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Preliminary Biochemical study of Aqueous, Ethanolic and Alkaloids Extracts of *Xanthium Spinosum L.*

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Abstract

An aquous hot and cold aquous extracts and ethanolic extracts beside alkaloids fraction for *Xanthium Spinosum L.* 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 and antifungal activities of extracts were tested by Agar wells diffusion method. Human blood cells were used to determine cytotoxicity for extracts in concentration 500 mg/ml.. No cytotoxic effects were observed by this concentration.

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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].

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

In Iraq, there are many plants and herbs that need 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 *Xanthium SpinosumL.*(which belongs to compositae family(Asteraceae)) extracts and their activity towards some clinical bacterial and fungal isolates .

Materials and Methods

Preparation of plant material

The plant *Xanthium Spinosum L.*(Al-Hisak) was collected from Qalat suker in Thi_Qar governorate at June and July months , gratefully classified by Dr.Abdul Ratha Akbar Alwan Al-Mayah at Biology Department, College of Science, University of Basrah, and washed well with tap water and then distilled water. The washed plant was placed on filter paper type whatman No.15 in dry place with good aerification and was turned over continuously to prevent decay of plant. Then it was kept on freezer till use.

Preparation of the Extracts

Preparation of hot aqueous and ethanolic extracts

Both hot aqueous or ethanolic extracts were prepared by extracting 20 gm of dry plant with 500ml of distilled water or ethanol using soxhelet extractor . The extraction was carried out for 24 hours. Then the extract was filtered and concentrated to one-quarter of the original volume by direct heating. The residual solution was left in Petri dishes 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[5].

preparation of the cold aqueous and ethanolic extracts

Both cold aqueous or ethanolic extracts were prepared by mixing 20 gm of dry plant with 500ml of distilled water or ethanol (95%). The mixture was stirred manually from time to time for 24 h at laboratory temperature, filtered and the filtrate was left in Petri dishes to dry at laboratory temperature. All the steps have been repeated many times to obtain the enough amount of crude extract. The crude extracts were collected and kept in the laboratory until use[5].

Extraction of Alkaloids

Dried plant (20gm) tissue was treated with 250 ml of 10% acetic acid in ethanol, and left for 24 hours, then filtered. The filtrate was concentrated to one-quarter of the original volume and precipitation of the alkaloid was made by drop wise addition of conc. NH_4OH.The residue was

collected by centrifugation, and washed with 1% NH_4OH. The washed residue was dissolved in a few drops of chloroform, and left to dry at laboratory temperature ,this represent a crude alkaloid[6].

Qualitative Tests

Several qualitative tests for extracts have been carried out to know their general chemical composition[6-15] . These tests were for alkaloids, glycosides, saponins, carbohydrates, tannins, ninhydrin, resins, vanillin-H_2SO_4, triterpenes, steroids, sodium fusion, solubility and pH test [9] .

Electronic Spectra Measurements

Electronic spectra were performed by UV Win 5.0 spectrophotometer at Chemistry Department, College of Science, University of Thi-Qar. It was used a quartz solution cell of 1 cm path length in the region (200-800)nm at the laboratory temperature. The solvents were distilled water and ethanol (95%), and the concentration of spectral solutions was 0.01 gm/60 ml[16].

Infrared Spectra Measurements

Infrared spectra were recorded on a FTIR-8400S shimadzu spectrophotometer at chemistry department, college of science, of Al-Mustansyria University.

Determination of Biological Activity

During this study obtained the bacterial and fungal isolates from mycology and bacteriology laboratory / college of science / university of Basrah.

1-Determination of Antibacterial Activity

The bacterial isolates, were cultured by streaking method on Nutrient Agar (NA) (Oxid) incubated 5 ml from one young colony of growth in age of 24 hours in Nutrient broth NB Oxide for 6 hours at 37^0C thus obtaining bacterial growth 10 cell/ml. After that 0.1 ml from growth was spread on Muller-Hinton (Difco), wells of 6mm diameter were made on surface of Agar and filled with 100µl extract of concentration 250, 500 mg/ml ,then Petri dishes were incubated at 37^0C for 24 hours .

2-Determination of Antifungal Activity

Agar wells diffusion method is used by doing digs on sabourauds dextrose Agar media as follows:

1- Dextrose sabourauds Agar media is used to activate the fungal isolates at 27^0C for 7 days to *Aspergillus flavus* and for 3 days to *Candida albicans* and *Cryptococcus neoformans.*
2- Fungal suspension (0.2 ml) of concentration 3×10^6 colony form unit/ml compared with Macfarland tube(1) is taken by using sterilized volumetric pipette and is poured on culture media. The suspension of *Aspergillus flavus* is then spread using L-shape sterilized by ethanol and flame. Then, the Petri dishes were left for 1 hour until the suspension dried but the yeast isolates was streaked on the culture media.
3- By using sterilized cork borer, wells were done on the media in diameter of 6mm and filled by 100µl of extract(in concentration 500mg/ml and 250 mg/ml).
4- The Petri dishes incubated at 27^0C for 7 days to *Aspergillus flavus* and 3 days to *Cryptococcus neofomans* and *Candida albicans* so the results were recorded by measuring the average of diameters inhibition zone[5,17,18].

Determination of cytotoxicity

Human blood cells were used to determine cytotoxicity of the two extracts according to ref.[19]. Concentration of 500mg/ml of extract in phosphate buffer saline were prepared for both extracts .Also negative control contain 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 four test tubes at 37^0C for 3 hours was made. Hemolysis was then followed

Results

Table (1): Physical properties for extracts of *Xanthium Spinosum L.*

No.	Extracts Type	Color	appearance	%Yield of Extracts	pH
1	Hot aqueous extract	Dark brown	Viscous	10.49	8-9
2	Cold aqueous extract	Dimmed green	Powder	11.93	9-10
3	Hot ethanolic extract	Bluish green	Viscous	33.9	8
4	Cold ethanolic extract	Green	Powder	0.47	6
5	Alkaloids	Blackish brown	Semi-powder	0.815	-

Table (2): Qualitative tests for extracts of *Xanthium Spinosum L*

No.	Tests	Hot aqueous extract	Cold aqueous extract	Hot ethanolic extract	Cold ethanolic extract	Alkaloids
1	Alkaloids test					
a	Dragendroff reagent	+	+	+	+	+
b	Wagner's reagent	+	+	+	+	+
2	Glycosides test	-	-	-	-	-
3	Saponins test					
a	Forming dense foam	+	+	+	-	
b	Mercury(111)chloride test	+	+	+	-	
4	Carbohydrates test					
a	Phenol-conc.H_2SO_4 test	+	+	+	+	
b	Molish test	+	+	+	+	
5	Tannins test					
a	Lead acetate test(1%)	+	+	+	+	
b	Ferric chloride test(1%)	+	-	+	-	
6	Ninhydrin test (1%)	+	-	+		
7	Resins test	-	-	+	+	
8	Fusion with Sodium test					
a	Sulfer test	-	-	-	-	-
b	Nitrogen test	+	+	+	+	+
9	Solubility tests					
a	Solubility in Water					
b	Solubility in Ether	+			-	-
c	Solubility in HCl (5%)	+	+	+	-	-
d	Solubility in NaOH(5%)		+	+	+	+
e	Solubility in $NaHCO_3$ (5%)				-	-

Table (3): Cytotoxicity for extracts of *Xanthium Spinosum L.* against red blood cells.

Concentration of extract	The hemolysis				
	Hot aqueous extraction	Cold aqueous extraction	Hot ethanolic extraction	Cold ethanolic extraction	Alkaloids
500mg/ml	-	-	-	-	-
250mg/ml	-	-	-	-	-
Negative control	-	-	-	-	-
Positive control	+	+	+	+	+

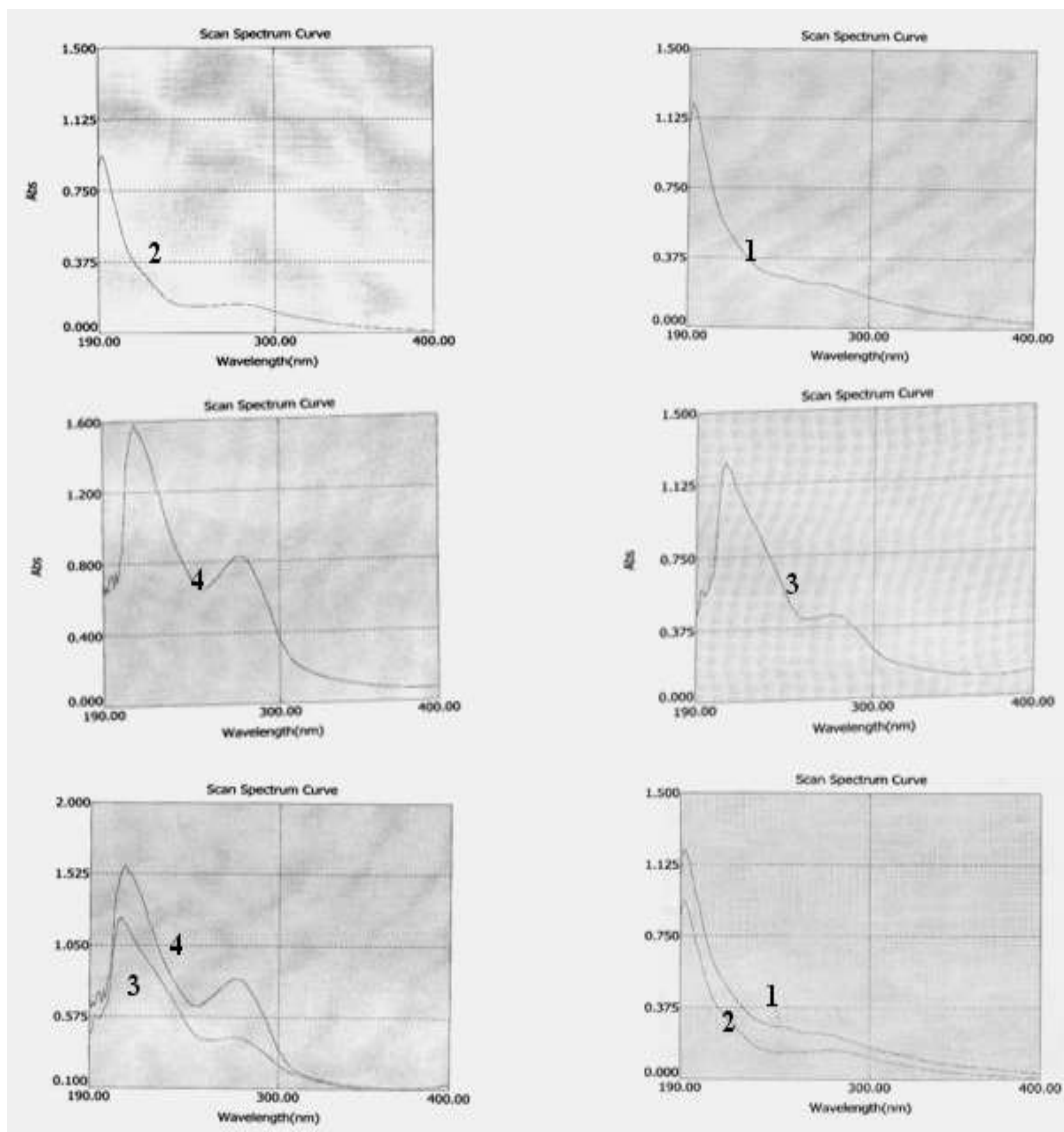
*Nagative control: Phosphate buffer saline
*Positive control: Tap water
*(-): No hemolysis
*(+): Hemolysis was found

Table (4) : Absorption data of hot and cold aqueous and ethanolic extracts of *Xanthium Spinosum L.*

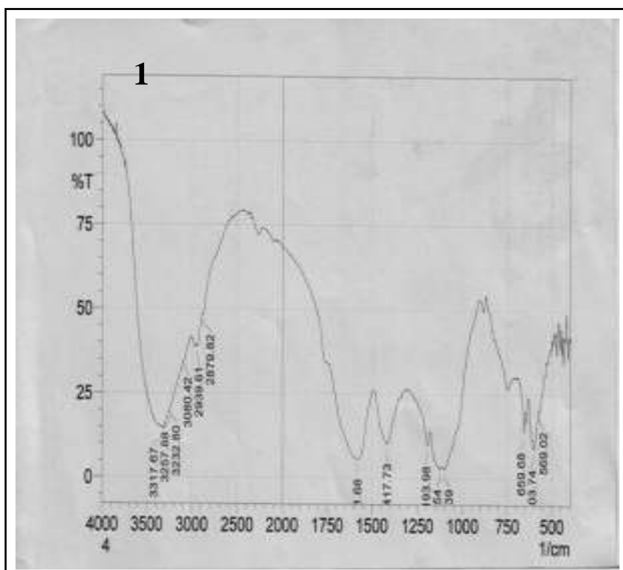
Extract	λ_{max} ,nm	Absorbance
Hot aqueous	192	1.203
Cold aqueous	192 278	0.936 0.152
Hot Ethanolic	208 274	1.237 0.439
Cold Ethanolic	211 275	1.567 0.834

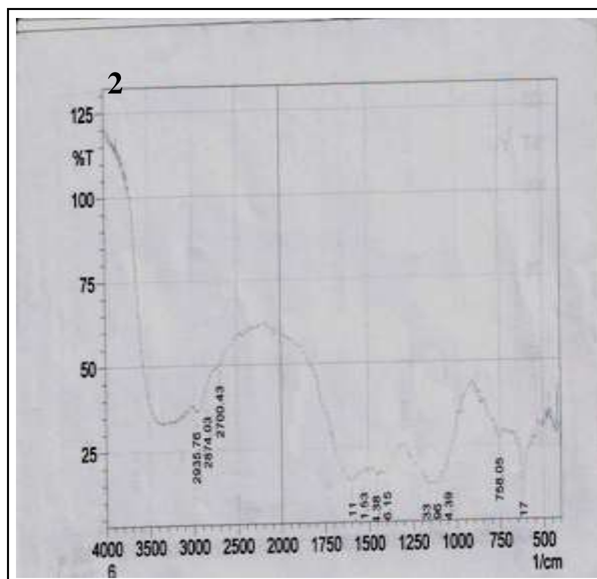
Table (5):FTIR data of extracts of *Xanthium Spinosum L*.

Extract	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. (cm^{-1})	AmideI&II (cm^{-1})	C-H bend &C-N Str. (cm^{-1})	Methylene Twisting &Wagging Vib. (cm^{-1})
Hot aq. Extract	3317.67 3257.88 3232.8 3080.42	2939.61	2879.82	-	1581.68	1417.73	1193.98 1124.54 1101.39
Cold aq. Extract	~3500- ~3000	2935.76	2874.03	-	1597.11(amide1) 1531.53(amide2)	1454.38 (C-H bend) 1406.15 (C-N Sto.)	1157.33 1112.96 1074.39
Hot Ethanolic Extract	3419.9 3363.97	2920.32	2850.88	1739.85	1670.41		
Cold Ethanolic Extract	3381.33 3356.25 3335.03 3219.3	2926.11	2852.81	1743.71	1.666.55(amide1) 1631.83(amide2)	1413.87 1381.08	1193.98 1165.04 1124.54 1078.24 1053.17
Crude Alkaloid	3223.16 3178.79	2922.25	2850.88	1724.42	1631.83	1410.01 (C-H bend) 1379.15 (C-N Sto.)	

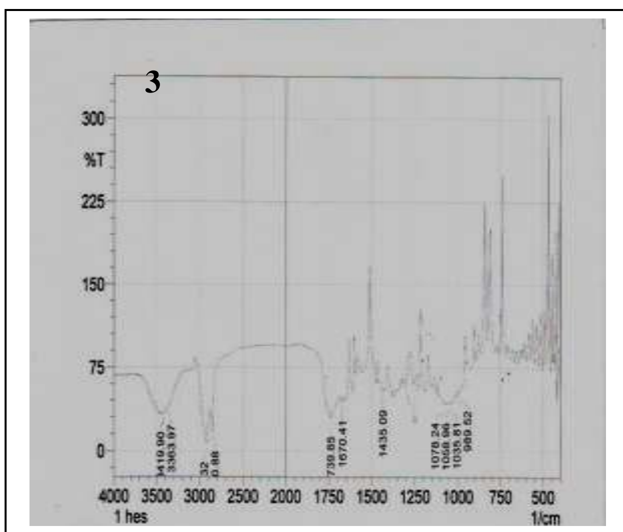


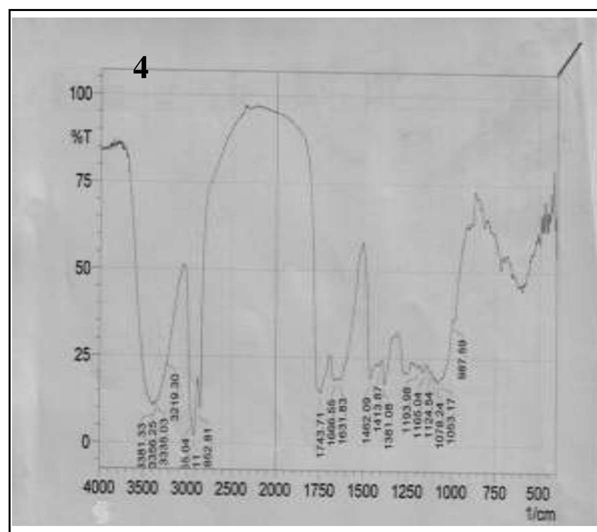
Figure(1):Electronic absorption Spectra for: (1) Hot aqueous extract, (2)Cold aqueous extract, (3) Hot ethanolic extract, (4) Cold ethanolic extract of *Xanthium Spinosum L.*



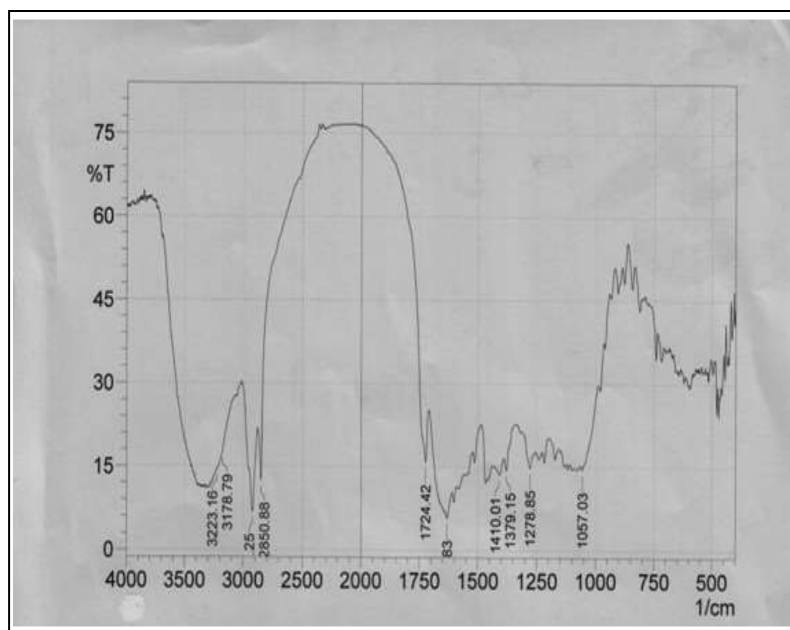


Figure(2): FTIR spectra for: (1) hot aqueous extract,(2) cold aqueous extract.

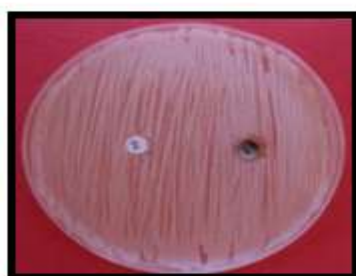




Figure(3): FTIR spectra for: (3) hot ethanolic extract,(4) cold ethanolic extract.



Figure(4): FTIR spectrum for **alkaloid** extract.



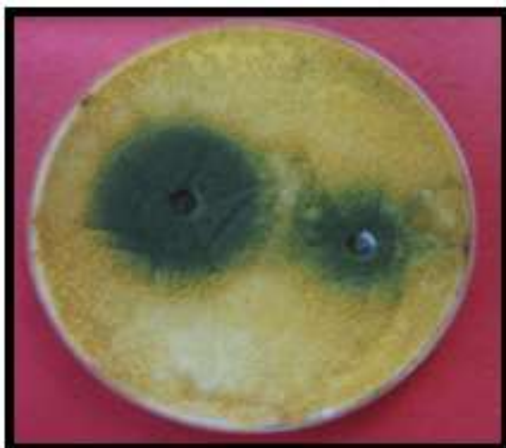
Staphylococcus aureus

Streptococcus pyogens

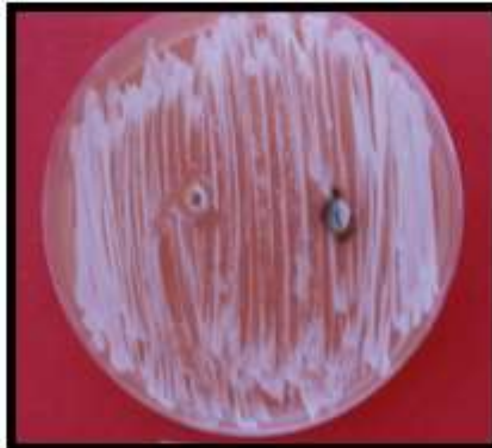
Escherichia coli

Klebsiella sp.

Figure (5): Sensitivity of bacterial isolates: *Staphylococcus aureus,streptococcus pyogenes,Escherichia coli,*and *klebsiella sp.* against alkaloids extracted from *Xanthium Spinosum L*. (1) in concentration 500mg/ml. (2):control (Chloroform).



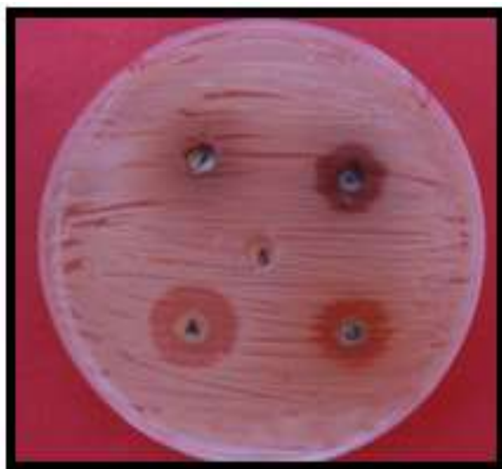
Aspergillus flavus



Candida albicans

Cryptococcus neoformans

Figure (6): Sensitivity of fungal isolates:*Aspergillus flavus,candida albicans*, and *cryptococcus neoformans* against alkaloids extracted from *Xanthium Spinosum L.* (1) in concentration 500mg/ml. (2):control (Chloroform).



Staphylococcus aureus

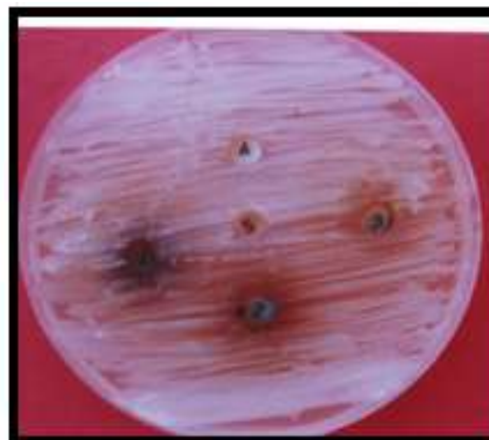
Streptococcus pyogens

Escherichia coli

Klebsiella sp.

Figure (7): Sensitivity of bacterial isolates: *staphylococcus aureus, streptococcus pyogens,Escherichia coli,*and *klebsiella sp*. against: 1-hot aqueous extract. 2- cold aqueous extract. 3- hot ethanolic extract. 4- cold ethanolic extract of *Xanthium Spinosum* in concentration 500mg/ml for all. 5- control: (Ethanol).

Aspergillus flavus



Candida albicans

Cryptococcus neoformans

Figure (8):Sensitivity of fungal isolates: *Aspergillus flavus, candida albicans,*and *Cryptococcus neoformans* against: 1- hot aqueous extract. 2- cold aqueous extract. 3-hot ethanolic extract. 4- cold ethanolic extract of *Xanthium Spinosum L.*in concentration 500 mg/ml for all. 5- control (Ethanol).

Table (6): Inhibition zone diameter average measured in mm for extracts of *Xanthium Spinosum L.*in concentration 500 gm/ml against some bacterial and fungal isolates.

No.	Bacterial and fungal isolates	Average of diameters inhibition zone in mm.					
		Hot aq. extract	Cold aq. Extract	Hot ethanolic extract	Cold ethanolic extract	Alkaloids	Average
1	*Staphylococcus aureus*	0	15	15	19	8	11.4
2	*Streptococcus pyogens*	18	2	19	23	8	14.4
3	*Escherichia Coli*	19	0	17	22	14	11.2
4	*Klebsiella Sp.*	20	0	16	21	15	14.4
5	*Aspergillus flavus*	0	0	0	0	0	0
6	*Candida albicans*	0	18	11	0	8	7.4
7	*Cryptococcus neoformans*	0	10	8	0	8	5.2
Average		8.14	6.42	12.3	12.14	8.71	

Table(7): Inhibition zone diameter average measured in mm for extracts of *Xanthium Spinosum L.* in concentration 250 gm/ml against some bacterial and fungal isolates.

No.	Bacterial and fungal isolates	Average of diameters inhibition zone in mm.					
		Hot aq. extract	Cold aq. extract	Hot ethanolic extract	Cold ethanolic extract	Alkaloids	Average
1	*Staphylococcus aureus*	0	15	10	15	8	9.6
2	*Streptococcus pyogens*	18	0	10	11	8	9.4
3	*Eschoichia Coli*	12	0	8	15	8	8.6
4	*Klebsiella Sp.*	13	0	10	12	0	7
5	*Aspergillus flavus*	0	0	0	0	0	0
6	*Candida albicans*	0	13	8	0	8	5.8
7	*Cryptococcus neoformans*	0	6	0	0	0	1.2
Average		6.14	4.85	6.57	7.57	4.571	

Discussion

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 [20] .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[21].

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[22].

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[23].

Many studies indicated to use of this plant as, antiparasital ,antiviral, antibacterial ,and antifungal because it contains many active compounds such as alkaloids ,phenols,resins,and which agree with results in our study [24](table 2).

We can note from table(1)that the extracts are of basic properties which indicate that biological activities are not due to acidity of extracts . we can also note that the existence of resins in alcoholic extracts and their absence in aqueous extracts may be the reason of the high inhibition activities for alcoholic extracts in comparing with aqueous extracts (tables 6&7).

The resins 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[25].

The present study agree also with[26] which showed that *Mastic lenticus* plant contained resins which are antimicrobial active. 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[27].

It is apparent from figure (1)and table (4)that the pattern of absorption bands is the same in all spectra and the bands of absorption spectra due to $\pi \rightarrow \pi^*$ transition[16].Spectrum of hot aqueous extract exhibited one band at 192 nm which remains in spectrum of

cold aqueous extract and other band with lower energy (at 278 nm) appears too. Both ethanolic extracts showed up two bands ,the first band at 208 nm is shifted to lower energy in cold ethanolic extract(at 211 nm) ,while the second lower energy band remains approximately itself in both ethanolic extracts. The bands in ethanolic extracts are of lower energy in comparing with bands in hot aqueous extract and that may be related to rather more rigid compounds in the last[27].For the same reason, the compounds in cold ethanolic extract are of lower rigidity than compounds in hot ethanolic extract[28].

The more relevant N-H, C-H, amideI&II stretching and C-H&C-N bendings,methylene twisting and wagging absorption bands are listed in table(5)[16].

The plant extracts may influence different microbes, so they called broad spectrum antibiotics or influence one specific pathogenic microbes, but not the other and here they called narrow spectrum antibiotics[29].

In general, the extracts influenced both gram positive and gram negative bacterial isolates but there are obvious variation in the effect on fungal isolates (tables 6&7and figures5&6).The yeast *Candida albicans* was more affected and the cause may be not to have a thick capsule surrounds its walls as in the yeast *Cryptococcus neoformans* or the cause due to existence of conidia ,thick walls, and high ability on a bearing unsuitable circumstances as in *Aspergillus flavus*[30] which showed up high resistance against the extracts.

In general, the variation in the sensitivity of fungi towards different plant extracts belong to fungi nature itself from where structure, its cellular membranes thickness, and its contents of lipids , proteins and the relation of these with mechanism of action of the active compounds of extracts [31].

The crude alkaloids were found of lower biological activity in comparing with other crude extracts. The cause may be the impurity and necessity of isolation and purification of an alkaloid compound and or the cause may be the microscopic creature itself that it has mechanisms enable it for resisting crude alkaloids[6].

The extracts don't show up any cytotoxicity (table3) when they tested by using human blood cells and these results of high interest on study of activity of antibacterial and antifungal 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 lab 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[32].

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[33].

المصادر العربية

1. حسين ، فوزي طه قطب(1981). النباتات الطبية، زراعتها ومكوناتها. دار المريخ للنشر، الرياض.
2. كريم ، فوزي محمد فرحان ، صالح احمد(1986). النباتات الطبية في الأردن. منشورات جامعة اليرموك. الاردن.
3. القاطع، جاسم محمد؛ النجار، جلال مصطفى وشرف، صابر محمد (1982). مقدمة في الكيمياء العضوية.الطبعة الاولى، جامعة صلاح

الدين.

4. انتريكين، جون ب وكيرونيز، نيكولاس د(1983). تشخيص المركبات العضوية. ترجمة د.موفق ياسين شندالة ود. روعة غياث الدين صالح. جامعة الموصل.
5. الحريشاوي، رواء محمد عبيد. رسالة ماجستير- كلية العلوم - جامعة البصرة. (2004).
6. الحسن،سفانة كاظم جاسم.رسالة ماجستير.كلية العلوم.قسم علوم الحياة.(2006).
7. الذهب، أزهار عمران لطيف. رسالة ماجستير-كلية العلوم - جامعة بابل (1998).
8. واثق ستار عبد الحسن. رسالة ماجستير-كلية التربية- جامعة البصرة(2003).
9. نجوى محمد جميل. رسالة ماجستير- كلية العلوم - جامعة البصرة. (2005).
10. هميم، سعد سلمان. رسالة ماجستير-كلية التربية- جامعة البصرة (2003).

Foreign References

11. Glombitza, K.W.; Makran,G.H.; M.rhon,Y.W.; Michel,K.J. and Motawi, T.K. (1994). Planta Med.60:244-247.
12. Lee,C.K.J.Pharm.21(1):62-66(1998).
13. Souri , E. ;Amin , G. ; Sharifabadi , A.D. ; Nazifi , A. and Firsam , H. (2004) J. of pharmaceutical Research. 3: 55-59 .
14. Harborn, J.B. Photochemical methods a guide to modern techniques of plants analysis.2nd ed. chapman and Hall, London, New York. (1984)
15. Saadalla,R.A.Biochemistry Practical ,manual.College of medicine,Basrah. (1980)
16. Meyer, E & Walter, A.J.Arch. Hydro boil. 13:161-177(1988).
17. Ahmed,M.; Nazil,S.& Anwar, N.M.J.Chem. Soc. Paki.11:213-217(1989).
18. Al-Khazraji, S.M.M.Sc.thesis. University of Baghdad (1991).
19. Richard, J.P.C.Natural products Isolation. Humana Press, Totowa, New Jersey. (1998)
20. Adedayo, O.;Anderson, W.A.; Moo-Young, M.; Sncickus,V.; patil, P.A.&Kolawole,D.O.Pharmaceutical Biology.39:1-5(2001).
21. Silverstein, R.M.; Bassler, G.C. and Morrill,T.C.Spectometric identification of organic compounds. 4th ed. John Wiley and Sons, Inc. USA. (1981)
22. Egorove,N.S.Antibiotics a Scientific approach.Mir Publisher,Moscow. (1985)
23. Hammer,K.A.; Carson,C.F. and Riley,T.V.Applied.Microbial.J.86:985 -990Pe..(1999)
24. Xian – gun, H.and urasella, M.J.Ethnpharm, 43 : 173-177(1994).
25. Bull,A.T.;Ward,A.C.and Good fellow, M.Microbiology and Molecular biology Reviews.64:573-606 (2003)
26. Carter,B.K.J.Bio.Science.46:260-271 (1996).
27. Ayush, K. and Herbert, P. S. Nat. Acad. Pre.27:1486-1513 (2005)
28. Rizk , A.M. and El-Ghazaly , G.A. applied Research Center , Doha , Qatar . 306 p (1995)
29. Al-Ani,A.B.J.of Al-Anbar.Univ. (1996)
30. Hancock,R.E.W and Wong,P.G.V. 26(1):48-52 (1984).
31. Collee, J.; Fraser, A.; Marmion, B.& Simon, A.14 th ed. Churchill Liverstone.New York.978 P (1996).
32. Ian,W.D.&Ian,W.S.Microbial Physiology.Black Well Scientific Publications.London. 12-18 (1976)
33. Ahmed, I.; Mehmood, Z. and Mohammad, F. J. Ethnopharmacole. 62:183-193 (1998).

دراسة بايوكيميائية اولية للمستخلصات المائية و الكحولية و القلويات الخام لـــ

Xanthium Spinosum L.

واثق ستار عبد الحسن

قسم الكيمياء- كلية العلوم- جامعة ذي قار

الخلاصة

تم تحضير المستخلصات المائية والكحولية الحارة والباردة بالاضافة الـــى مســـتخلص القلويـــدات لـــــــ *Xanthium Spinosum L.* لقداجريت الكشوفات النوعية للكشف عن التراكيب الكيميائية العامة لها كذلك تم الحصول على اطياف الاشعة المرئية وفوق البنفسجية . اختبرت فعاليات المستخلصات ضد البكتريا والفطريـــات باستخدام طريقة الانتشار بالاكار.استخدمت كريات دم الانسان لتحديد السمية الخلويـــة للمستخلصـــات بتركيـــز 500 ملغم / مل ولم تلاحظ تأثيرات سمية باستخدام هذا التركيز