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THE CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF

SUAEDA SP. AQUEOUS AND ALCOHOLIC EXTRACTS

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SUMMARY

An aqueous, cold and hot ethanolic extracts fraction for stems and leaves *Suaeda* sp. have been prepared. Qualitative tests have been carried for detection of their general chemical composition, and UV- visible spectra were also obtained and demonstrated that the absorption bands is the same in all three extracts and the bands due to $\pi \rightarrow \pi^*$ transition, the absorptionfor both ethanolic extracts (hot and cold) are very similar,IR absorption bands exhibit the similarity in frequenciesthis emphasize on similarity in structures for ethanolic extracts.IR absorptions with accordance to chemical qualitative

tests.The yield percentage of extracts was higher with using hot water than hot and cold ethanol. The antibacterial activity of the extracts was tested by the well diffusion method, the hot ethanolic extract at the concentration 10% was the most effective against the bacteria used in this study.*Klebsiella pnumoniae* followed by *Proteus vulgaris* were highly affected by the three extracts, while *Escherichia coli*, *Salmonella typhi*, and *Vibrio choelerae* did Human blood was used to .not show any sensitivity against these extracts determine cytotoxicity for the extracts in the 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.

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

Introduction

Medicinal plants are important elements of traditional medicine in virtually all cultures. The idea that certain plants had healing potential was known long before human being discovered the existence of pathogens. Medicinal plants which have been used by human being to treat common infectious diseases are important elements of traditional medicine. During the last years, traditional medicine has not been limited to specific culture. It has been used in developing countries as well as its using extended to developed countries (1).Huge numbers of antimicrobial agents have been discovered, the pathogenic microorganisms are developing resistance against these agents day by day. In third world countries, irrational use of antimicrobial agents is a major cause of such resistance. In recent years, attempts have been made to investigate the indigenous drugs against infectious diseases (8). Researches in the field of indigenous plants are a significant aspect of developing a safer antimicrobial principle through isolation, characterization, identification and biological studies (12).The genus *Suaeda*, which is belonging to the family (Chenopodiaceae), comprises approximately 110 species which distributed in tropical to subtropical regions in the world. Medicinally this plant is used for the treatment of the wounds. It is laxative, diuretic and emetic. It increases the menses, ophthalmic diarrhea, eyesight and excess doses cause abortion and induce vomiting (13).The present study was undertaken to determine the chemical composition and antibacterial activity of *Suaeda* sp. collected from Basrah city, Iraq.

2 –Materials and Methods

2-1 Preparing plants for study*:* -

The plants (stem and leaves) were collected from Abul - Khasseb region in Basrah city south of Iraq in April 2005, and dried them at room temperature in dark condition and milled them electronically to a coarse powder and stored.

2-2 Preparing the extracts:-

Both hot aqueous and ethanolic extracts were prepared by putting 20 gm of plant powder in vessels made of extraction thimble filter paper, these were put inside soxhelet extractor by using 250 ml of distilled water and ethanol (95%).The extraction was carried out for 24 hours, and then the solvent extract was filtered and concentrated at 70° to half. The residue solution was left in Petri dishes to dry at laboratory temperature. The extract powders were collected and kept in the laboratory until use. (16)

Cold ethanolic extract was prepared by mixing 20 gm of plant with 250 ml of ethanol (95%) with stirring for 24 hours at laboratory temperature. Then the extracts were filtered .The filtered was left in Petri dishes to dry at laboratory temperature. The extract powder were collected and kept in the laboratory until use. (6)

2-3 Qualitative tests: -

Several qualitative tests have been carried out to know the general chemical species which is found in the extracts .Test solutions were prepared according to Harborn and Cowan, (6,4), kept in dark container at -18 ^{o}C until use. These tests involved:Alkaloids, glycosides ,saponins ,carbohydrates ,flavonoids , tannins , ninhydrin,resins,fuocoumarins ,triterpenes ,steroids ,unsaturation ,ethanolic rhodaminB, solubility and pH test according to Harborn and Al-Huraishawi (6,2) .

2-4: Electronic spectra measurements: -

Electronic spectra were obtained on specord 40 analytic jena AG U.V.visible spectrophotometer ,made in Germany at College of pharmacy ,in 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 was 0.005 gm / 5 ml for both aqueous and ethanolic extract ants.

2-5: Infrared spectra measurements

Infrared spectra were recorded on Buck Scientific Inc.Infrared spectrophotometer model 500 at chemistry Department College of education of Basrah University.

2-6: Determination of cytotoxicity for aqueous and ethanolic extracts:

Human blood was used to determine the cytotoxicity (10 samples were used, 4 female and 6 male, the results were identical) of the three extracts according to Xian-guo, *et al*. (18) .Concentration of 500mg / 5 ml of phosphate buffer saline for each extract was prepared. Also positive control contained only phosphate buffer saline and negative control (tap water, which contains salts that cause shrinkage to the blood) have been used. Then, 0.2 ml of blood was added to sterile test tube contained 0.8 ml of extract to reach a total volume of 1 ml. The same was made for both controls. The five test tubes were incubated at 37^{o} C for 3 hours to observe hemolysis.

2-7: Microbial cultures:

The following bacterial isolates 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

These isolates were isolated from Iraqi soil and water, identified in Marine Science Centre, Marine bacteria Laboratory then used in the study.

2-8: Antibacterial activity:

The antibacterial activity was determined by the well diffusion method according to NCCLS (9). Three to five identical colonies from each plate were lifted within a sterile wire loop and transferred to test tube containing 5 ml trypticsoy 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}C FU/ ml, McFarland standard were prepared by mixing 995.5 ml of sulfuric acid 1%

with 5.00 ml of Barium chloride 1%. 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 60^0 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, 6 mm 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 37^0 C for 18 hours. Subsequently, the plates were examined for bacterial growth inhibition and the inhibition zone diameter measured to the nearest millimeter.

Results:-

Table 1: - Physical Properties for *Suaeda* sp. extracts.

No. of extract	Type of extract	Color of extract	%of extract	Appearance of extract	Taste of extract	pH of extract*
1	Hot aqueous extract	Brown	21 %	Powder	Salty	6.7 – 6.8
2	Hot ethanolic extract	Dark - green	7 %	Viscous	Bitter	4.8– 4.9
3	cold ethanolic extract	Dark - green	5 %	Viscous	Bitter	4.6 - 4.7

*= (0.2gm / 10 ml) at 38 ^{o}C

Table 2: Solubility in common solvents at 38 ^{o} C

Solvent	**Extract 1**	Extract 2	Extract 3

		Very slightly soluble	Slightly soluble
Acetone	**Not soluble**	Slightly soluble	Slightly soluble
Benzene	Not soluble	Slightly soluble	Slightly soluble
Butanol	Not soluble	Slightly soluble	Very slightly soluble
$CHCl_3$	Not soluble	Slightly soluble	Very slightly soluble
CCl_4	Not soluble	Soluble	Very soluble
	Very slightly soluble	Slightly soluble	Slightly soluble
Ethanol	Not soluble	Not soluble	Not soluble
Ether	Not soluble	slightly soluble	Soluble
Hexane	Very slightly soluble	Very slightly soluble	Very slightly soluble
Methanol	Very soluble		
Water			

Table3: U.V. visible absorption data for *Suaeda* sp. Extracts

extract	Λ Max$^{(nm)}$	Absorbance
Hot aqueous	214, 268	3.7356, 1.8293
Hot ethanolic	216,272,406	2.8591,1.1480, 0.3843
Cold ethanolic	218, 273, 400	2.7773,0.9686,0.4816

Table (4): IR data of extracts of Suaeda sp.

extract	O-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 aqueous	3400	2950	2930	1640	1640	1410 1325	1320,1340
Hot ethanolic	3360	2925	2850	1730	1630	1400	1050,1100
cold ethanolic	3350	2925	2850	1730	1630	1400	1040,1080

Table 5: Specific test for aqueous and ethanolic extracts of Suaeda

Reagents	Extract 1	Extract 2	Extract 3
Carbohydrates :			
by phenol conc.-H_2SO reagent	+	+	+
by Molish reagent	+	+	+
Glycosides			
A- before the hydrolysis	-	-	-
B- after the hydrolysis	-	-	-
Tanins			
by lead acetate (1%)	+	+	+
by Ferric chloride (1%)	+	+	+
Ninhydrin (1%)	+	+	+
Phenols			
A- by $FeCl_3$	+	+	+
B- by Folin reagent	+	+	+
Bayer reagent(unsaturated test)	+	+	+
Oxygen test	+	+	+
Triterpens & strols by Liberman Burchard reagent	−	+	−
Triterpenoids test	+	+	+
Triterpenes & steroids test	+	+	+
Alkoloids test			
A- by Dragendroff reagent	-	+	+
B- by Mayer reagent	-	+	+
C –by Wagner reagent	-	+	+
D – by ninhydrin reagent	-	+	+
Flavonoids			
A - by KOH	-	-	-
B - by H_2SO_4	-	-	-
Coumarin		-	
Fuocoumarins	-	-	-
Resins		+	
Saponins	-	-	-
Lipids by ethanolic rhodamine B (0.5%)	-	-	-
Solubility: in HCl (5%)	+	-	-
in NaOH (5%)	+	+	+
in $NaHCO_3$ (5%)	+	+	+

Table 6: - Results of cytotoxicity for hot aqueous and ethanolic extracts

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

(-) = No Lysis .
(+)= Hemolysis is found.

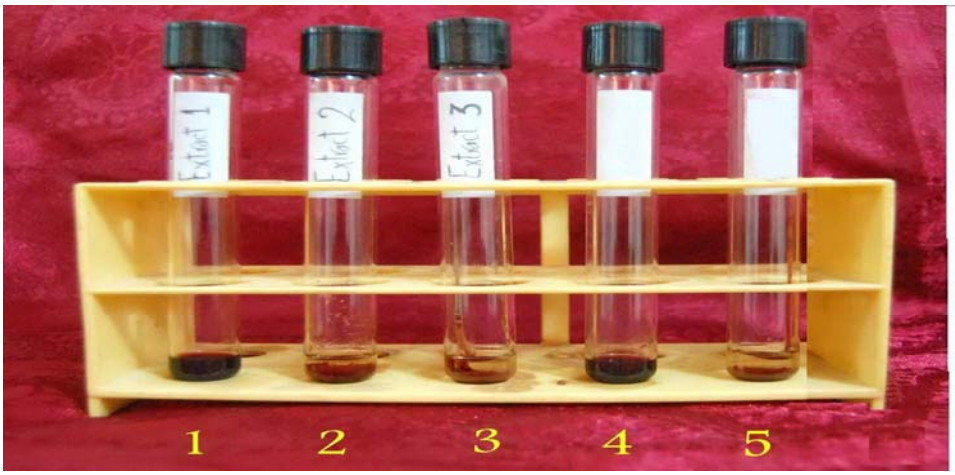
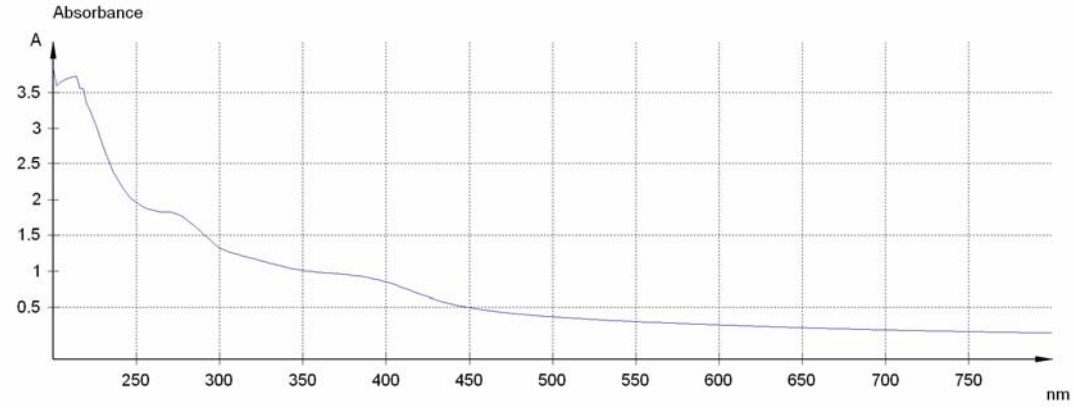
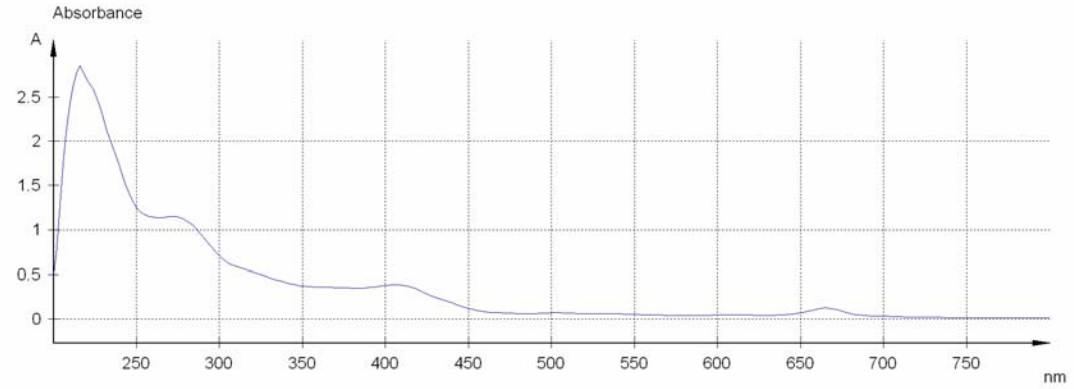


Figure 1 : Cytotoxicity of aqueous and ethanolic extract for *Suaeda* sp. against red blood cells :
1 – Hot aqueous extract.
2 - Hot ethanolic extract.
3 – Cold ethanolic extract.
4 – Tap water.
5 – Normal saline.

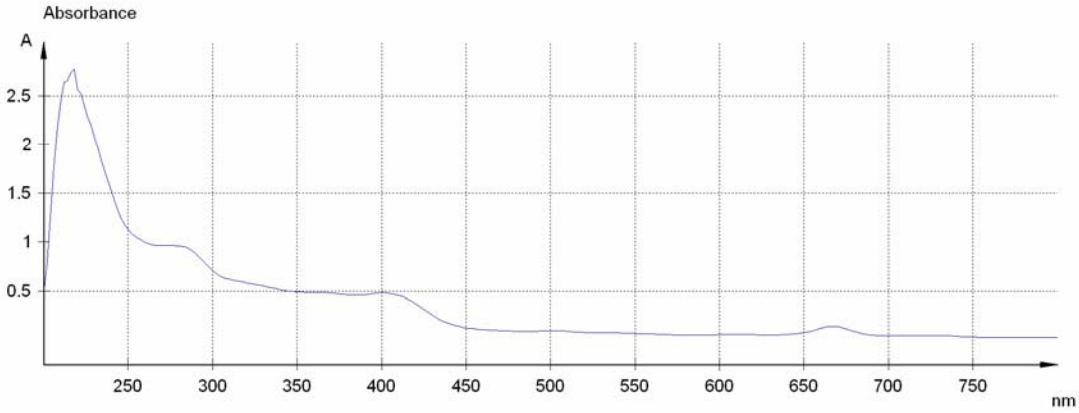
Extract 1



Extract 2



Extract 3



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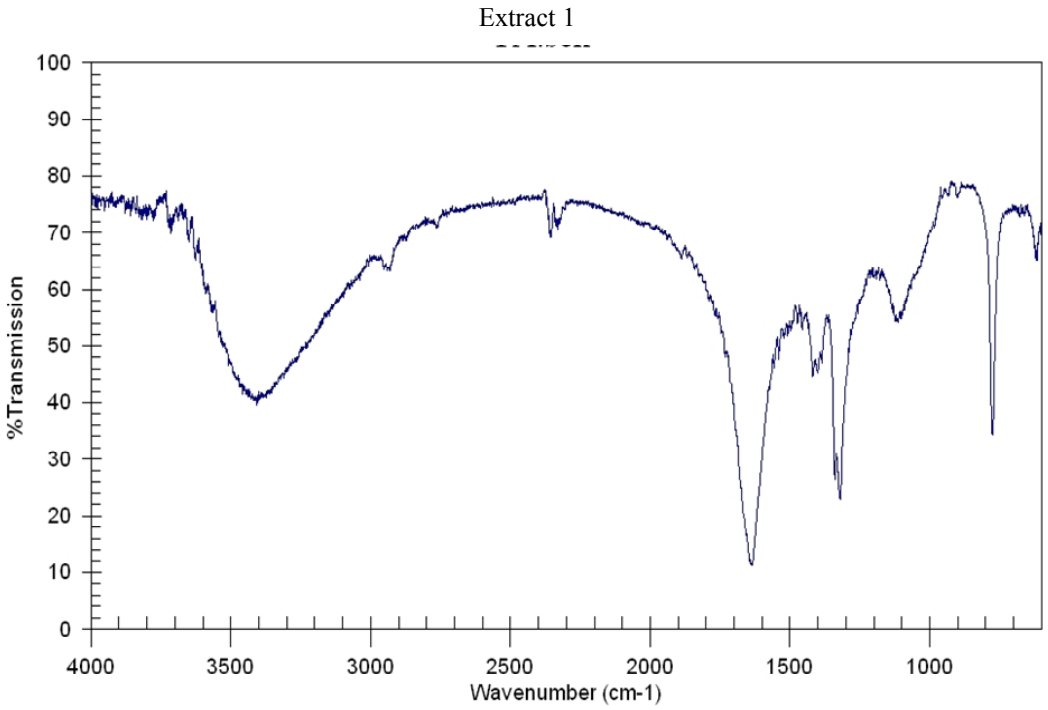
and cold ethanolic extracts respectively to Sauvad sp.



Suaeda

Fig.(2): IR spectra for hot aqueous extract of Saueda

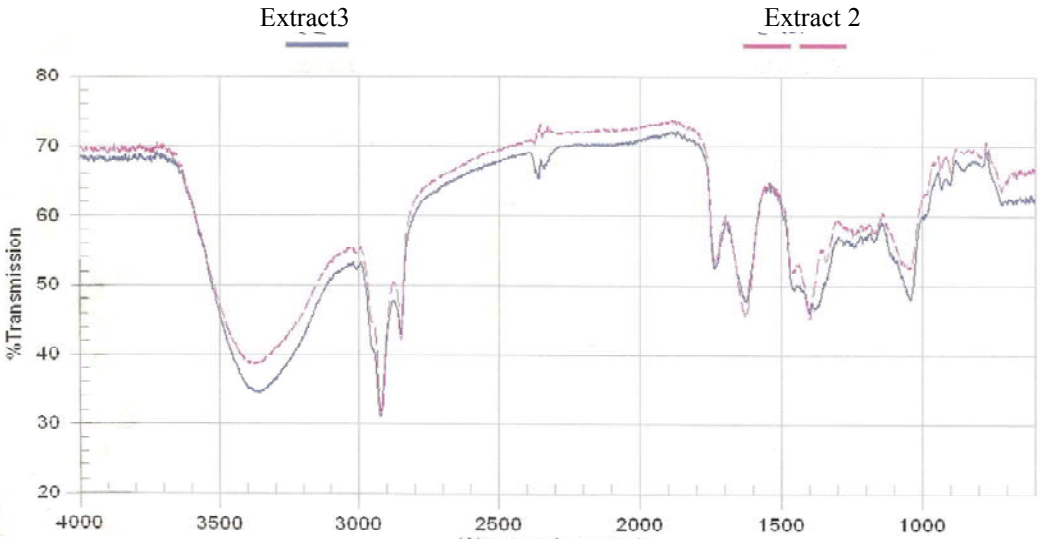


Fig. (3): IR spectra for hot and cold ethanolic extract of *Sueda*

P.vulgaris	6.0	6.0	6.0	7.0
K.pnumoniae	6.0	6.0	7.0	8.0
S.typhi	6.0	6.0	6.0	6.0
S. dysenteriae	6.0	8.0	10.0	11.0
V.choelerae	6.0	6.0	6.0	6.0

*Numbers represent diameters of inhibition zones measured in mm

Table-8-Antibacterial activity of *Suaeda* sp. cold ethanolic extract

Bacterial species	concentration%			
	2.5	**5.0**	**7.5**	**10**
S.aureus	6.0	6.3	7.5	8.0
E. coli	6.0	6.0	6.0	6.0
P.aeroginosa	6.0	6.0	6.0	6.0
P.vulgaris	6.0	7.0	9.0	10.0
K.pnumoniae	7.0	9.0	10.0	12.0
S.typhi	6.0	6.0	6.0	6.0
S. dysenteriae	6.0	6.0	7.0	7.5
V.choelerae	6.0	6.0	6.0	6.0

*Numbers represent diameters of inhibition zones measured in mm

Table-9-Antibacterial activity of *Suaeda* sp. hot ethanolic extract

Bacterial species	concentration%			
	2.5	**5.0**	**7.5**	**10**
S.aureus	9.0	11.5	12.0	14.0
E. coli	6.0	6.0	6.0	6.0
P.aeroginosa	6.0	6.5	8.0	10.5
P.vulgaris	8.0	9.5	10.5	14.0
K.pnumoniae	11.5	13.0	15.5	17.0
S.typhi	6.0	6.0	6.0	6.0
S. dysenteriae	7.5	9.0	10.7	12.3
V.choelerae	6.0	6.0	6.0	6.0

*Numbers represent diameters of inhibition zones measured in mm

Discussion:-

The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries, and moreover, the use of herbal remedies has risen in the developed countries in the last decade. In this manner, plants continue to be a rich source of therapeutic agents. It will be used for the treatment of bacterial infection. The need of the hour is to screen a number of plants that are traditionally used and also to evaluate their probable phyto constituents (11).

Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study that plant extract prepared with ethanol as the solvent provided more constituent antimicrobial activity. We can observe from Table (1) that the alcoholic extracts are acidic and bitter, the yield percentage of extracts was higher with using hot water than hot ethanol and cold ethanol, so hot water was more efficiencies for extracting than the ethanol in case of this plant. As expected the three extracts dissolve in polar solvents and do not dissolve in non polar or low polar solvents as shown in Table (2).

It is apparent from figure (2) and Table (3) that the pattern of absorption bands is the same in all three spectra and the bands of absorption due to $\pi \rightarrow \pi^*$ transition this emphasized on presence of conjugated electronic system ,may be aromatic or unsaturated system (15,17). The absorption for both ethanolic extracts (hot and cold) are very similar, the band with lower energy at 406, 400 nm (in hot and cold ethanolic extracts respectively) are nearly consistence with the green color of ethanolic extracts.

The more relevant IR absorption bands are listed in Table (4) exhibit the similarity in frequencies, this emphasizes on similarity in structures for ethanolic extracts (hot and cold) – whereas the bands in 3400 cm^{-1} attributed to OH stretching frequency in alcohols or phenols (3500-3200cm^{-1}), the stretching frequencies of N-H band (3400-3250cm^{-1}) in amine, imine, and amides (1°, 2°, 3°) can sometimes be confused with these of hydrogen bonded OH frequencies, but primary amines show two bands the unsymmetrical stretching , secondary amines absorb weekly.

The bands in the range (2590-2850 cm^{-1}) attributed to C-H stretching in alkenes or attributed to C-H in aldehyde group which is doublet (two bands between 3000-2600 cm^{-1}) or to OCH_3 (2900-2800 cm^{-1}), and the intensities are medium.

The bands between (1700-1500 cm^{-1}) may be attributed to N-H bending in primary amines (1650-1580cm^{-1}) primary amide (1670-1620 cm^{-1}) which is as doublet or attributed to C=C stretching in alkenes (1680-1600cm^{-1}) or to C=O stretching (1650-1590cm^{-1}), in secondary amide CONH (1570-1510 cm^{-1}) tertiary amide (1670-1620 cm^{-1}) and in unsaturated aldehyde or ketone (1710- 1665 cm^{-1}), and in ά ß – unsaturated (1700-1665cm^{-1}), and in aliphatic esters appears at(1700-1735 cm^{-1}) , that of ά ß – unsaturated ester appears at (1730-1715 cm^{-1}). Also C=N appears in range (1690-1640 cm^{-1}), and nitrate RONO, $RONO_2$ (1700-1600 cm^{-1}).

The bands in fingerprint region (1500-700 cm^{-1}) attributed to C-H bending (1470-1450 cm^{-1}), OH bending (1400-1250 cm^{-1}), C-N stretching, N-O stretching O=S=O (1400-1300 cm^{-1}), C=S (1200-1050 cm^{-1}) O=S (1110-1000 cm^{-1}).

The band below 1000 cm^{-1} in hot aqueous extract may be related to C-H bending in aromatic or alkynes or to C-Cl (800-700 cm^{-1}).

Results of chemical composition of the three extracts used in this study indicated that *Suaeda* sp. seems to posses some antimicrobially active compounds including triterpenoids and other compounds of phenolic nature or with free hydroxyl group, which are classified as active antimicrobial compounds (14). The antimicrobial activity may be indicative of the presence of some metabolic toxins or broad spectrum antibiotic compounds, *Suaeda* sp. posses amounts of tannins (table 5). Antimicrobial property of these substances is well established. (7, 4, 5).

Table 7 showed the results of antibacterial activity of *Suaeda* sp. against the bacterial isolates used in the study. From these results one can observe that the majority of the bacterial isolates did not affected by the aqueous extract of *Suaeda* sp.except for *Klebsiella pneumoniae* (diameter of inhibition 8.0 mm at 10% concentration) followed by *Proteus vulgaris* (diameter of inhibition 7.0 mm at 10% concentration). The six other bacterial species did not show any sensitivity towards the aqueous extract of *Suaeda* sp. The results indicate that the effect of the aqueous extract is relatively low; this might have resulted from the lack of solubility of the active constituents in aqueous solution. Active compound(s) may be present in insufficient quantities in the crude extract to show activity with the dose level employed. Lack of activity can thus only be proven by using large

doses, Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents (3).With no antibacterial activity, extracts may be active against other bacterial species which were not tested, these results were in agreement with (10) but they were in disagreement with (13).

Results of antibacterial activity of cold ethanolic extract of *Suaeda* sp. had been evaluated *In vitro* against the bacterial pathogens in Table 8.This extract showed higher antibacterial activity against the bacterial species as compared with the aqueous extract; the level of antibacterial activity is a function of the investigated concentration. The most effected concentration was 10%, while the less effective concentration was 2.5%, four of the eight bacterial species used in this study showed different degrees of sensitivity towards this extract, *K. pneumoniae* was the most effected bacterial species (diameter of inhibition zone was 12.0 mm at 10% concentration) followed by *P. vulgaris* (diameter of inhibition zone was 10.0 mm at 10% *dysenteriae* showed differences in their *aureus* and *S.* concentration), *S.* sensitivity against this extract, while the rest four bacterial species used in this study did not show any sensitivity towards the cold ethanolic extract of *Suaeda* sp.

Table 9 showed the results of *In vitro* antibacterial activity of hot ethanolic extract towards the bacterial species used in this study, from these results it can be easily seen that this extract was the most effective among the three extracts examined, five bacterial species were effected by this extract, the most effected bacterial species was *K. pneumoniae* (diameter of inhibition zone was 17.0 mm at 10% concentration) followed by *P. vulgaris coli*, *S.* (diameter of inhibition zone was 14.0 mm at 10% concentration), *E. choelerae* were not effected by this extract, the activity of this *typhi*, and *V.* extract may be attributed to the presence of alkaloids and other compounds which appear to be concentrated in the hot ethanolic extract.

This work may provide essential information in the selection of plant extract for further isolation of constituents responsible for the activity against the studied species, thereby aiding to explore an antibacterial lead that is helpful in combating the diseases caused by the studied pathogens.

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التركيب الكيميائي و الفعالية البكتيرية للمستخلصات المائية و الكحولية لنبات الطحمة *Suaeda* sp.

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الخلاصة

حضرت المستخلصات المائية والكحولية الباردة و الحارة لسيقان وأوراق نبات الطحمة *Suaeda* sp. وأجريت الكشوفات النوعية لتحديد التركيب الكيميائي العام ، كذلك استحصلت أطياف المرئية وفوق البنفسجية وأظهرت أن حزم الامتصاص هي نفسها في المستخلصات الثلاثة وهذه الحزم تكون نتيجة انتقالات $\pi^* \rightarrow \pi$. الامتصاصية للمستخلصات الكحولية الحارة والباردة متشابهة جدا كما اظهرت امتصاصية حزم IR تشابها في الترددات مما يؤكد على التشابه في تركيب المستخلصات الكحولية الحارة والباردة.توافقت امتصاصية IR مع الكشوفات النوعية الكيميائية . كانت النسبة المئوية للمستخلص المائي أعلى من المستخلصات الكحولية الحارة والباردة. اختبرت الفعالية البكتيرية للمستخلصات بطريقة الانتشار بالأكارحيث أظهرت كل من بكتريا *Klebsiella pneumoniae* و *Proteus vulgaris* التأثر الأكبر بهذه المستخلصات فيما لم تتأثر أيا من العزلات *Vibrio choelerae* و *Salmonella typhi* و *Escherichia coli* بالمستخلصات وقد كان المستخلص الكحولي الحار لنبات الطحمة بتركيز 10% هو الأشد فعالية ضد البكتيريا المستخدمة في الدراسة. واستخدم دم الإنسان لتحديد السمية الخلوية للمستخلصات بتركيز 500ملغم/مل ولم تلاحظ تأثيرات سمية باستخدام هذا التركيز إذ تعتبر هذه الخطوة الأولى في ايجابية العمل وفي الاستمرارية في اختبار المستخلصات على الإنسان.