Preliminary Biochemical Study of Hot Aqueous and Ethanolic Extracts of *Phoenix dactylifera*

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Abstract

An extraction process was conducted to obtain both hot aqueous and ethanolic extracts for *Phoenix dactylifera* (fiber of palm tree).Qualitative tests have been carried for the both extracts to determine general chemical species , uv.visible spectra were obtained . The anti-bacterial activity of both extracts against some gram positive and gram negative bacteria was tested .Human blood cells were used to determine cytotoxicity for both extracts.

1.Introduction

In the recent years, the scientific thinking has gone towards curing a lot of various diseases by using the popular medicine known as (herbal medicine) for activity ,safety, and economic factors [1].

Recently, the interest in planting medical plants and investing them has increased through describing them as a natural source to make the remedy and substitution of them by active materials chemically different [2].

Apart from the scientific reasons which showed the manufactured materials in the lab often have bad side effects and might not be discovered unless after using the remedy for a long time [3].

Further more , the study of the activity of many medical plant extracts as anti-microbes has led to important results which are the activity of most plant extracts because of their influence on an target ,not that one which is affected by anti-manufactured living [4].

So ,in Iraq and which contain many plants and herbs that we have to do our best to discover these fortunes which enrich the science. So, we think that we worked simple preliminary study on *phoenix dactylifera* (fiber of palm tree) that

important and widespread trees in southern of Iraq and testing an activity towards some germ isolated.

2. Materials and Methods

2.1:Preparing Plant Part For Study

The plant part which is *phoenix dactylifera* (fiber of palm tree) was collected and classified (1) .Then it was put on filter paper type whatman No.15(England) in dry place with good ventilation and was turned over continuously to prevent decay of plant.

2.2:Preparing the Extracts

Both hot aqueous and ethanolic extracts were prepared by putting 20 gm of plant part in vessels made of filter paper, these vessels were put inside soxhelet extractor by using 500 ml of distilled water and ethanol (95%) .The extraction was carried out for 24 hours.Then the extract was filtered and concentrated by direct heating to half.The residue solution was left in petridishes to dry at laboratory temperature.All the process has been repeated many times to obtain the enough amount of extract cruds. The extract cruds were collected and kept in the laboratory until use[5] .

(1): The plant part was classified by Dr.Abdul Ratha Alwan at Biology Department, College of Science, University of Basrah.

2.3:Qualitative Tests

Several qualitative tests have been carried out to know the general chemical species which is found in the two extracts. Test solutions were prepared according to [6-15] and kept in blank glass vessels inside freezer until use. These tests were involved: alkaloids, glycosides, saponins, carbohydrates, flavonoids, tannins, ninhydrin, resins, fuocoumarins, vanillin-H_2SO_4, triterpenes , steroids, ethanolic rodamin-B(0.5%), unsaturation, sodium fusion and solubility tests.

2.4:Electronic Specta Measurement

Electronic spectra were obtained on an Helios v4-60U. Visible spectrophotometer at Physics Department, College of Science,in University of Basra. 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/30 ml for both hot aqueous and ethanolic extracts.

2.5:Determination of Antibacterial Activity

Tthe bacteria isolated were cultured by streaking method on Nutrient Agar (NA)(Oxiod) inoculated 5 ml from one young colony of growth in age of 24hours .In Nutrient both (NB) Oxoid so incubated 6 hours at 37°C for obtaining bacterial growth 10 cell/ ml, after that 0.1 ml was spreaded from growth on Muller-Hinton (Difco), formed well on surface of Agar by sterilized cork porer in 6mm filled the wells in culture media by extract in size of 0.1 ml [5]; incubated the petridishes at 37°C for 24 hours .So the results were recorded by measuring the average of diameters inhibition zone.

2.6:Determination of Cytotoxicity for Aqueous and Ethanolic Extracts

Human blood cells were used to determine cytotoxicity of the two extracts according to [16]. Concentration of 500 mg/ ml of phosphate buffer saline for both extracts were prepared. Also positive control contained only phosphate buffer saline and negative control (tap water) have been used. Then, 0.2 ml of blood cells were added to sterile test tube containg 0.8 ml of extract to reach a total volume of 1 ml. The same process was made for both control. Incubated the four test tubes at 37° C for 3 hours. So noted the hemolysis, later.

3. Results

Table (1): Physical properties for hot aqueous and ethanolic extracts.

NO.	Extract Type	Color	Shape
1	Hot aqueous extract	Blackened brown	viscous
2	Hot ethanolic extract	Reddish brown	powder

Table (2): Anti-bacterial activity of hot aqueous and ethanolic extracts against bacteria with concentrations 500, 750, and 1000 mg/ml.

NO.	Bacteria	Average of diameters inhibition zone in mm. for						Average
		Hot aqueous extract			Hot ethanolic extract			
		Concentration mg/ml			Concentration mg/ml			
		500	٧٥٠	1000	500	٧٥٠	1000	
١	Escherichia coli	٠	٨	١٤	١١	١٥	٢٠	١١.٣٣
٢	Klebsiella pneuamonia	٠	١٠	١٦	٠	١٠	١٥	8.5
٣	Staphylococcus aureus	٠	٩	٢٢	١٠	١٥	١٧	١٢.١٦
٤	Streptococcus pyogens	٠	٧	١٩	٠	١٠	١٣	٨.١٦
Average		٠	8.5	١٧.٧٥	٥.٢٥	١٢.٥	١٦.٢٥	

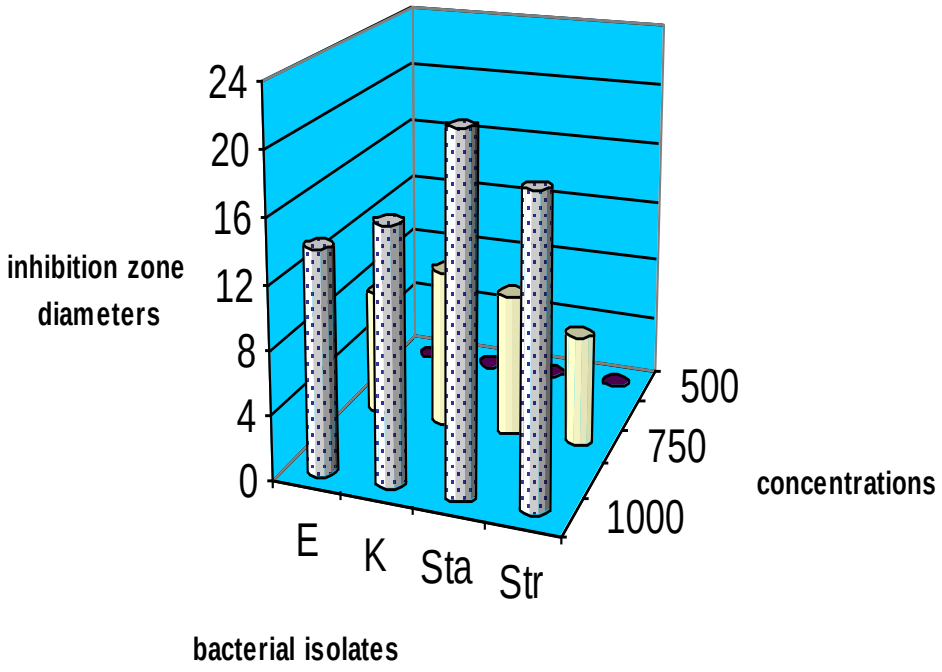


Figure (١): The average of inhibition zone diameters for alcoholic extract of Phoenix *dactylifera* against four bacteria isolated.

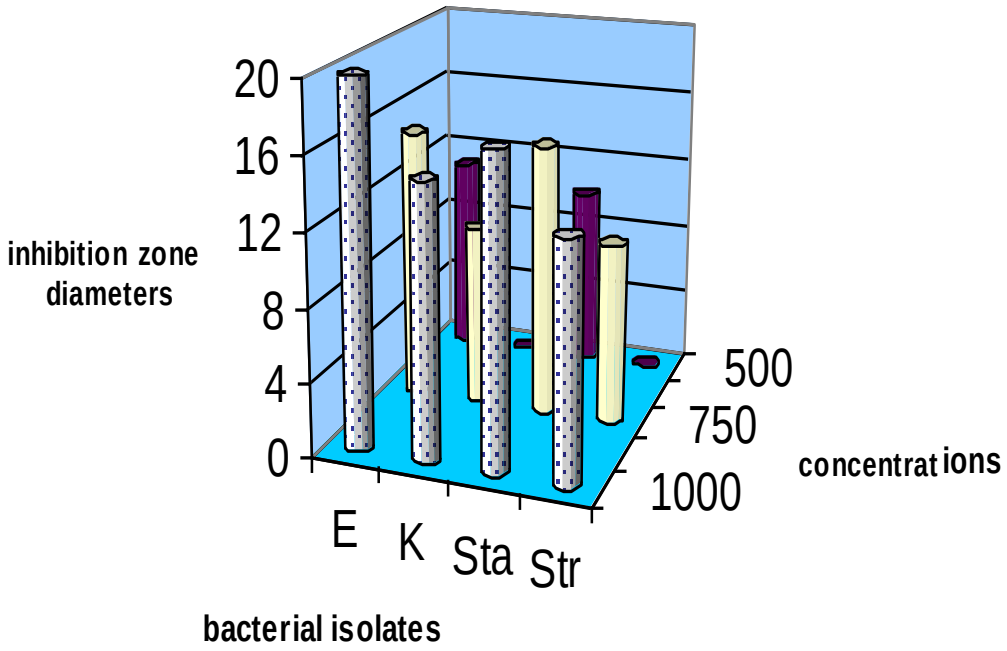
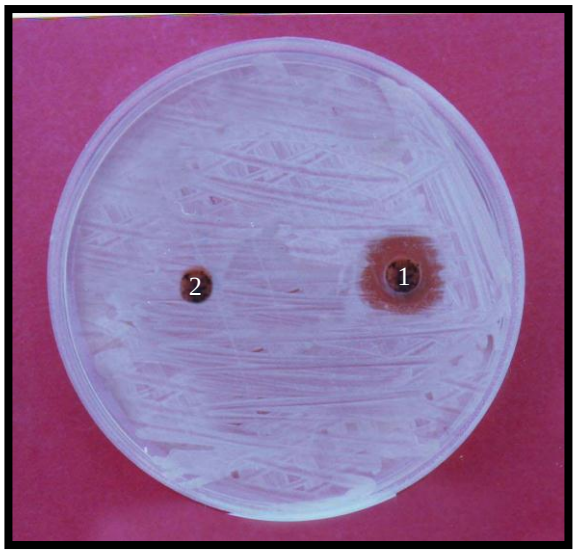
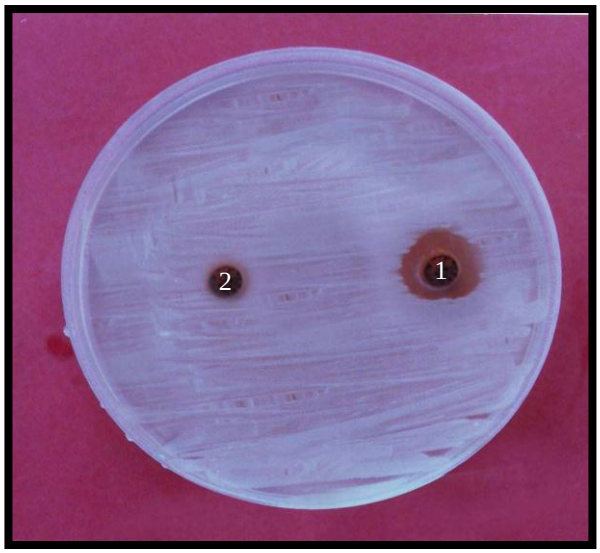


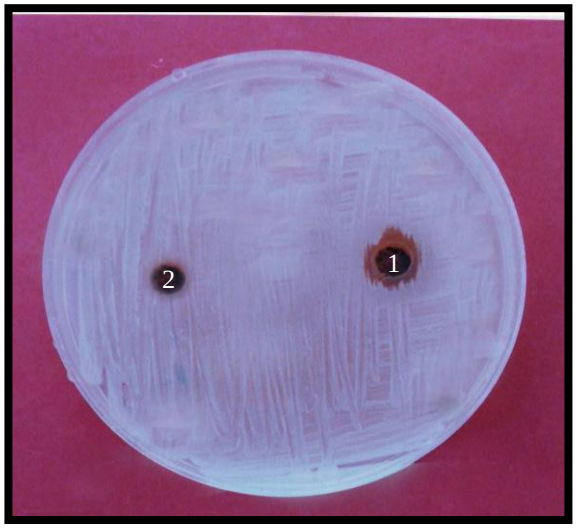
Figure (2): The average of inhibition zone diameters for aqueous extract of Phoenix *dactylifera* against four bacterial isolated.



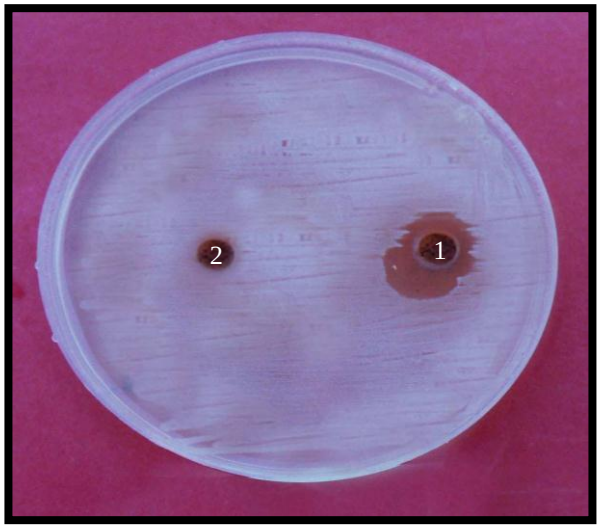
Klebsiella pneumoniae



Streptococcus pyogenes



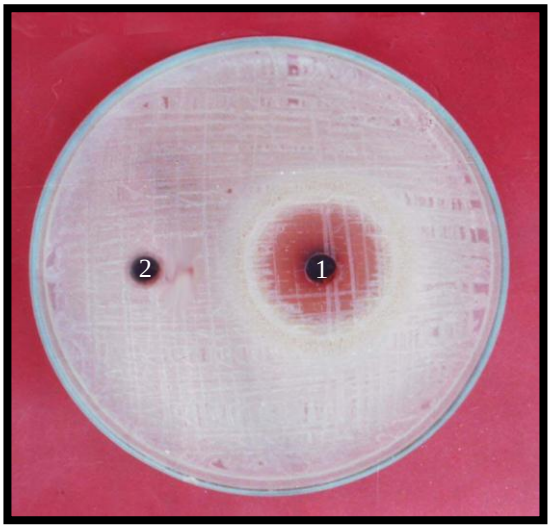
Staphylococcus aureus



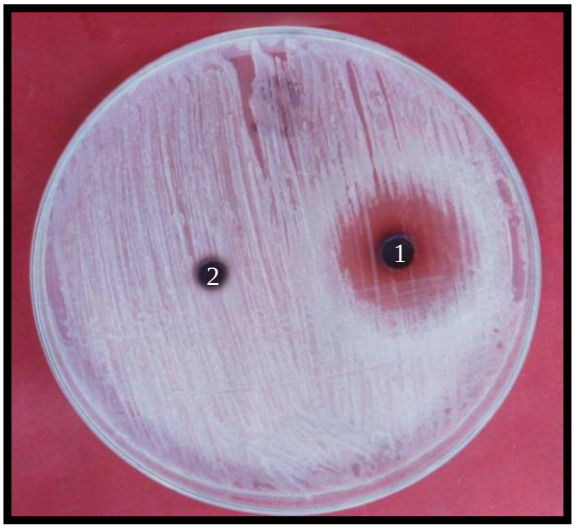
Escherichia coli

Figure (3): The anti-bacterial activity of *Phoenix dactylifera* alcoholic extract against four bacteria isolated in concentration 1000 mg/ ml

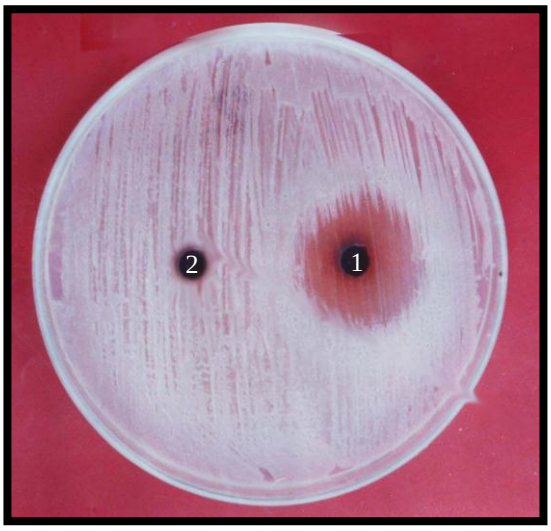
(١): alcoholic extract
(2): Control



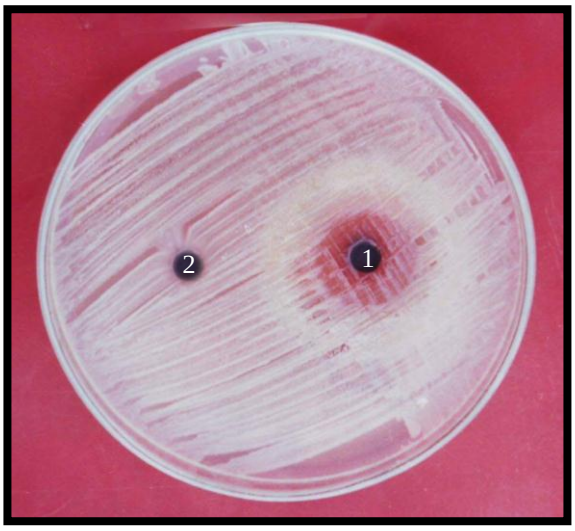
Klebsiella pneumoniae



Streptococcus pyogenes



Staphylococcus aureus



Escherichia coli

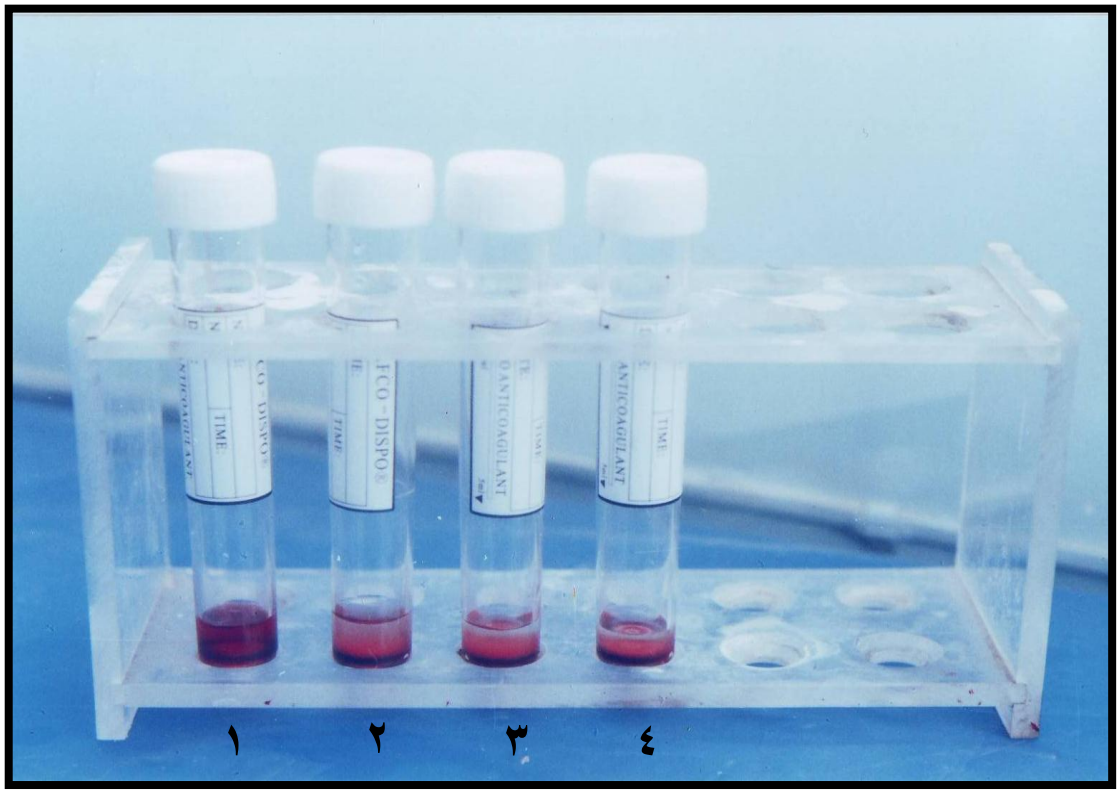
Figure (4): The anti-bacterial activity of *Phoenix dactylifera* aqueous extract against four bacteria isolated in concentration 1000 mg/ml

(1) : aqueous extract
(2) : Control

Table(3): Results of cytotoxicity for hot aqueous and ethanolic extracts

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

- (-): No Lyses.
- (+): Hemolysis was found.



Figure(5): Cytotoxicity of Phoenix dactylifera alcoholic and aqueous extracts against red blood cells :
1- Tap water
2- Normal saline
3- Alcoholic extract
4- Aqueous extract

Table(4): Qualitative tests for both hot aqueous and ethanolic extracts

NO.	*Specific Tests*	*Hot aqueous extract*	*Hot ethanolic extract*
1	**Alkaloids test**		
a	**Dragendroff reagent**	+	+
b	**Wagner's reagent**	+	+
2	**Glycosides test**		
a	**Before glycoside hydrolysis**	+	+
b	**After glycoside hydrolysis**	+	+
3	**Saponins test**	+	+
a	**Forming dense foam**		
b	**Mercury(III) chloride test**		+
4	**Carbohydrates test**		
A	**Phenol-Conc.H_2SO_4 test**	+	+
B	**Molish test**	+	+
5	**Flavonoids test**		
A	**Ethanolic KOH test**	-	+
B	**H_2SO_4 test**	-	+
6	**Tannins Test**		
A	**Lead acetate test (1%)**	+	+
B	**Ferric chloride test(1%)**	+	+
7	**Ninhydrin test (1%)**	+	-
8	**Resins test**	-	-
9	**Fuocoumarins test**	-	+
10	**Vanillin-H_2SO_4 test**	-	-
11	**Triterpenes and Steroids test**	+	+
12	**Ethanolic Rodamine-B(0.5%) test**	-	-

13	Unsaturated test		
A	Bromine test	+	+
B	Bayer test	+	+
14	Fusion with Sodium test		
A	Sulfur test	-	-
B	Nitrogen test	+	+
C	Phosphour test	-	-
15	Solubility test		
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(5): Absorption data of hot aqueous and ethanolic extracts

Extract	$\lambda_{max,nm}$	*Absorbance*
Hot aqueous	205 275	2.911 1.08
Hot Ethanolic	235 275 320	2.875 1.723 1.03

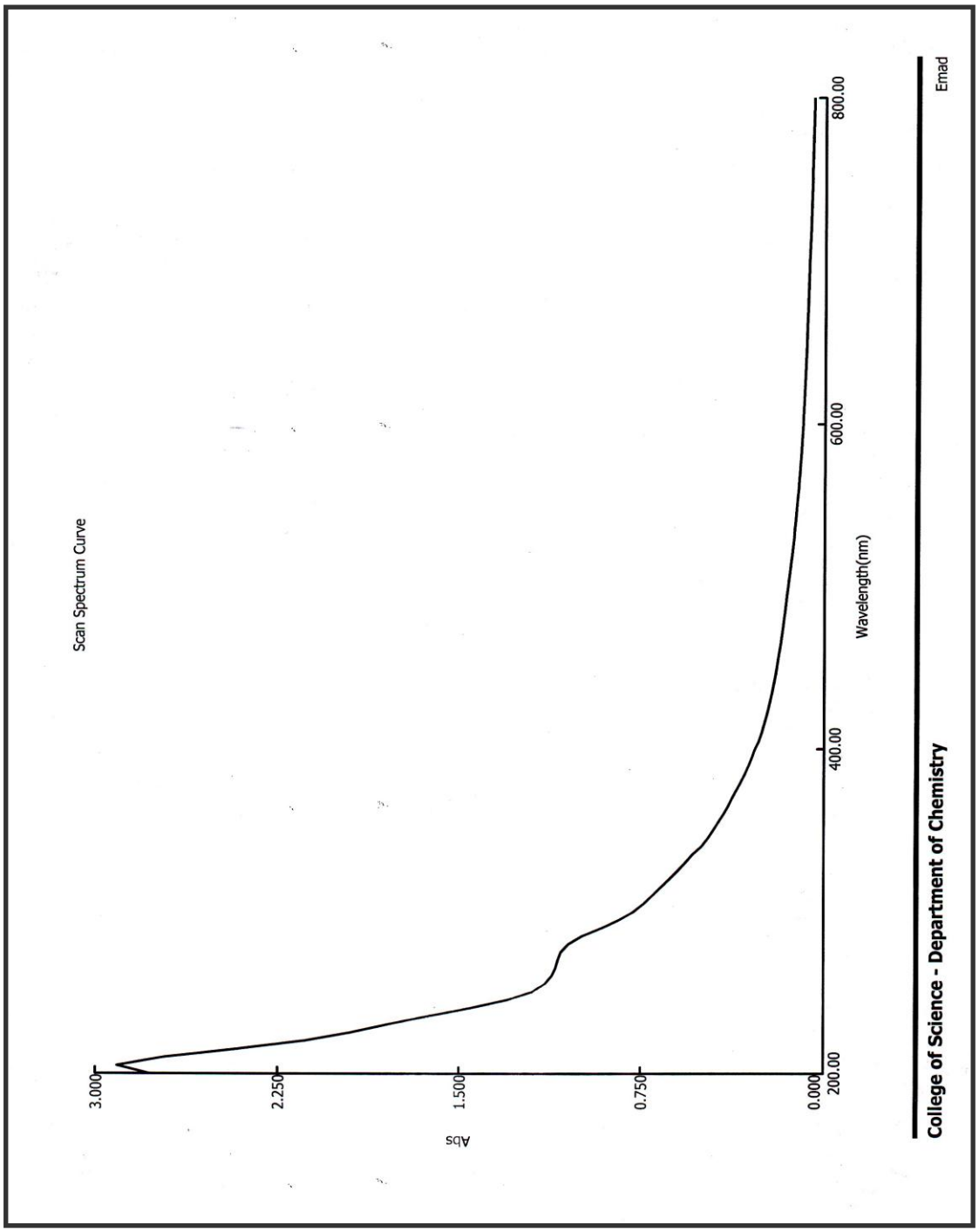


Figure (6): Electronic absorption spectrum for hot aqueous extract.

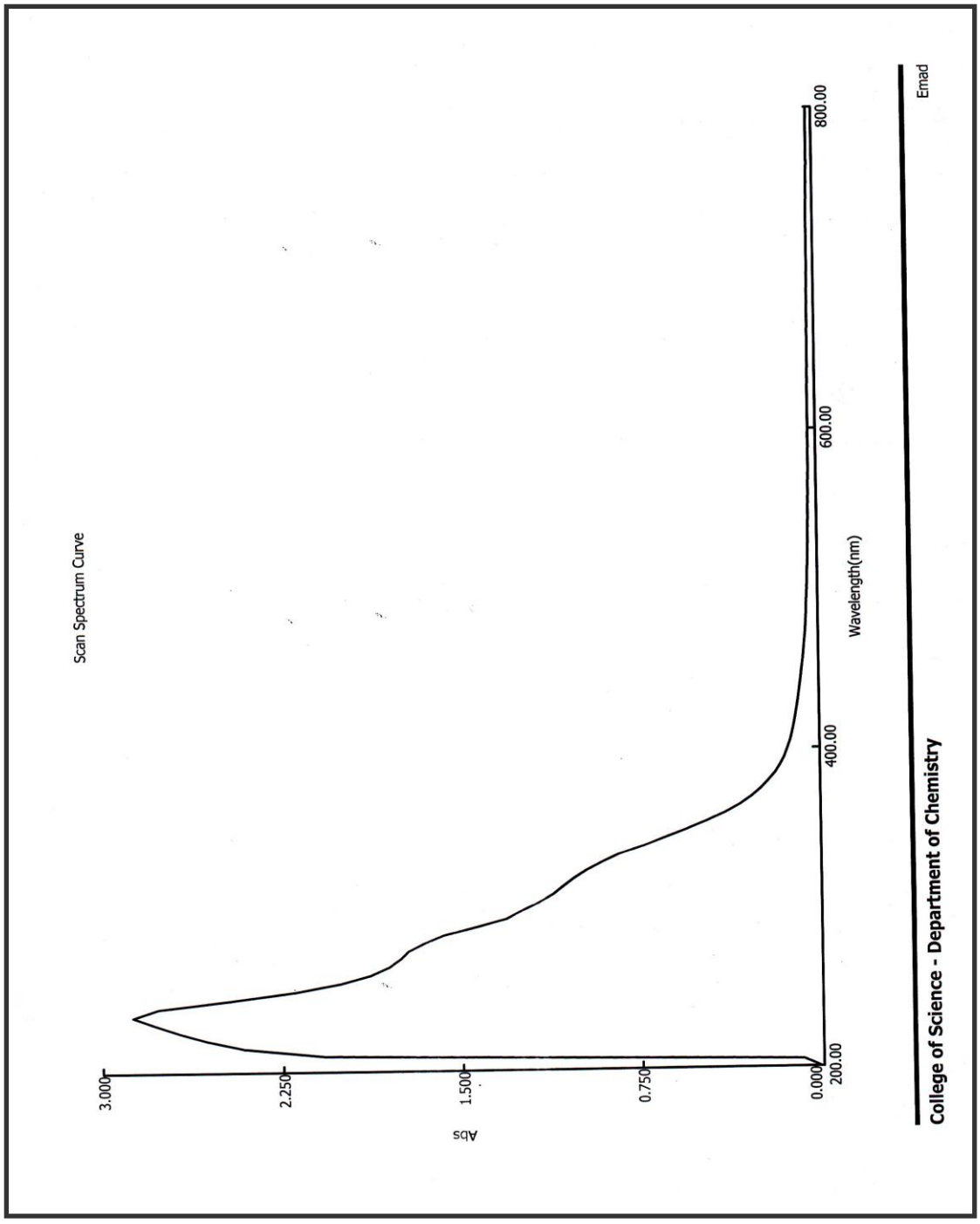


Figure (7): Electronic absorption spectrum for hot Ethanolic extract.

Discussion

In the recent years, it has been noticed an increase in the resistance of micro-living for chemically synthetic antibiotic due to the huge use for it [17] and that interprets the forward looking of many researchers to find an alternative sources for curing the resistance cases ,the new ingredients have variant methods in their influence will be a solution for the problem of drug resistance [18].

The best source is the plant because of it's abundance in nature and contains medical active materials [19]. So the current study headed to test some fiber of palm tree extracts where palm tree is one of widespread plants in large areas in Basrah and recognizing the active ingredients in it.

According to the results of our current study,table (4) showed that both extracts contained most the active species which are being exposed : alkaloids , saponins , tannins , triterpenes , and steroides and these results in agreement with refrence [20] which refered that polar solvents (water and alcohol) are able to extract polar biochemically active compounds.

It is apparent from figures (3&4) and table (5) that the bands of both absorption spectra due to π- π^* transitions [21] and the pattern of absorption bands is the same in both spectra with presence of additional band at 320 nm in spectrum of ethanolic extract which is absent or almost concealed in spectrum of aqueous extract.

The presence of the band at 275 nm in both spectra may be related with the same chemical species .Also it is showed that π- π^* transition need more energy to occur in spectrum of aqueous extract and that may be related to rather more rigid compounds [22].

Both extracts were biochemically more active against gram positive bacteria (*Staphylococcus aureus & Streptococcus pyogens)* compared with gram negative bacteria (*Escherichia coli & Klebsiella pneuamoniae)* (table (2) and figures(1&2)).This variation may be due to the difference in composition of the exterial cell that gram negative bacteria contains cell membrane has an independant separate line represented in lipopolysaccharide which is shared complicated proteins that has the capability to deter the passage of plant extract into the cell [23].

The hot ethanolic extract was biochemically more active than hot aqueous extract.The variation in effect degree of plant extract types on microlivings is due to different factors ,the important of them are : type of extract , method of extraction , polarity of solvent, and bacterial type affected by extract or this variation in effect of the two extracts may be interpreted depending on what active compounds which both extracts contain ,also apart from the differences in cell composition , genetic factors, and nature of target in tested bacteria affected by extract [24].

The variation in activity may be due to losing some active compounds during extraction process or during storage of extract which lead to decrease of extract activity .Volatile oils , Glycosides or other compounds were lost during hot extraction as noted in table (4) where the Glycosides and amino acids are absent in hot ethanolic extract while phenolic compounds (flavonoids &foccumarines) are also absent in hot aqueous extract [25].

The alcohol solvent can be more efficient in extracting active compounds which are more solouble in it rather than in water such as flavonoids and foccumarines [26].These compounds affect on bacterial cell by incorporating with cell protein and precipitation it, these compounds also are good solvents for lipids of bacterial cell wall leading to wall lyses, and coming out cell composition, then death of the cell [27].

Generally, both extracts types showed up the effect on the four tested isolated and the reason may be that the other active compounds such as alkaloids which infiltrate cell membrane of bacteria and control active sides for enzymes inside germ cell,these sides are essential for growth and reproduction and this causes the death of germ cell [28].

The activity may attribute to that the plant contains many organic acids: strearic, oleic, matrices, and plasmatic acids [29] which lead to deform protein of the bacterial cell [30] .

Chemical study has carried out to know active compounds for this plant part which indicates that it contains flavonoids, glycosides, phenols, and organic acids, seperated and characterized by using both high performance liquid chromatography technique and mass spectroscopy [31].

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