethanol (95%) respectively. The extraction was carried out for 24 hours . Then the extract was filtered and concentrated to half of the orginal volume by direct heating . The residue solution was left in petridishes to dry at laboratory temperature . These steps have been repeated many times to obtain enough amount of crude extracts. Crude extracts were collected and kept in the laboratory until use[9].

2-3:**preparation of the cold aqueous and ethanolic extracts**

Cold ethanolic extract was prepared by mixing 20 gm of plant powder with 250 ml of ethanol(95%) with stirring for 24 hours at laboratory temperature, filtered and the filterate was left in petridishes to dry at laboratory temperature . All the steps have been repeated many times to obtain the enough amount of crude extract. The crude extract was collected and kept in the laboratory until use[10].

2-4: **Qualitative tests**

Several qualitative tests have been carried out to know their general chemical composition. These tests were for Alkaloids, glycosides ,saponins ,carbohydrates ,flavonoids , tannins , ninhydrin,resins,fuocoumarins ,triterpenes ,steroids ,unsaturation ,ethanolic rhodami B , solubility and pH test[10-19]..

2-5: **Electronic spectra measurements**

Electronic spectra were performed by an Helios v4-60 UV-visible spectrophotometer at Physics Department ,College of Science, University of Basrah. It was used a quartz solution cell of 1 cm path length in the region (200 – 700) nm at the laboratory temperature. The solvents were distilled water and ethanol (97%) and the concentration spectral solutions was 0.005 gm / 5 ml for both aqueous and ethanolic extractants [20].

2-6:**Infrared Spectra Measurements**

Infrared spectra were recorded on Buck Scientific Inc. Infrared spectrophotometer,model 500 at chemistry department, college of education, of Basrah University.

2-7:**Microbial cultures**

The following bacterial species were used to test the activity of the extracts:

1. *Staphylococcus aureus*
2. *Escherichia coli*
3. *Pseudomonas aeroginosa*
4. *Proteus vulgaris*
5. *Klebsiella pneumoniae*
6. *Salmonella typhi*
7. *Shigella dysenteriae*
8. *Vibrio choelerae*

2-8:**Antibacterial Activity**

The antibacterial activity was determined by the well diffusion method according to [9,21,22]Three to five identical colonies from each plate were lifted within a sterile wire loop and transferred to into a tube containing 5 ml tryptic soy broth (TSB). the turbidity of each bacterial suspension was adjusted to reach an optical comparison to that of a 0.5 McFarland standard , resulting in a suspension containing approximately 1 to 2 x 10^8 CFU/ ml.

Müller- Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface . This procedure was repeated by streaking 2 more times , rotating the plate approximately 60^{o} each time to ensure even distribution of the inoculum . As a final step, the rim of the agar was also swabbed. After allowing the inoculum to dry at room temperature , 6mm diameter wells were bored in the agar. The plant extract was checked for antibacterial activity by introducing 50 µL of 2.5%, 5%, 7.5% and 10% concentrations into duplicate wells. The plates were allowed to stand at room temperature for 1 hour for extract to diffuse into the agar and then they were incubated at 37^{o} C for 18 hours. Subsequently, the plates were examined for bacterial growth inhibition and the inhibition zone diameter measured to the nearest millimeter.

2-9: **Determination of cytotoxicity for aqueous and ethanolic extracts**

Human blood cells were used to determine cytotoxicity (10 samples were used ,4 female and 6 male ,the results were identical) of the three extracts according to [23]. .Concentration of 500mg/ml in phosphate buffer saline for both extract were prepared . Also negative control contained only phosphate buffer saline and positive control (tap water) have been used. Then, 0.2 ml of blood cells were added to sterile test tube containing 0.8 ml of extract to reach a total volume of 1 ml. The controls were treated in the same way. Incubation of the five test tubes at 37^{o} C for 3 hours .Hemolysis was then followed.

3: Results

Table(1): Physical Properties for extracts of *Lycium sp*

No. of extract	Type of extract	Color	%Yield of Extract	Appearance	Taste	pH*
1	Hot aqueous extract	Brown	10%	Powder	Sweet	7 - 7.1
2	Hot ethanolic extract	Dark - green	25%	Gummy	Bitter	5.8 - 5.9
3	cold ethanolic extract	Bright - green	7%	Viscous	Bitter	5.9 - 6.1

* **:**pH of solution of 0.2gm extract / 10 ml of solvent at 38 ^{o}C.

Table(2): Solubility for extracts of *Lycium sp.* in common solvents at38 ^{o} C

Solvent	Hot aqueous extract	Hot ethanolic extract	Cold ethanolic extract
Acetone	Not soluble	Very slightly soluble	Slightly soluble
Benzene	Not soluble	Slightly soluble	Slightly soluble
Butanol	Not soluble	Slightly soluble	Slightly soluble
$CHCl_3$	Not soluble	Slightly soluble	Very slightly soluble
CCl_4	Not soluble	Slightly soluble	Very slightly soluble
Ethanol	Very slightly soluble	Soluble	Very soluble
Ether	Not soluble	Slightly soluble	Slightly soluble
Hexane	Not soluble	Not soluble	Not soluble
Methanol	Very slightly soluble	slightly soluble	Soluble
Water	Very soluble	Very slightly soluble	Very slightly soluble

Table(3): UV.Visible absorption data for extracts of *Lycium sp*

Extract	λ_{max},nm	Absorbance
Hot aqueous	269	1.651
Hot ethanolic	235 , 273	2.650 , 1.664
Cold ethanolic	270 , 404	0.780 , 0.256

Table (4):FTIR data of extracts of *Lycium sp*

Extract	O-H Str.Vib. & N-H Str.Vib. (cm^{-1})	asymm.str. vib of C-H(cm^{-1})	symm.str.vib .of C-H(cm^{-1})	C=O Str. Vib. (cm^{-1})	N-H bend. (cm^{-1})	O-H bend. &C-H bend. (cm^{-1})	C-O Str.Vib. &C-N Str.Vib. (cm^{-1})
Hot aq. Extract	3729,3521,3400	3937.5	-	1620		1417	1146,1125
Hot Ethanolic Extract	3360	2917	2833.5	1646		1458.5,1396,1250,1208.5,1062.5	
Cold Ethanolic Extract	3360	2917	2833.5	1729		1458.5,1354,1292,1062.5	

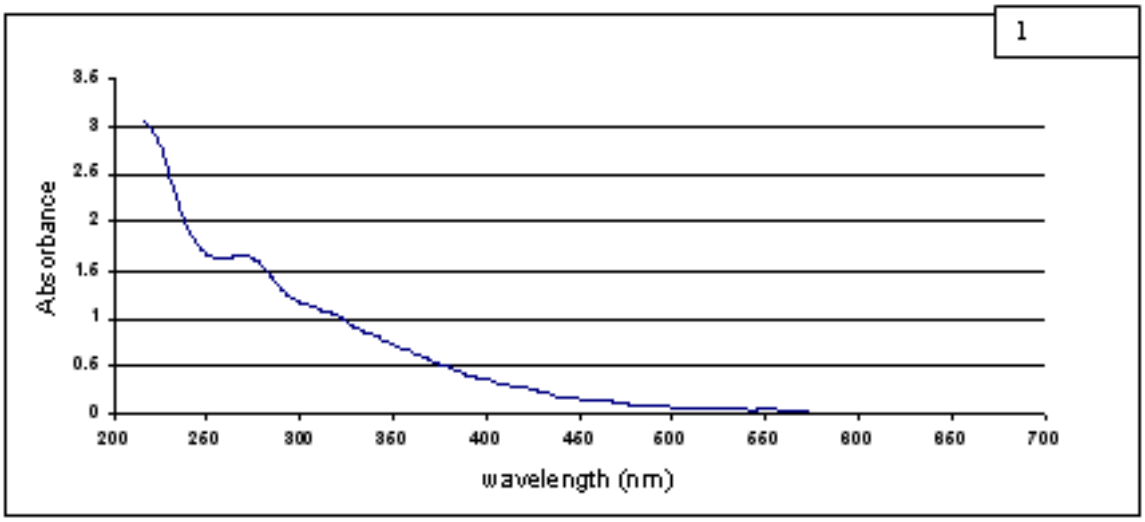
Table(5): Qualitative tests for extracts of *Lycium sp.*

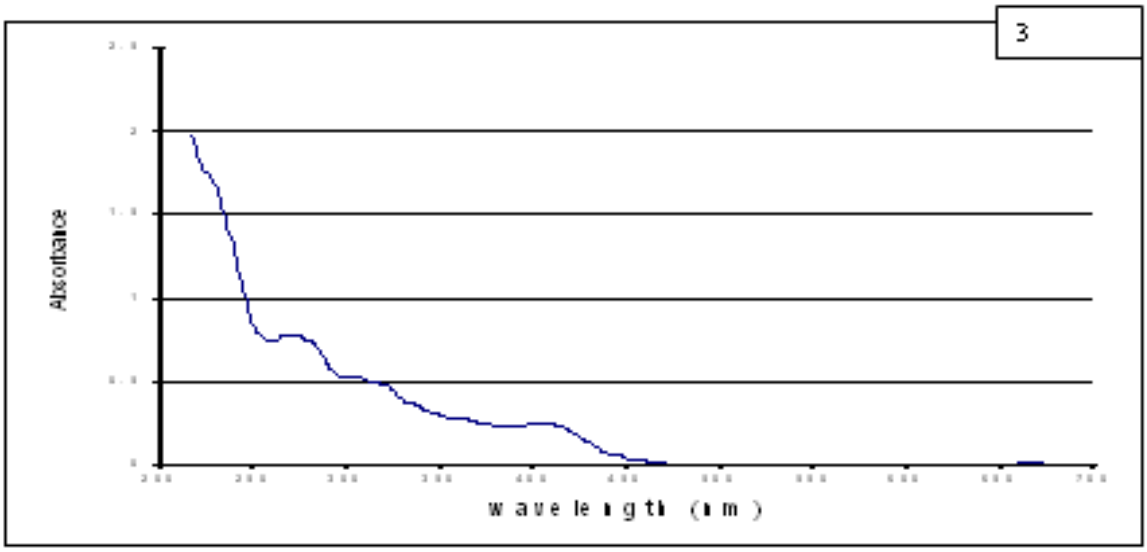
Tests	Hot aqueous extract	Hot ethanolic extract	Cold ethanolic extract
Carbohydrates tests :			
A-by phenol conc.-H_2SO reagent	+	+	+
B-by Molish reagent	+	+	+
Glycosides tests:			
A- before the hydrolysis	-	-	-
B- after the hydrolysis	-	-	-
Tannins Tests:			
A-by lead acetate (1%)reagent	+	+	+
B- by Ferric chloride (1%)reagent	+	+	+
Ninhydrin (1%) test	+	+	+
Phenols tests:			
A- by $FeCl_3$ reagent	+	+	+
B- by Folin reagent	+	+	+
Unsaturated test by Bayer reagent	+	+	+
Oxygen test(by preparation of ferrox salt)	+	+	+
Triterpens & strols tests by Liberman Burchard reagent	–	+	–
Triterpenoids tests	+	+	+
Triterpenes & steroids tests	+	+	+
Alkoloids tests:			
A- by Dragendroff reagent	-	+	+
B- by Mayer reagent	-	+	+
C –by Wagner reagent	-	+	+
D – by ninhydrin reagent	-	+	+
Flavonoids tests:			
A - by KOH	-	-	-
B - by H_2SO_4	-	-	-
Coumarin test		+	
Fuocoumarins test	-	-	-
Resins test		+	
Saponins test	+	+	+
Lipids test by ethanolic rhodamine B(0.5%)	-	-	-
Solubility tests : in HCl (5%)	+	-	-
in NaOH (5%)	+	+	+
in $NaHCO_3$ (5%)	+	+	+

Table (6) : cytotoxicity for extracts of *Lycium sp.*

The hemolysis				
Hot aqueous extract	Hot ethanolic extract	cold ethanolic extract	Tap water	Phosphate buffer saline
+	-	-	+	-

(-) = No hemolysis . (+)= Hemolysis was found .





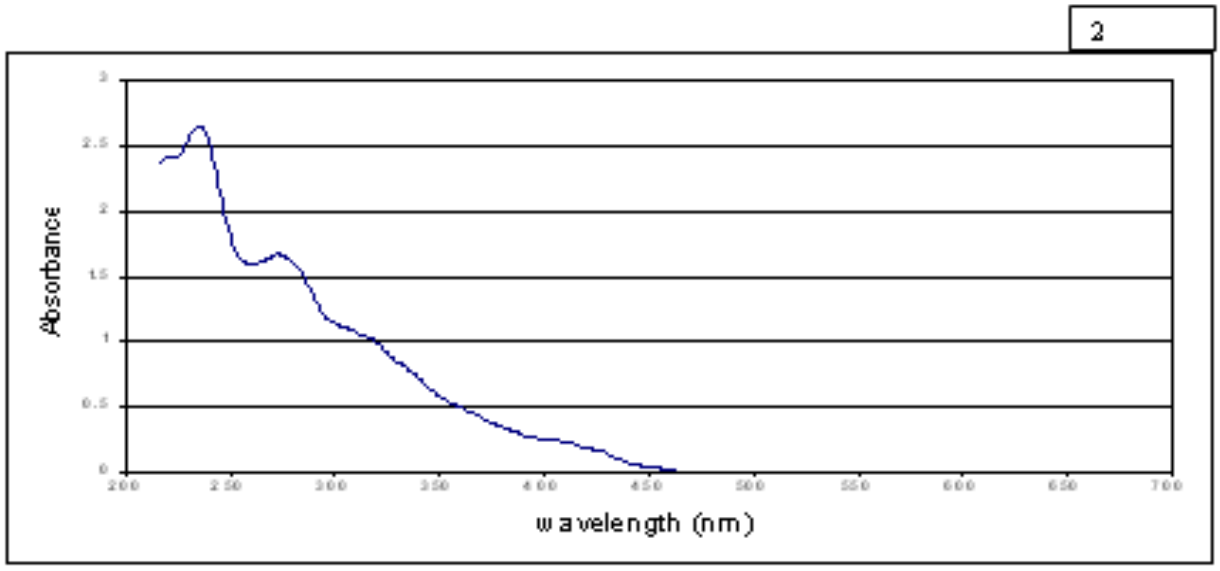


Fig.(1) : Electronic absorption spectra for:(1) Hot aqueous extract,(2) Hot ethanolic extract, (3) Cold ethanolic extract of *Lycium sp.*

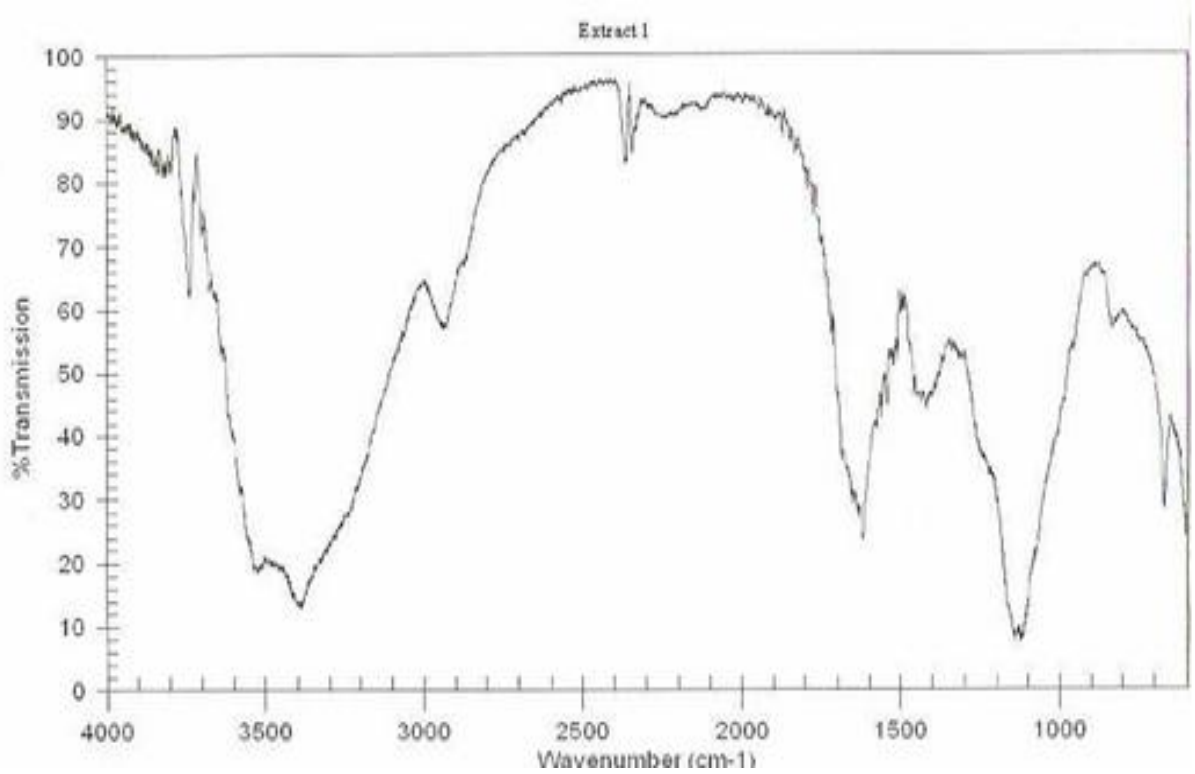


Fig . (2) : IR spectrum for hot aqueous extract of *Lycium sp*

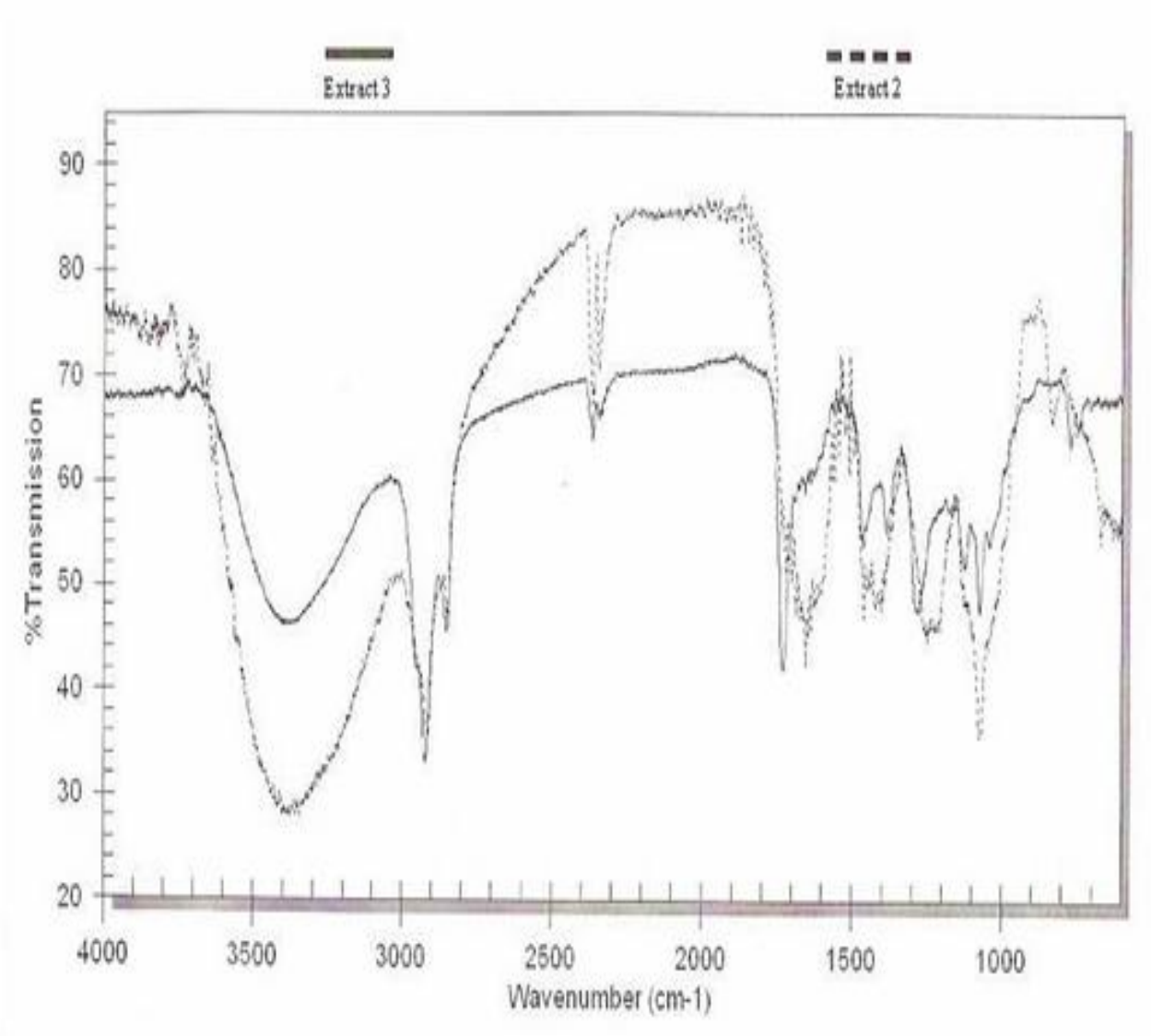


Fig.(3):IR spectra for hot and cold ethanolic extract of *Lycium sp*

Table(7):Inhibition zone diameter average measured in mm. for hot aqueous extract of *Lcium sp.*against some bacterial isolates.

Bacterial isolates	Average of inhubition zone in mm.				
	Concentration% of extracts				Average
	2.5	5	7.5	10	
S.aureus	6.0	6.0	6.0	6.0	6.0
E.Coli	6.0	6.0	6.0	6.0	6.0
P.aeroginosa	7.0	8.5	9.0	12.0	9.125
p.vulgaris	6.0	6.0	6.0	6.0	6.0
K.pnumoniae	6.0	6.0	6.0	6.0	6.0
S.typhi	6.0	6.0	6.0	6.0	6.0
S.dysenteriae	6.0	8.0	10.0	11.0	8.75
V.choelerae	6.0	6.0	6.0	6.0	6.0
Average	6.125	6.5625	6.875	7.375	

Table(8):Inhibition zone diameter average measured in mm. for cold ethanolic extract of *Lcium sp.*against some bacterial isolates.

Bacterial isolates	Average of inhubition zone in mm.				
	Concentration% of extracts				Average
	2.5	5	7.5	10	
S.aureus	6.0	6.3	7.0	8.5	6.95
E.Coli	6.0	6.0	6.0	6.0	6.0
P.aeroginosa	6.0	6.0	6.0	6.0	6.0
p.vulgaris	6.0	6.0	6.0	6.0	6.0
K.pnumoniae	6.0	7.2	8.4	9.0	7.65
S.typhi	6.0	6.3	6.0	8.0	6.575
S.dysenteriae	7.0	8.1	9.0	10.3	8.6
V.choelerae	6.0	6.0	6.0	8.0	6.5
Average	6.125	6.4875	6.8	7.725	

Table(9):Inhibition zone diameter average measured in mm. for hot ethanolic extract of *Lcium sp.*against some bacterial isolates.

isolates	Concentration% of extracts				Average
	2.5	5	7.5	10	
S.aureus	7.0	8.1	9.3	10.0	8.6
E.Coli	6.0	6.0	6.0	6.0	6.0
P.aeroginosa	6.0	6.0	6.0	6.0	6.0
p.vulgaris	6.0	7.1	7.8	8.3	7.3
K.pnumoniae	6.3	7.0	8.5	9.2	7.75
S.typhi	6.5	7.3	7.9	8.2	7.475
S.dysenteriae	7.0	8.1	9.0	12.0	9.025
V.choelerae	6.2	7.0	7.7	8.5	7.35
Average	6.375	7.075	7.775	8.525	

4:Discussion

We can note from table(1) that the extracts are of acidic properties which may be one of the causes of biological activities for these extracts against bacterial isolates. We can also note that the yield percentage of extract was higher on using hot ethanol than hot water and cold ethanol, so hot ethanol was more efficiencies for extracting than the other two solvents in case of this plant .As expected the three extract dissolve in polar solvent (ethanol,methanol,water,…etc.) and do not dissolve in nonpoloar or low polar solvents(hexane,ether,benzene,chloroform, CCl_4,Acetone…etc.) because they consist of polar compounds (table (2)).

It is apparent from figure (1) and table (3) that the pattern of absorption bands is the same in all three spectra and the bands of absorption spctra due to $\pi \rightarrow \pi^*$ transition [20].Spectrum of cold ethanolic extract exhibited one band at 269 nm which nearly remains in spectrum of hot aqueous extract and other band with lower energy(at 404 nm,which is nearly consistence with the bright-green color of cold ethanolic extract) appears too.The band of 270 nm in spectrum of cold ethanolic extract is shifted to lower energy (at 274 nm) in hot ethanolic extract and other band with lower energy (235 nm) appears also in spectrum of hot ethanolic extract.

The more relevant O-H, N-H, C-H,C=O,C-O,C-N stretchings and N-H,O-H bendingsabsorption bands are listed in table(4)[20].

From table (7) , one can observed that the majority of the bacterial isolates did not affect by the aqueous extract of *Lycium sp.* exept for *P.aeroginosa* (inhibition zone diameter average was 12mm at 10% concentration) followed by *Shigella dysenteriae*(inhibition zone diameter average was 11mm at 10% concentration). The concentration of 2.5% of the aqueous extract was less effective ,

while the more effective concentration was 10% of the aqueous extract.

Results of antibacterial activity of cold ethanolic extract of Lycium sp.had been evaluated *in vitro* against eight bacterial species in Table (8), this extract showed higher antibacterial activity against the bacterial species as compared with the water extract, the level of antibacterial activity is a function of the investigated concentration. The most effective concentration was 10 % while the less effective concentration was 2.5 % , five of the eight bacterial species used in this study showed different degrees of sensitivity towards this extract , *S.dysenteriae* was the most effected bacterial species (inhibition zone diameter average was 10.3 mm at 10% concentration) followed by *Klebsiella pnumoniae* (inhibition zone diameter average was 9 mm at 10% concentration)**.**The species *S. typhi* , *S.aureus* and *V.choelerae* were less effected and showed differences in their sensitivity against this extract.

The species *P. aeroginosa, E.coli* and *P. vulgaris* did not show any sensitivity against the cold ethanolic extract ,also both *E.coli* and *P. vulgaris* did not show any inhibition against the hot aqueous extract and cold ethanolic extracts respectively.

Table (7) showed the results of *In vitro* antibacterial activity of hot ethanolic extract towards the bacterial species used in the study, from these results it can be easily seen that this extract was the most effective among the three extracts examined. All bacterial species were effected by this extract except *E. coli* and *P. aeroginosa* , the most affected bacterial species was *S. dysenteriae* (inhibition zone diameter average was 12 mm at 10% concentration) followed by *S. aureus* (inhibition zone diameter average was 10 mm at 10% concentration).

So , hot ethanolic extract was the most effective one , the activity of it may be attributed to the presence of alkaloids ,triterpens,sterols,coumarin and resins which appear to be concentrated in the hot ethanolic extract .The hot ethanolic extract contained resins which are very complex chemical structures results from oxidation of different types of odorant oils or gummous materials ,insoluble in water, but dissolve in organic solvents such as ether and alcohol[24]. These compounds has special chemical affinity for reacting with cell ingredients or may have specific accepters at in the bacterial and fungal cell membrane and also there are suitable carriers which carry resins molecules to inside of the cell to inhibit the action of enzymes ,coenzymes, and other biological active molecules[25].

The cold ethanolic extract of *Lycium sp.* showed somewhat less antibacterial activity. On the contrary the aqueous extract did not show effective antibacterial activity at same concentrations , this is in agreement with [26], except for *P. aeroginosa* which was effected only by the aqueous extract , this may be attributed to the presence of saponin, the antibacterial constituent that the chemical composition of three extracts identified it only in the water extract. This is in agreement with [27].

The most effected bacterial species by the three extracts of *Lycium* sp. was *S. dysenteriae* followed by *S. aureus,* it is well known that these two food borne pathogens which can cause food poisoning , this explains why the internal use for *Lycium* sp. leaf syrup as anti- stomachache and anti- inflammatory drug [28].

The extracts don't show up any cytotoxicity (table(6)) when they tested by

using human blood cells and these results of high interest on study of activity of antibacterial plant extracts where some compounds of plant are toxic in their nature[2].

The use of human blood cells for testing cytotoxicity of compounds or plant extracts in laboratory is simple method ,un costly, and its results are fast and it is considered the first step in the work positively and in the continuity of testing the extracts on human by pharmacy and medicine colleges[29].

Successful prediction of botanical compounds from plant materials is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use primarily water as the solvent but in our study we found that plant extract in organic solvent (ethanol) provided more consistent antimicrobial activity compared to those extracted in water. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay.

In the shadow of wide use of classic plants as antibacterial and antifungal drugs where they have usually wide antibacterial and antifungal range and also they don't have any cytotoxicity on host cells , these plants became very important upon their use in preparing aseptic or antiseptic materials or their use as one of components of chemical drugs[30].

The results of the present study support the folkloric usage of the studied plants and suggest that some of the plant extracts posses compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to the isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

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دراسة كيموحيوية تمهيدية لثلاث مستخلصات من نبات العوسج

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*** كلية العلوم – جامعة ذي قار

الخلاصة

حضرت المستخلصات المائية الحارة والكحولية الباردة والحارة لـ *Lycium sp.* وأجريت الكشوفات النوعية لتحديد التركيب الكيميائي العام،كذلك أستحصلت أطياف ألمنطقة ألمرئية وفوق ألبنفسجية.اختبرت ألفعالية ألضدمايكروبية للمستخلصات بطريقة ألانتشاربالأكار.استخدمت خلايا ألدم ألحمراء لتحديد ألسمية ألخلوية للمستخلصات بتركيز 500 ملغم/مل ولم تلاحظ تأثيرات سمية باستخدام هذا التركيز،إذيعتبرهذاالخطوة الاولى في إيجابية العمل وفي الاستمرارية في اختبار المستخلصات على الانسان من قبل كليات الصيدلة والطب .