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Hypoglycemic Action of Synergistic Interaction of Phenolic Compounds Isolated from Iraqi Phoenix dactylifera (Breim) Leaflets in Alloxan – Induced Diabetic Rabbits

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Abstract
Diabetes mellitus is a multifactorial disorder characterized by hyperglycemia resulting from increased hepatic glucose production . The current study was carried out to investigate the hypoglycemic action of synergistic interaction of phenolic compounds isolated from Iraqi *Phoenix dactylifera* (Breim) leaflets . Infra - red and gas chromatography – mass spectroscopies were applied for identification of the phenolic compounds which are represented by alpha – tocopherol , 2-hydroxy-5-methylisophthalaldehyde , 3-hydroxy –tyrosine and 4-n-propylresorcinol . The phenolic compounds recorded a significant decreasing after 4 hours (280.1 mg / 100 ml) and highly significant decreasing at 6 hours (238.3 mg/ 100 ml) and 24 hours (134.5.mg / 100 ml) after oral administration in hyperglycemic rabbits . The phenolic compounds had no toxic effect on red blood cells since no hemolysis was found towards these cells, this means phenols Isolated can be used safely to treat diabetes mellitus disease instead of insulin drug but this work demands further clinical and pharmaceutical studies .
Keywords:*Phoenix dactylifera* (Breim) , phenolic compounds, synergistic interaction , hypoglycemic action , significant decreasing , anti – diabetic plants .

1. Introduction

Diabetes mellitus disease is a chronic disorder characterized by elevated blood glucose levels and disturbance in carbohydrates , fats and proteins metabolism . This biochemical multifunctional disorder results from lack and failure in secretion of insulin hormone leading to increase of hepatic glucose production then hyperglycemic case was happened (Patience *et at* , 2014 ; Raman *et at*,2012) .

Therefore this dangerous disease is now becoming a common metabolic problem resulting from the inability of our body's response to high glucose level . Hyperglycemic patients experience various vascular complications such as atherosclerosis , diabetic nephropathy , retinopathy and neuropathy . Development of synthetic drugs is one of the source of treatment of diabetes mellitus but the main problem to use these drugs and antibiotics , is the side effects which these drugs have . The current available synthetic drugs used to cure the hyperglycemia case , are sulphonylurea , biguanide , thiazolidinedione and alpha - glycosidase inhibitors compounds (Mohammed and Al-Maliki,2014 ; Nwaegerue *et at*,2007) .

Medicinal plants are the benefical substances to treat diabetes mellitus disease because they have various active chemical compounds as secondary metabolites are naturally present in the different parts of these plants . These chemical compounds are represented by phenolic compounds , alkaloids , glycosides , saponins , steroids and terpenes . The biochemical importance of these active compounds come from presence of many functional groups in the chemical structures for these chemical metabolites (Surya and John ,2001 ; Soladoye *et at* , 2012). Many medicinal plants possess hyperglycemic properties , most claims are anecdotal and few of them have received adequate medical or biochemical evaluation . Several reviews about the medicinal plants were carried out in the management of diabetes mellitus and these plants have been reported . So the importance of active chemical compounds as natural products belong to their biochemical ability to treat different diseases including diabetes mellitus which is represented by presence of blood glucose in high levels (Bnouham *et at*, 2006 ; Robert *et at*,2006 ; Mohammed and Al-Maliki , 2014).

Natural phenols are phytochemical compounds are abundant in medicinal plants as secondary metabolites and they are considered as active chemical compounds because their ability to treat various diseases . Phenols which are present naturally protect the plants against biological and environment stresses . Because of their necessary protective biochemical functions , they are ubiquitous in medicinal plants and so are found in almost all food groups (Sambanthamurthi *et al*, 2011 ; Al- Maliki ,2012) .

Chemically , phenols are aromatic compounds contain many hydroxyl groups in their chemical structures and they are classified into metabolic subclasses are represented by flavonoids , tannins , coumarins , lignans and simple phenols . Metabolitic phenolic compounds are biochemically synthesized in secondary metabolism process by shikimic acid pathway , therefore these active compounds are one of largest constituents of secondary plants compounds . Simple phenols consist of aromatic ring in which a hydrogen atom is replaced by hydroxyl group , also the simplest phenolic compound are C_6 structures consisting of aromatic ring with hydroxyl group attached . These include pyogallol and hydroquinone (Gorinstein *et al*, 2004 ; Al- Maliki , 2011 ; Brandt and Molagaurd , 2001) .

Various and several phenols were used as analgesic medicine , relief of headaches , depressant on central nervous system , influence on prostaglandin metabolism , anticlotting agents, anti- coagulant , antispasmostic and antifertility agents .The medicinal action of secondary phenolic compounds belongs to presence of hydroxyl group as a functional group in chemical structures of these phytochemicals , so this active group has ability to bind with biochemical molecules in living organisms especially human being (Fukuda and yoshida ,2004 ; Pengelly , 2004) .

Different medicinal plants such as *Achilea santolina L. ,Capparis spinosa L, Enuka sative , Ocimum sativum L., Piper longum , Urtica piluliffera L., Brassica oleracea , Var capitata , Prospis Juliflora and Allium cepa* were used to treat hyperglycemia case and they have been made hypoglycemic actions so this decreasing in blood high glucose levels led to find a herbal drugs substituents instead of insulin (Mohamed and Al-Maliki ,2014 ; Mohammed *et at* , 2009; Proestons *et at*,2006).

Phoenix dactylifera L. (Breim date palm) belongs to Arecaceae family , is the one of foods plant supporting and providing the dates and it is used in sweets industry . The date palm involves many varieties depending on the shape and the organoleptic features of it's fruits . Six hundreds varieties of *phoenix dactylifera* L . species world wide are found . The drugs derived from this plant are depended on presence of active natural chemical compounds that can promote health and alleviate harm. Some of varieties of date palm contain various phyto -chemicals and enzymes that act as antioxidants to maintain growth and metabolism . From these metabolic compounds , phenolic compounds such as flavonoids , phenolic acids and tannins that have a biochemical role as antioxidants , anticancer , antitumor , antibacterial and antihyperglycemic agent (Laouini *et at*,2013 ; Ahmed *et at* ,1995 ; Rubem and Vera , 2003 ; Pietta ,2000) . The current study has aimed to investigate and evaluate the hypoglycemic action of phenolic compounds synergistic interaction which were isolated and identified from Iraqi *Phoenix dactylifera* L. (Breim species) leaflets .

2. Materials and Methods

2.1 Plant material

Phoenix dactylifera L .(Breim species) leaflets were collected from a farm in Msheigeeja village in Abu Al-Khaseeb region at Basrah governorate in Iraq in October 2015 . The date palm plant was classified and identified by a botanist in biology department in college of education for pure sciences at university of Basrah in Iraq . The plant leaflets were cleaned , washed by tap water then by distilled water to remove soil and other dirts , dried in dark place , ground as powder by electrical mill and kept in dark containers at room temperature until the day of use .

2.2 Preparation of cold aqueous extract of Breim species leaflets

Fifty grams of dried ground leaflets of Breim species were stirred in 500 ml of distilled water by using magnetic stirrer apparatus for 16 hours . The precipitate was removed by filtration process by using Buchner funnel then the filtrate was concentrated under vacuum by using f reeze drier to get the crude . By the same method cold ethanolic extract was prepared but by using ethanol as a solvent (Harborne ,1984 ; Bruneton , 1995).

2.3 Preparation of hot aqueous extract of Breim date palm leaflets

Fifty grams of dried ground leaflets of Breim species date palm were refluxed in 500 ml of distilled water for 16 hours then the filtration process was achieved and the precipitate was removed . The filtrate was concentrated under vacuum by using freeze drier to get the crude . By the same method hot ethanolic extract was prepared but by using ethanol as a solvent (Harborne,1974; Waterman and Mole ,1994) .

2.4 Preliminary Qualitative Analysis of Phoenix dactylifera Breim Leaflets Extracts

The hot and cold ethanolic and aqueous extracts were underwent sevral qualitative tests to know kind of their active chemical compounds by using different chemical reagents as the following :

1. **Alkaloids test :** It was carried out by using Dragendroff , Myer and Wagner reagents (Harborne,1984 ; Ahmed *et at*, 1989) .

2. **Phenols test :** It was achieved by using ferric chloride (1% w/v) reagent (Lau *et at* , 1989)

3. **Flavonoids test :** It was made by using (5N) ethanolic potassium hydroxide reagent (Croteau *et at*,2000).

4. **Tannins test :** It was carried out by using (1% w/v) lead acetate reagent (Croteau *et at* ,2000 ; Harborne,1984). **5**. **Carbohydrates test :** It was detected out by using Molisch's reagent (Harborne,1984 ; Harborne,1974) .

6. **Glycosides test :** It was achieved by using Bendect 's reagent (Al –Saadi and Al – Maliki ,2014) .

7. Saponins test : It was carried out by using (5% w/v) mercuric chloride (Harborne,1984).

2.5 Gas Chromatography – Mass Spectrum technique:

The chemical compounds of cold and hot aqueous and ethanolic extracts were separated and identified by using

gas chromatography - mass spectrum technique in laboratory of GC – MS in college of agriculture at university of Basrah in Iraq . The analysis was carried out by using shimadzu GC - MS – QP 2010 ultra system having automatic sampler CTC analysis combi PAL robotic arm . The specification of used capillary column is Agilent 190915 – 433 : 1548 , 52894 HP – SMS ,50% phenyl methyl silox . The diluted samples (1/100 v/v in hexane) of 2ml were injected .

2.6 Isolation of Phenols from of *Phoenix dactylifera* Breim leaflets

Fifty grams of Breim leaflets ground was mixed with 250 ml of (2 % v/v) hydrochloric acid and the mixture was put in waterbath for one hour at 90°C then the mixture was stirred on magnetic stirrer for two hours. Filtration was achieved by using Buchner funnel and precipitate was removed then filtrate was treated with 200 ml of diethylether with the same volume of filtrate . The mixture was placed in waterbath at 30° C for 50 minute , then it was concentrated by using rotary evaporator at 70C° then crude phenols were gotten (Stalikas , 2007) .

2.7 Thin Layer Chromatography of Phenolic Compounds

Phenolic compounds isolated which are abundant in *phoenix dactylifera* (Breim) leaflets , were separated depending on thin layer chromatography (TLC) analysis by using 50 ml of isolated phenols that was toulerenced on alumina pate (2 × 8 cm) coated by silica gel. Butanol – acetic acid – water solvent were used as eluent with ratio (0.5 : 2.5 : 7) . The separation time was 45 minute , then TLC was dried and the spots were developed by iodine vapor , UV – Lamp at 233 nm and (1%) ferric chloride . Rate of flow (R_f) values were measured for all separated compounds (Harborne,1984) .

2.8 Column Chromatography of Phenolic Compounds

Phenolic compounds which were separated by TLC , they were separated by column Chromatography (CC) technique to isolated each phenol alone . Glass column was used with optimization are , radius (1cm) , length (50cm) , the stationary phase (silica gel) and the mobile phase (Butanol – acetic acid – water) solvent were used a s eluent with ratio (0.5 : 2.5: 7) . The separation process time was 45 min and the volume of each separated was 3 ml , then TLC was applied for each phenolic compound separated by CC, then rates of flow (R_f)values were calculated for all phenols and the correspondence process was achieved between R_f values in both TLC and TLC of CC (Al-Saadi and Al-Maliki ,2015) .

2.9 Forrier Transform Infra – red spectroscopy of separated phenols

Infra -red spectra were recorded for all phenolic compounds isolated and separated from *phoenix dactylifera* (Breim) leaflets by using FTIR- 8400S spectrophotometer type Japan, SHIMADZU The sample were prepared by mixing with potassium bromide (KBr) as a disk and the spectral range for measuring was ($4000cm^{-1}$ -500 cm^{-1}) .

2.10 Experimental Animals

Healthy rabbits weighing 1.5 – 2 Kg were purchased from local market in Basrah Governorate at Iraq . Rabbits were kept in clean polypropylene cages and they fed standard antibiotic free diet and were kept in fasting case for 24 hours before doing the all experiments

2.11 Alloxan induced diabetic rabbits and spectral method of determination of blood glucose

Hyperglycemia rabbits were gotten by injection of alloxan monohydrate dissolved in sterile normal saline then this process lead to happen the hyperglycemia case in experimental animals. Prepared alloxan was carried out immediately after preparation and was administrated at period of 48 hours by using 150 mg/Kg dose of rabbit weight and injected via marginal ear vein under light by using 1 ml syringe after that 20% w/v of glucose sugar dissolved in drinking water was administrated orally for diabetic rabbits , then were kept in fasting case for 18 hours after seven days from the last administration .Blood glucose concentrations were recorded in diabetic rabbits by using glucose oxidase peroxidase enzymatic colorimetric (GOD – PAP) method by using glucose measurement device provided by measuring strips (Wasfi *et at,1994*) .

2.12 Effect of oral administration of phenolic compounds in hyperglycemic rabbits

Twelve fasted hyperglycemic rabbits were divided into two groups. The first one was given 3ml of normal saline and considered as a control group while the second group was given 0.3 g /3ml normal saline dose of phenolic compounds for 1Kg of rabbit body weight and this group was considered as treatment group . Then blood glucose concentrations were measured spectrophotometrically at times equal to (0,2,4,6 and 24 hours) after oral administration (Manohar *et at* , 2012).

2.13 Estimation of cellular toxicity of phenolic compounds synergistic interaction

Cellular toxicity of phenolic compounds isolated and identified from *phoenix dactylifera* **(** Breim) leaflets , was measured by using 200mg of phenols extract dissolved in 10 ml of normal saline, then various concentrations (1:1 , 1:10, 1: 100, 1: 1000 v/v) were prepared . The control group was normal saline only , after that 0.8 ml of each concentration was put in a sterile test tube of (Ependroff tube) type contains anti – cloating substance . Then 0.2 ml of fresh human blood was add into each tube therefore the final volume was 1.0 ml , after that the tubes were incubated at 37°C for 30 minutes and finally they were centrifuged for 5 minutes (Kilani – Jaziri *et at*,2011) .

2.14 Statistical Analysis

The statistical analysis was achieved for all experiments concerning diabetic rabbits by using one way ANONV method of variance analysis by SPSS version 14-2006 in order to test presence of significant differences between control and treatment mean (Wasfi *et at*,1994) .

3. Results and Discussion

Phoenix dactylifera **(**Breim**)** is considered one of medicinal plants in addition to the nutrition value of its fruits because it contain many different active natural chemical compounds. The extraction percentages of cold and hot aqueous ,ethanolic and phenolic extracts are illustrated in table (1) .

Table (1) Extraction percentages of all extracts isolated from *phoenix dactylifera* Breim leaflets .

No	Extract type	Extract weight (gm)	Extraction percentage (%)
1.	Hot aqueous	2.89	5.78
2.	Cold aqueous	4.98	9.96
3.	Hot ethanolic	4.150	8.30
4.	Cold ethanolic	4.375	8.75
5.	Phenols	2.62	5.24

It is noticed that cold aqueous extract had the highest extraction percentage (9.96%) which was more than other extracts . Whereas the phenols extract recorded the lowest extraction percentage (5.24) , therefor the explanation of this case belongs to presence of more chemical compounds qualitatively and qualitatively in cold aqueous extract but the compounds were in low quantity in phenols extract . The extraction percentages were calculated as in the following law: . Weight of plant crude (gm)

$$\text{Extraction percentage (\%)} = \frac{\text{. Weight of plant crude (gm)}}{\text{Weight of powder plant (gm)}} \times 100$$

Several various active chemical compounds were extracted , isolated , separated and identified from different varieties of *Phoenix dactylifera* L. such as phenollic antioxidants and flavonoids (Praveen ,2002 ; Marles and Farnsworth,1995).

The preliminary qualitative tests results of cold and hot ethanolic and aqueous extracts are indicated in table (2).

Table (2)Phytochemical results of qualitative analysis of extracts isolated from phoenix dactylfera (Breim) leaflet

Reagent	Extract type				Test result	Test result
	Cold aqueous	hot aqueous	Cold ethanolic	hot ethanolic		
Drarogendroff	-	-	-	+	Formation of orange precipitate	Alkaloids are present
Molisch	+	+	+	+	Formation of violet ring	Carbohydrates are present
Benedict	++	+	+	+	Formation of red precipitate	Glycosides are present
Ferric chloride (1%)	++	++	+	++	Appearance of bluish- green colour	Phenols are present
Ethanolic KOH(5N)	+	-	+	-	Formation of yellow precipitate	Flavonoids are present
$HgCl_2$ (5%)	+	-	+	-	Formation yellow precipitate	Saponins are present
lead acetate (1%)	+	+	-	-	Formation of light brown – white precipitate	Tannins are present

It was found that cold aqueous extract contain carbohydrates , glycosides , phenols ,flavonoids ,tannins and saponnins but the hot aqueous extract have carbohydrates , glycosides , phenols and tannins . Also the cold

aqueous ethanolic extract showed positive result regarding to existence of carbohydrates ,glycosides , phenols , flavonoids and saponnins whereas the hot ethanolic extract indicated presence of carbohydrates , glycosides , alkaloids and phenols . Therefore abundance of natural compounds as phytochemicals in medicinal plants including *Phoenix dactylifera*(Breim) leaflets insures the biochemical and medicinal activity of this plant . The difference of abundance of active chemical compounds in study plant belong to variance the type of solvent polarity and temperature required for dissolving the phytochemicals.

Therefore , the importance of these phytochemical natural active chemical compounds in Iraqi *Phoenix dactylifera* (Breim) leaflets , represents the high biochemical activity of this medicinal plant in treating many different diseases including diabetes mellitus (Laouini *et al* ,2013 ; Stalikas,2007).

Phenols of Iraqi *Phoenix dactylifera* (Briem) leaflets were isolated and purified by using hydrochloric acid (2%v/v) as a solvent and diethylether as an extractor for these active chemical compounds . The medicinal importance of these phytochemicals belong to their ability in treatment of various diseases such as cancer, hyperlipidemia , hyper - thyroidism and diabetes mellitus (Raman *et al* ,2012 ;Bratosikova e*t al* ,2003).

3.2Thin Layer Chromatography (TLC) results :

The chemical results of application of thin lager chromatography used for separation of phenolic compounds abundant in phenols extract indicated appearance of five spots with rates of flow equal to 0.35,0.43 , 0.73 and 0.81 this ensures presence of five active chemical compounds belong to phenols family in phoenix dactylifera (Breim) leaflets as in the table (3) .

Table (3) Thin layer chromatography results of phenolic compounds separated from phoenix dactylifera leaflets.

Eluent system	Reagent	Spot numbers	Result	Rf values	Conclusion
Butanol – HAC – H_2O (0.5 : 2.5 : 7)	Eyes	4	Light green	0.35, 0.43, 0.73, 0.81	Pure compounds
	UV-Lamp	4	Light violet	0.35 ,0.43 , 0.73 , 0.81	Presences of double bond coniugation system
	I_2- Vapor	4	Brown	0.35, 0.43, 0.73,0.81	Presences of orangic compounds
	$FeCl_3$(1%)	4	Bluish- green	0.35, 0.43, 0.73 ,0.81	Presences of phenolic compounds

The differences of R_f values of four phenolic compounds belong to variance in many factors such as their polarity , molecular weight and stereochemistry . Also using of TLC as an analytical technique for separation of phytochemical active compounds including phenols , ensures importance and advantage of this chromatographic method in isolation and purification of these compounds because it has many features such as accuracy and good separation (Harborne ,1984).

3.2 Column Chromatography (CC) results

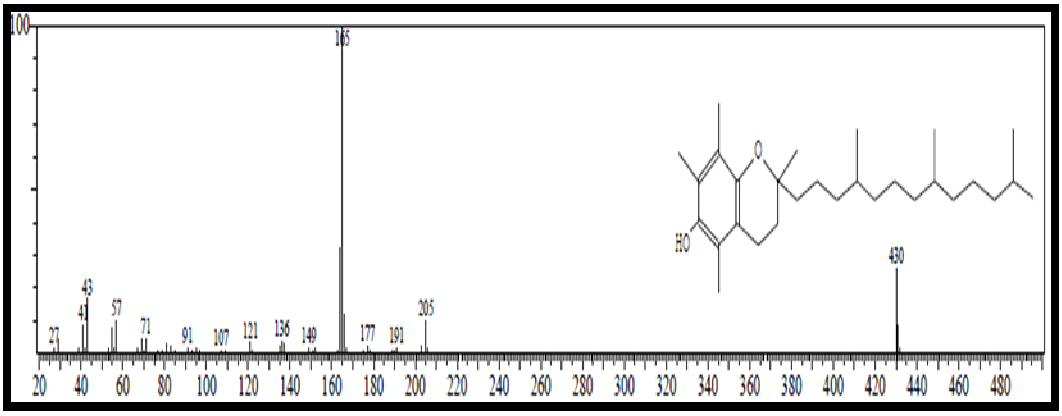
The chemical results of separation of phenolic compounds by column chromatography method led to isolation of each phenolic compounds isolation by using chromatographic separation column which was optimized and the separation was carried out by collecting each active chemical compound then application of TLC for each one and correspondence of TLC in both separations . The rates of flow (R_f) values were measured according to sequential separation for first , second , third and fourth compounds as in table (4) .

Table (4) : TLC results of phenolic compounds separated by column chromatography.

Phenolic compound number	Phenols developer	Rate of flow (R_f)value	Status
1	$FeCl_3$(1%)	0.35	Pure phenolic compound
2	$FeCl_3$(1%)	0.43	Pure phenolic compound
3	$FeCl_3$(1%)	0.73	Pure phenolic compound
4	$FeCl_3$(1%)	0.81	Pure phenolic compound

3.3 Mass Spectroscopy of phenolic compounds

Mass spectrum was recorded for each phenolic compound isolated and separated from Iraqi *Phoenix dactylifera* (Breim) leaflets by using shimadzu GC-MS-Qp2010 ultra system spectrophotometer. The spectra of phenols indicate the abundance , m/z values and structural and functional peaks so the active phenolic compounds isolated , separated and identified by mass spectroscopy are alpha –tocopherol , 2-[4-n-propylresorcinol] , 3-hydroxy tyrosine and 2-hydroxy-5- methylisophthaldehyde as in the figures (1,2,3, and 4)



Figure(1): Mass spectrum of alpha-tocopherol and its chemical structure

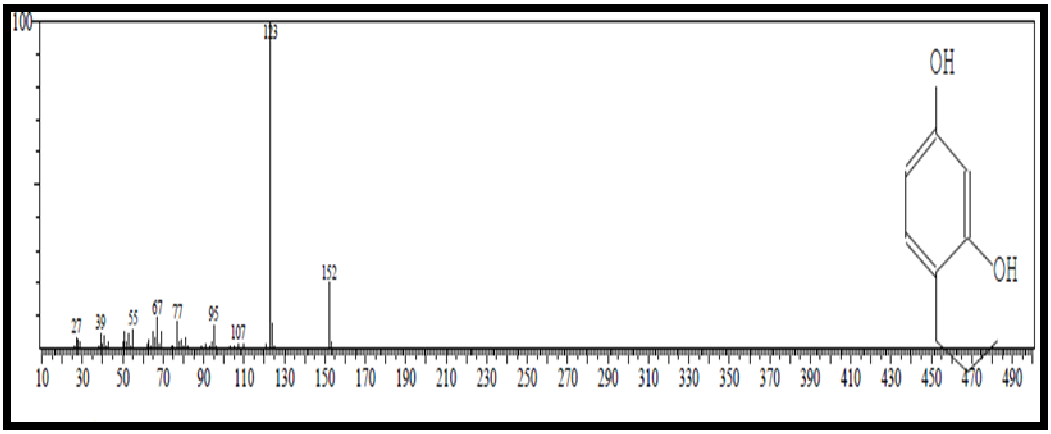


Figure (2): Mass spectrum of 2-[4-n-propylresorcinol] and its chemical structure

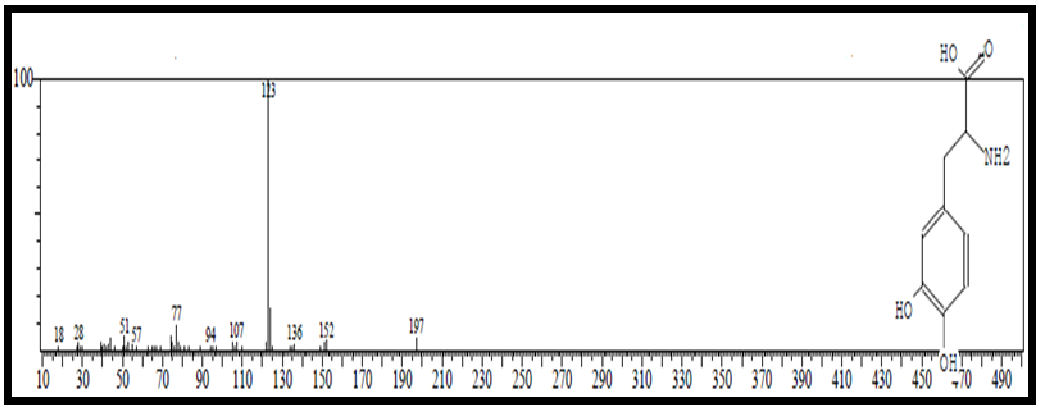


Figure (3): Mass spectrum of 3-hydroxy tyrosine and its chemical structure

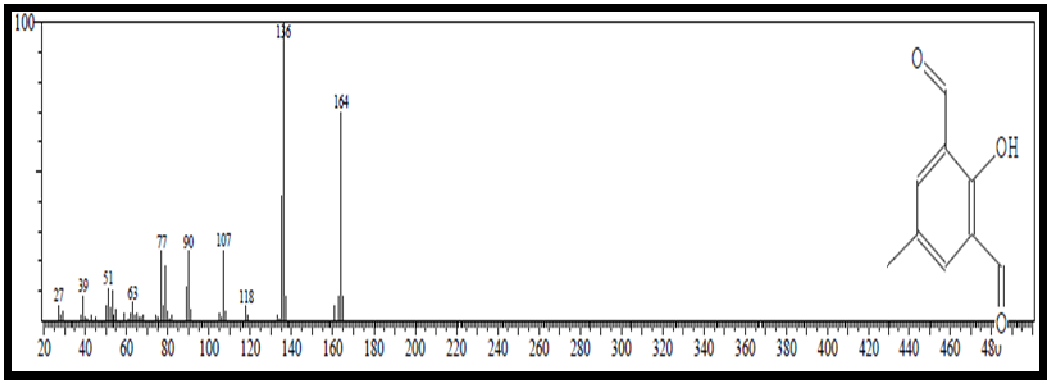


Figure (4): Mass spectrum of 2-hydroxy-5- methylisophthaldehyde and its chemical structure

3.4 Forrier- Transform Infra-red Spectroscopy of phenolic compounds:

FT-IR-spectra of four phenolic compounds isolated and identified from *Phoenix dactylifera* (Breim) leaflets , which are represented by alpha - tocopherol, 2-[4-n-propylresorcinol], 3-hydroxy tyrosine, and 2-hydroxy-s-methyl isophth- aldehyde are illustrated in figures (5,6,7 and 8).

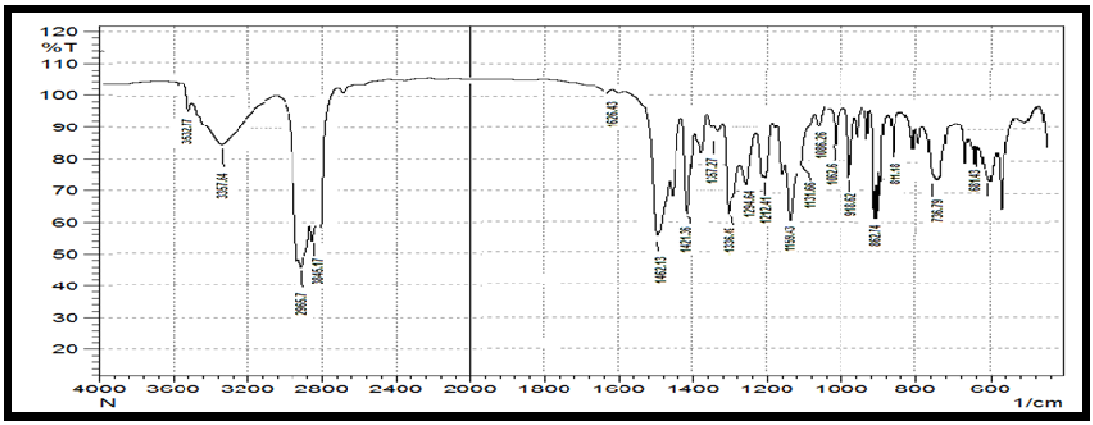


Fig (5):FT-IR-spectrum of alpha-tocopherol compound

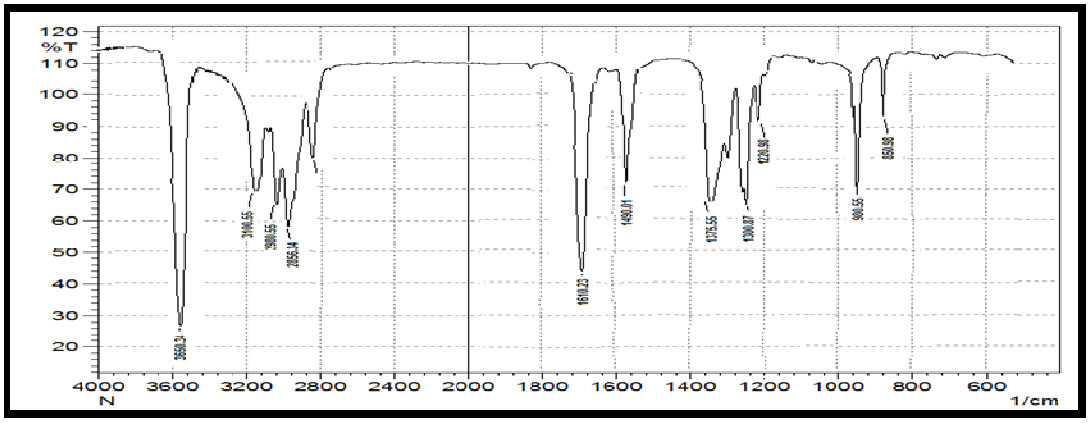


Fig (6):FT-IR-spectrum of 2-[4-n-propylresorcinol]compound

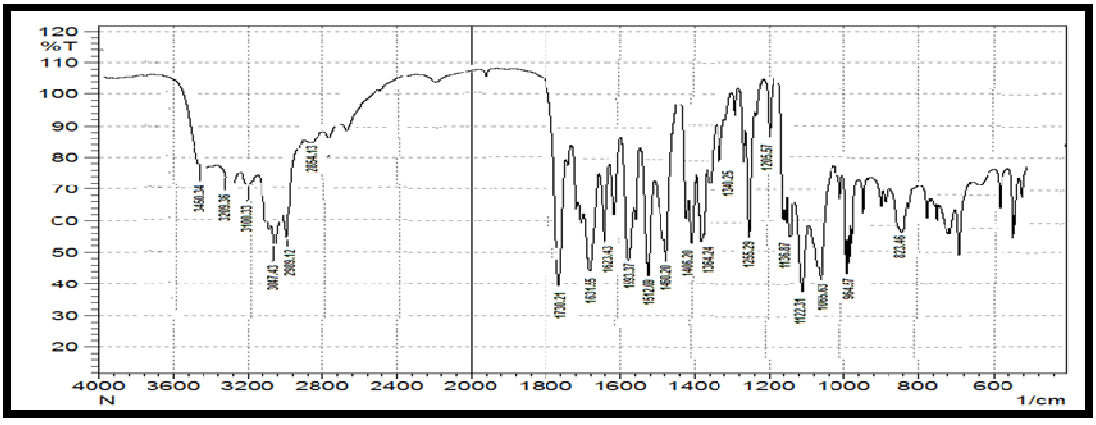


Fig (7):FT-IR-spectrum of 3-hydroxy tyrosine compound

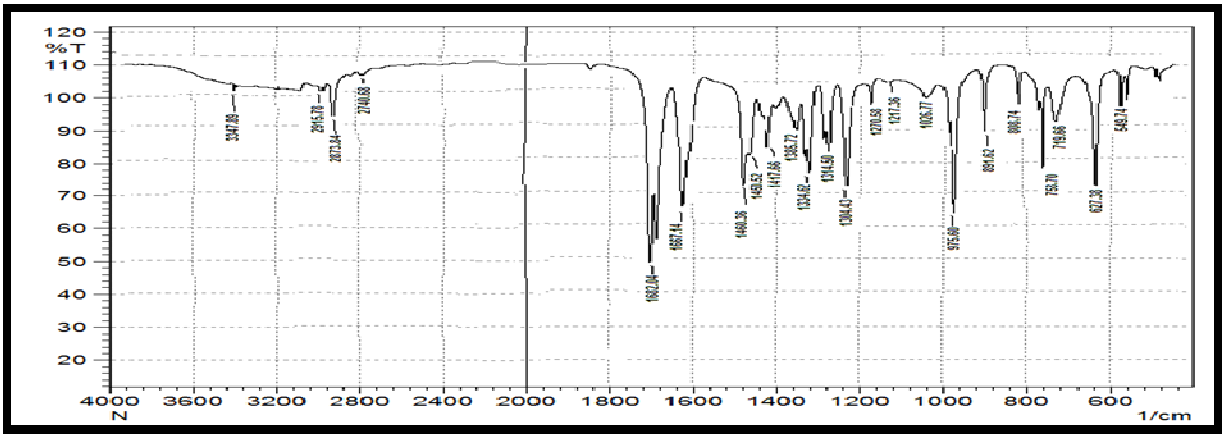


Fig (8):FT-IR-spectrum of 2-hydroxy-5- methylisophthaldehyde compound

From all IR- spectra belonging to the four phenolic compounds isolated from *Phoenix dactylifera* (Breim) leaflets , different structural and functional groups are present in chemical structures of these phytochemical compounds such as hydroxyl (-OH) , aromatic C=C , aliphatic C-H , C-O ,C=O ,aromatic CH_2 , aliphatic C= C groups which appear in various wavenumbers according to their absorbance's.

3.5 Hypoglycemic Action of phenolic compound synergistic interaction in alloxan – induced diabetic rabbits

The effect of oral administration of four phenolic compounds isolated from *Phoenix dactylifera* leaflets regarding decreasing blood glucose concentrations in alloxan –induced diabetic rabbits , is shown in table (5). It was noticed that the synergistic interaction of these active natural compounds led to significantly decrease after 2 hours ($p^{*}<0.05$) (325.50mg /100ml $\pm$4.77) from oral administration and also a significant decreasing was observed at 4 and 6 hours ($p^{**}<0.01$) with values equal to (280.67$\pm$2.7 and 238.33 $\pm$8.19) respectively whereas the highest significant lowering was recorded at 24 hours (p***<0.001) with value equal to (134.50 mg/100ml $\pm$4.88).

Table (5): Hypoglycemic action of oral administration of phenolic compounds isolated from *Phoenix dactylifera* (Breim) leaflets in fasted hyperglycemic rabbits.

Extract Dose (gm/kg)	N	Blood glucose conc. (mg/100 ml)				
		0 hrs	2 hrs	4 hrs	6 hrs	24 hrs
Control 3ml normal saline	6	378.14± 4.80	361.67± 3.61	348.67± 5.98	331.67± 3.30	269.00± 3.26
0. 3g/3ml (phenols)	6	392.33± 4.716	325.50* ± 4.77	280.67**± 2.70	238.33**± 8.198	134.50***± 4.88

Also it was noticed that blood glucose concentrations have been decreased with increase of time from start of oral administration of phenolic compounds dose. The hypoglycemic action of phenols isolated from *Phoenix dactylifera* (Breim) leaflets belongs to biochemical ability of these active phytochemicals to reduce gluconeogenesis and increase glycogenesis processes in the liver and also decrease the gluconeogenesis pathway in the liver and muscles (Chung- Hung *et al* , 2012:Singh,2012).

Alloxan substance inhibits glucokinase enzyme which is responsible for conversion of glucose to glucose-6-phosphate,therefore phenols as phytochemical active compounds are able to induce reactive oxygen species (ROS) formation resulting in the selective necrosis of beta cells . Also metabolitic phenolic compounds act as antioxidants have ability to capture different free radicals so these natural phytochemicals protect the insulin hormone from these reactive radicals , then decrease oxidation of this hormone (Stalikas,2007; Abdallah *et al*,2011; Subha *et al,*2004). Various experimental studies were studied about different medicinal plants such as *Barleria lupulina, capparis sepiaria, Galega officinalis and konjac mannan* were commonly used as remedy for diabetes mellitus(Hand and Chawla ,1989; Lemus *et al* ,1999).

3.5 Cytoxicity Investigation results of phenolic compounds

The results of cotoxicity investigation of all phenolic compounds isolated from phoenix dactylifera (Briem) leaflets are shown in table (6) .

Table (6): Cytoxicity Investigation results of phenolic compounds isolated from phoenix dactylifera (Briem) leaflets.

Phenolic compounds conc. (mg/ml)	Hemolysis
1:1	NT-
1:10	NT-
1:100	NT-
1:1000	NT-
Control negative (Blood + Ringer solution)	NT-
Control positive (Tap water + Blood)	T+++

NT =Non Toxic , T = Toxic

It was noticed that the concentrations (1:1, 1:10 , 1:100 , 1:1000) mg / ml didn't give any hemolysis against red blood cells , so , the phenolic compounds isolated from *Phoenix dactylifera* (Breim) leaflets can be used safely to treat diabetes mellitus disease .

4. Conclusions

The phenolic compounds isolated , separated ,identified from *Phoenix dactylifera* (Breim) leaflets showed an excellent activity for decreasing of hyperglycemia case in fasted alloxan – induced diabetic rabbits which result from the effect of biochemical synergistic interaction of these natural active compounds. Therefore the phenols isolated can be used safely to treat diabetes mellitus disease instead of insulin and intibiotics but this work demands further clinical and pharmaceutical studies

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