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Original Article

Evaluation of Hypoglycemic Effect of Two flavonoidic Compounds Isolated from Iraqi Phoenix dactylifera (Gintar) Leaflets in Alloxan-Induced Diabetic Rabbits

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ABSTRACT

Diabetes Mellitus (DM) is a chronic metabolic disorder disease resulting from lack in action of insulin hormone. Thin Layer Chromatography (TLC) results showed presence of two flavonoidic spots from phoenix dactylifera(Gintar) leaflets have rate of flow equal to 0.35 , 0.4 . So These compounds were separated by column chromatography (CC) then they were identified by infra-red spectrum and gas chromatography-mass spectrum technique and they were phenol,2,2'-methylene bis[6-(1,1-dimethyl)-4-methyl and phenol,2,2'-methylene bis [6-methoxy-3-(2-propenyl). Hypoglycemic effects of these active compounds were investigated and estimated in normalglycemic and hyperglycemic rabbits. The blood glucose levels in fasted normal rabbits were 137.66, 117.5, 106.88, 100.50 and 80.66 mg/100ml at 0,2,4,6 and 24 hr respectively from oral administration time. Also the blood glucose levels in fasted diabetic rabbits were 375.83, 315.50, 213.66, 152.35 and 145.50 mg/100ml at 0,2,4,6 and 24 hr respectively. The two flavonoids had no toxic effect on hemolysis of red blood cells so these active compounds can be used safely to treat diabetes mellitus disease.

Keywords: Hypoglycemic Effect, flavonoidic compounds, Diabetic rabbits, *Phoenix dactylifera* (Gintar), Mass spectrum

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INTRODUCTION

Diabetes mellitus disease is a chronic complex metabolic disorder which is characterized by elevated blood glucose concentration and disturbance resulting from various biochemical disorders in carbohydrates metabolism. It is known that quantity of glucose sugar is controlled and regulated by insulin hormone secreted from β-cells in Langerhans islets abundant in pancreas orang (1,2) . Also diabetic patients, from both type 1 and 2 exhibit abnormal antioxidant status, auto – oxidation of glucose sugar as monosaccharide and an increase in glycosylated proteins. Glucose in plasma either is gotten from dietary sources or is either resulting from the glycogenolysis process (catabolism of glycogen as polysaccharide in the liver) or glyconeogenesis process (anabolism of glucose in the liver and kindly from non-carbohydratic biochemical sources such as pyruvate, lactate, glycerol and amino acids (3,4).

Diabetes mellitus is considered as a dangerous common metabolic problem resulting from the inability of body's response to high glucose concentrations therefore diabetic patients experience different vascular complications such as atherosclerosis, hyperglycemic nephropathy and retinopathy. Hyperglycemia case is an accepted and well investigated reason of diabetes related eye disease, so many studies exist which implicate hyperglycemia as a key factor involved in visual disorder. This complex disease has affected millions of people all over the world (5,6,7).

Medicinal plants are the natural sources to produce active chemical compounds biochemically through secondary metabolism by using many various different chemical pathways catalyzed by specific enzymes. The existence of these active metabolites in plant is very important to protect the plant from dangerous effects. Also occurrence of these natural chemical compounds, has led to treat various diseases because the excellent ability of these natural products as therapies for health disorders. In medicinal plants, there are various components as chemical compounds such as phenols, flavonoids, tannins, alkaloids, glycosides, saponins, steroids, terpenes and essential oil(8,9).

Flavonoids are chemical classes belong to phenolic compounds and consist of many subclasses such as flavones, isoflavones, flavanonol, flavonoles, flavonones, flavan-3-ol and chalcones. Because of hydroxyl group in their chemical structures so they are hydroxylated phenolic substances are naturally and biochemically produced by secondary metabolism pathways. Presence of functional hydroxyl groups in flavonoidic compounds make these natural components are capable of having medicinal activity as antihyperglycemic, antibacterial, antifungal, antitumor and anticancer agents(10).

The medicinal significance of flavonoids as active metabolites compounds comes from their biochemical ability and activity for treatment of many different diseases such as diabetes mellitus and cancer. Also these natural compounds isolated from medicinal plants have no side effects so they can used safely as therapies for various diseases. Chemically flavonoidic compounds are based on at least two phenol rings linked via a heterocyclic pyrane ring. Also flavonoids occur as glycosides and methylated derivatives(11,12).

Phoenix dactylifera is one of the species belongs to date palm abundant in different countries and it belongs to Arecaceae family. It is known that *Phoenix dactylifera* (date plam) is considered as an essential food for different peoples at the world, and also various species of this medicinal plant, is used in sweets industry *Phoenix dactylifera* plant includes many varieties depending on the shape and organoleptic properties of its fruits(13,14). The current plant contains about 200 genera with around 3000 species. Gin tar is one of the *Phoenix dactylifera* species which is grown in Iraq country especially at Basrah governorate. Various and numerous studies indicated biochemical importance of the preventive effect of *Phoenix dactylifera* against different environmental chemical compounds which lead to toxic effects for human and animal tissues(15).

Different active chemical compounds such as phenols, gallic acid, caffeic acid, cumaric acid, vanillic acid, p-hydroxy benzoic acid, procyanidin and isoquercetrin were isolated, separated and identified from *Phoenix dactylifera*. Also some vitamins such as C , A and E were isolated as antioxidants from the same plant(16,17).
Therefore the present study has aimed to investigate and evaluate the hypoglycemic effect of flavonoids isolated from Iraqi *Phoenix dactylifera*(Gintar species) leaflets in diabetic rabbits induced by alloxan.

MATERIALS AND METHODS

Study plant: *Phoenix dactylifera* L.(Gintar) leaflets were gotten and collected from Mushaigeeja village in Abu Al-Khaseeb region at Basrah governorate in Iraq, September 2016. The plant was taxonomied and identified by a bontanist in biology department at Education College for pure sciences in Basrah University. The leaflets of date palm were cleaned and washed using tap water

then by distilled water to remove dusts and dirt's, After that the leaflets were dried in dark place, ground as powder by electrical mill and kept in dark containrs at room temperature until the day of use.

Preparation of cold aqueous extract of *Phoenix dactylifera* (Gintar) leaflet: Fifty grams of dried powder of *Phonenix dactylifera* (Gintar) leaflets were mixed with 500 ml of distilled water then the mixture was stirred on magnetic stirrer by using magnetic bar for 16 hours. The filtration process was carried out and the precipitin was removed by using Buchner funnel then the filtrate was dried and concentrated under vacuum instrument and the crude was gotten. By the same method, cold ethanolic extract was prepared but by using ethanol as a solvent (18).

Preparation of hot aqueous extract of *Phoenix dactylifera* (Gintar) leaflets: Fifty grams of dried ground leaflets of date palm were dissolved in 500ml of distilled water and refluxed for 16 hours. After filtration of mixture, the precipitate was removed and the filtrate was dried and concentrated under vacuum apparatus to get the crude. By the same method cold ethanolic extract was prepared but by using ethanol as a solvent (19).

Preliminary qualitative tests of *Phoenix dactylifera* (Gintar) leaflets extracts: The cold and hot aqueous and ethanolic extracts of date palm leaflets were underwent numerous qualitative tests by using various chemical reagents as the following:

1. Phenols tests : It was applied by using ferric chlorid (1% w/v) reagent(18).
2. Flavonoids test: It was achieved by using (5N) ethanolic potassium hydroxide reagent(20).
3. Alkaloids test: It was carried out by using Dragendroff, Myer and Wagner reagents (21).
4. Tannins test: It was applied by using (1% w/v) lead acetate reagent (22).
5. Glycosides test: It was achieved by using Benedict reagent (18).
6. Saponins test: It was carried out by using (5% w/v) mercuric chloride (20).
7. Reducing sugars test: It was applied by using Benedict reagent (19).

Gas chromatography-mass spectrum (GC-MS) technique: The technique of GC-MS was used successfully for cold and hot ethanolic and aqueous extracts to separate and identify the active chemical compounds abundant in these extracts by using gas chromatography –mass spectrum instrument at college of agriculture in university of Basrah. The optimal and standard conditions of instrument were represented by shimadzu GC-MS-QP 2010 ultra-system has 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.(1/100v/v in hexane) was used as diluted sample (2 ml) were injected.

Isolation of flavonoids from *Phoenix dactylifera* (Gintar) leaflets: Fifty grams of dried ground leaflets of *Phoenix dactylifera* (Gintar) were mixed with 250ml of (80% v/v) methanol then the methanolic mixture was stirred at room temperature by using magnetic stirring for 24 hours. The mixture was filtered by Buchner funnel and the precipitate was removed. Then 25ml of (1% w/v) lead acetate was added into filtrate, after that the mixture was filtered and the filtrate was removed by Buchner funnel then the precipitate was treated with 25ml of acetone and 30ml of concentrated hydrochloric acid. Filtration process was performed for the mixture and the filtrate was evaporated to give 3.01gm which was dissolved in 25ml of distilled water, then it was extracted by using ethyl acetate (3x50) by using separation funnel. The combined quantities of product from extraction were dried to yield 1.8 gm (18,23)

Thin layer chromatography of flavonoids isolated from *Phoenix dactylifera* (Gintar) leaflets: The isolated flavonoids were separated depending on thin layer chromatography (TLC) analysis by using 50ml of flavonoids of leaflets. Then it was tolerance on alumina plate (2x8cm) coated by silica gel. The eluent, butanol-acetic acid – water was used as mobile phase with ratio equal to (2: 2: 6). The separation process time was 45min then the spots were separated and the TLC was dried then the spots were developed by iodine vapor, UV-lamp at 233nm and (1%) ferric chloride. Rates of flow (R_f) values were calculated for all spots (18, 19).

Column chromatography of flavonoidic compounds: The flavonoidic compounds which were separated TLC, they were separated by column chromatography (CC) analysis to separate an isolate each flavonoidic compound alone. The separation was achieved by using glass column with standard separation conditions were represented by radius (1cm) , length (50cm) and the stationary phase was (butanol – acetic acid – water) solvent with ratio equal to (2 : 2 : 6) . The separation time was 45 min and the volume of each separation was 3 ml then TLC was carried out for each flavonoidic compound separated by column chromatography after that rates of flow (R_f) values were measured for each flavonoid, and the correspondence case was applied between R_f values

for both TLC and TLC of column chromatography (24).

Forrier Transformation Infra-red spectroscopy of flavonoid compounds: Infra-red (IR) spectra were recorded for each flavonoidic compound separated by column chromatography by using FT-IR -8400 S spectrophotometer type shimadzu, Japan. The samples were prepared by mixing them with potassium bromide (KBr) as a disc and the spectral range for measuring was (4000-500 cm^{-1}) .

10-Experimental Animals: Twelve healthy rabbits weigh (1.5-2.5 kg) were bought from main local market in Basrah at Iraq. Then they were kept in clean iron cages and fed standard antibiotic free diet after that the rabbits were underwent fasting condition for 24 hours before proceeding the experiments.

Induction of Hyperglycemia in rabbits : The fasted rabbits were induced by alloxane monohydrate dissolved in sterile normal saline and this process has led to appearance the hyperglycemia case in research animals. Alloxan solution was used immediately after preparation and was administrated at period of 48 hours by using dose of 150 mg/kg of rabbit weight then injected via marginal ear vein under light by using 1ml syringe. After that 20% w/v of glucose sugar was administrated orally for diabetic rabbits and were kept in fasting case for 18 hours after seven days from the last administration (25).

Estimation of Blood Glucose concentrations in Alloxan Diabetic Rabbits: The blood glucose concentrations in hyperglycemic rabbits were measured by using glucose oxidase peroxidase enzymatic colorimetric (GOD – PAP) method depending on device of measurement of blood glucose provided by measuring strips. (26)

Hypoglycemic Effect of flavonoidic compounds in Diabetic Rabbits: Twelve fasted hypoglycemic rabbits were divided into two groups , the first one was administrated 3ml of normal saline and considered as control group whereas the second group was administrated orally 0.3 gm/ml dose of flavonidic compounds for 1 kg of rabbit body weight and this group was considered as treatment group. Blood glucose concentrations were measured at times 0,2,4,6 and 24 hours after oral administration (27) .

Determination of cytoxicity of flavonoidic compounds isolated from *Phoenix dactylifera* (Gintar) leaflets: The cytoxicity of flavonoidic compounds mixture isolated from phoenix dactylifera (Gintar) leaflets was recorded by dissolving 200mg of flavonoids in 10ml of normal saline , Then different concentrations represented by (1:1 , 1:10 , 1:100 and 1:1000 v\v) were prepared . Normal saline was considered as a control group, after that 0.8 ml of each concentration was added into a sterile test tube is called Ependroff tube contains anticlotting substance. Then 0.2 ml of fresh human blood was added into each tube so the final volume became 1.0 ml and the all tubes were incubated at 37^{o}C for 30 minutes. Lately all test tubes were centrifuged for five minutes (28).

Statistical Analysis: The procedure of statistical analysis was carried out for all experiments regarding fasted normal and hyperglycemic rabbits depending on one way ANOVA method of variance analysis by SPSS version to test presence of significant differences between control and treatment mean (25).

RESULTS AND DISCUSSION

The percentage of extraction of cold and hot aqueous and ethanolic extracts and flavonoids are indicated in table (1).

The extraction percentages were measured according to the following equation.

Extraction percentage (%) = weight of plant crude (gm) / weight of powder plant (gm) *100

Phoenix dactylifera plant is considered as an important medicinal plant to treat various diseases so different drugs can be synthetically made and used from this plant. Many studies ensured presence of numerous active chemical compounds in the different parts of *Phoenix dactylifera* such as phenols, alkaloids, flavonoids, anthocyanins and glycosides (29,30). The extraction percentages of cold ethanolic , hot ethanolic and flavonoids extracts were 12.2% , 9.16% , 7.16% , 8.88% and 6.0% respectively so these percentages are considered significantly very good compared with a previously study (2).

The extraction percentage belonging to cold aqueous extract is the greatest this ensures abundance of high quantities of active metabolic compounds in *Phoenix dactylifera* (Gintar) leaflets. Alsothe flavonoids extract had the lowest extraction percentage.

Results of phytochemical qualitative analysis of extracts isolated from *Phoenix dactylifera* (Gintar) leaflets: The phytochemical results of qualitative analysis of cold aqueous, hot aqueous, cold ethanolic and hot ethanolic extracts isolated from *Phoenix dactylifera* (Gintar) leaflets are shown in table (2). It was noticed that the carbohydrates alkaloids, phenols and flavonoids are found in cold aqueous, hot aqueous, cold ethanolic,

hot ethanolic extracts and glycosides are abundant in hot aqueous extract only whereas tannins, terpenes and steroids are not found in all extracts.

The phytochemical qualitative analysis of *Phoenix dactylifera* (Gintar) leaflets results indicated presence of different active chemical compounds which were represented by phenols, flavonoids, alkaloids, glycosides and saponins. Many different studies proved existence of phenols and flavonoids in chemical extracts belonging to *Phoenix dactylifera.* So the importance of natural metabolic compounds illustrates the biochemical activity of date palm plant (31,32) .

Thin Layer Chromatography (TLC) Results of Flavonoids Separated from *Phoenix dactylifera* (Gintar) leaflets: Thin layer chromatography (TLC) analysis was carried out for separation of flavonoidic compounds existing in flavonoids extract belonging to *Phoenix dacteylifera* (Gintar) leaflets as in table (3). Two spots were appeared through separation in TLC have rates of flow (R_f) values equal to 0.35 and 0.40 therefor these spots indicate presence of two flavonoidic compounds.

Thin layer chromatography (TLC) proved presence of two flavonoidic compounds in flavonoids extract where these active compounds have different rete of flow (R_f) values because various factors affecting the separation process such as polarity, molecular weight and stereochemistry. Therefore TLC analysis is considered as an excellent technique since it has various advantages such as accuracy and characterized separation (18). Also the two flavonoidic compounds were separated by column chromatography to obtain each flavonoidic alone then thin layer chromatography was carried out and rates of flow (R_f) values were corresponded.

Column chromatography (CC) results of flavonoidic compounds: The two flavonoidic compounds were separated alone by using column chromatography with optimal conditions by collecting each compound then thin layer chromatography were applied for each flavonoidic compound and rates of flow were corresponded in both separations as in table (4) . The R_f values of flavonoidic compounds represted by 0.35 and 0.4 were the same R_f values when column chromatography was applied and this conditions ensure separating two active chemical compounds belong to flavonoids family (19).

Mass Spectroscopy of flavonoidic compounds: Mass spectrum of the two flavonoidic compounds were recorded by using Shimadzu GC-MS-QP2010 ultra system spectrophotometer are phenol,2,2' – methylene bis[6-(1,1-dimethyl)-4- methyl and phenol, 2,2' - methylene bis [6- methoxy-3-(2-propenyl)] as in figures (1 and 2)

Mass spectra indicated that the isolated flavonoidic compounds are phenol 2,2'- methylene bis [6-(1,1-dimethyl) -4- methyl] and phenol,2,2'-methylene bis [6-methoxy- 3-(2-propenyl)]. The mass spectra represent the abundance, m/z values and structural and functional groups. So the using of mass spectroscopy showed excellent features regarding to fine chemical structure of isolated flavonoids from *Phoenix dactylifera* (Gintar) leaflets.

Forrier – Transform Infred – red spectroscopy of flavonidic compounds: FT-IR spectra for the two flavonidic compounds represented by phenol,2,2' – methylene bis[6-(1,1-dimethyl)-4-methyl] and phenol, 2,2' - methylene bis [6-methoxy-3-(2-propenyl)] are shown in figures (3 and 4)

Also FT-IR spectra of the two flavonoidic compounds indicated presence of the structural and functional groups existing in the chemical structures through different peaks obtained from these spectra. The functional group represented by hydroxyl (-OH) is characterized group abundant in phenolic compounds especially flavonoids. Also presence of hydroxyl groups in aromatic system ensures occurrence of flavonoids having phenolic groups. The appearance of aromatic (C=C), aliphatic (C-H), (C-O), (C=O), aromatic (CH_2), aliphatic (C=C) groups as structural groups proves chemical structures obtained by mass spectra.

Results of hypoglycemic effect of phenol,2,2' – methylene bis[6-(1,1-dimethyl)-4- methyl] in normalglycemic and hyperglycemic rabbits: The blood glucose concentrations were measured in fasted normalglycemic rabbits by using 0.3 gm/3ml dose of the flavonoidic compound represented by phenol,2,2' – methylene bis[6-(1,1-dimethyl)-4- methyl as allustrated in table (5)

The blood glucose concentrations recorded by phenol,2,2'-methylene bis[6-(1,1-di-methyl) -4-methyl] compound were 133.50 , 129,66 , 121.83 , 118.16 and 101.66 mg/100ml at 0 , 2 , 4 , 6 and 24 hrs from oral administration in fasted normalglycemic rabbits . There was a significant decreasing at 4 and 6 hrs while high significant decreasing was recorded after 24hrs from oral administration. Also, hypoglycemic effect results of phenol, 2,2'- methylene bis [6-(1,1- dimethyl) -4-methyl] in fasted alloxan- induced diabetic rabbits are indicated in table (6). In Alloxan-induced diabetic rabbits, the biood glucose concentrations were recorded equal to 337.66 , 316.12 , 278.00 , 208.16 and 167.00 mg/100ml after 0 ,2 ,4 , 6 and 24hours respectively from oral administration o

flavonoidic compound dose . The hypoglycemic effect here is very clear where a decreasing was measured at 2hrs and significant decreasing was noticed at 4 and 6hrs but the highest significant lowering was recorded at 24hrs. This decreasing to glucose concentrations belonge to biochemical activity of phenolic compounds including flavonoids to reduce gluconeogenesis and increase glycogenesis pathways in the liver and also flavonoidic compounds decrease gluconeogenesis pathway in the muscles and liver (33,34).

The biochemical action of alloxan is represented by inhibition of glucokinase enzyme responsible for transformation of glucose to glucose-6-phosphate so the flavonoidic compounds increase glycogenesis process to reduce blood glucose (35).

Results of hypoglycemic effect of phenol, 2,2'-methylene bis [6-methoxy-3-(2-propenyl) in normal and hyperglycemic rabbits: The blood glucose concentrations were recorded in fasted normalglycemic rabbits by using 0.3gm/3ml /1kg dose of phenol,2,2'-methylene bis-[6-methoxy-3-(2-propenyl)] as ollustrated in table (7). The hypoglycemic effect of phenol, 2,2'-methylene bis [6-methoxy- 3-(2-propenyl)] in normal glycemic rabbits was investigated . The blood glucose concentrations recorded were 131.5, 119.66, 112.66, 92.50 and 87.00 mg/100ml at 0 , 2 , 4 , 6 and 24hours respectively . Asignificant decreasing was recorded after 4 and 6 hrs from oral administration of this flavonoidic compounds and also high significant lowering was noticed at 24hours. Also the hypoglycemic effect results of phenol,2,2'-methylene bis-[6-methoxy-3-(2-propenyl)] in fasted alloxan-induced diabetic rabbits are indicated in table (8).

In regard to blood glucose concentrations in hyperglycemic rabbits , it was found that the values of concentrations were 339.82 , 318.00 , 224.83 , 191.00 and 135.16 mg/100ml after 0 , 2 , 4 , 6 and 24hours from oral administration of the dose of phenol,2,2'-methylene bis [6-methoxy- 3-(2-propenyl)] compound . A decreasing was recorded at 4hrs and a significant decreasing was noticed at 6hrs whereas the highest significant lowering was recorded at 24 hrs.

Flavonoids as phytochemical active compounds have chemical ability for induction of reactive oxygen species (ROS) formation resulting from selective necrosis for Beta cells. Also phenolic compounds including flavonoids behave as antioxidants possess the capability to capture many various free radicals and this process lead to protect insulin hormone from danger of these reactive species (36,37) . Numerous various studies were achieved about the biochemical role of hypoglycemic action different active compounds isolated from various medicinal plant such as Dodonaoab viscosa, Gymnema sylvestre, Momordica charantia, Berberis lyceum (38,39,40), Catheranthus roseus (41) and Barssica oleracea var. capiptata (Brassica ceae) (42) .

Cytoxicity results of the two flavonoidic compounds mixture: The effect of cytoxicity of phenol,2,2' – methylene bis[6-(1,1-dimethyl)-4-methyl and phenol,2,2'-methylen bis-[6-methoxy-3-(2-propenyl)] compounds mixture, is indiated in table (9).

Cytoxicity of the two flavonoidic compounds mixture was investigated and estimated depending on red blood cells hemolysis method where it was found that the concentrations represented by 1:1 , 1:10 , 1:10 and 1:1000 mg/ml didn't show any hemolysis process towards red blood cells therefore these active chemical compounds isolated from *Phoenix dactylifera* (Gintar) leaflets are not toxic . Then these compounds be used safely for cure of hyperglycemia case represented by diabetes mellitus disease. Some health reports determined cytoxicity of different chemical compounds isolated from various medicinal plants (43,44) .

Conclusions

The flavonoidic compounds represented by phenol,2,2'-methylene bis[6-(1,1-di-methyl) -4-methyl] and phenol ,2,2'-methylene bis [6-methoxy-3-(2-propenyl)] have showed excellent hypoglycemic effects in normal and alloxan-induced diabetic rabbits . So these natural active chemical compounds can be used instead of insulin drug as therapies for diabetes mellitus disease. Also the two flavonoidic compounds have no toxic effect against hemolysis of red blood cells therefore they can be carried out safley to treat hyperglycemia case.

Table (1): Extraction percentage of *Phoenix dactylifera*(Gintar) leaflet extracts .

No.	Extract type	Extract weight	Extraction percentage (%)
1.	**Cold aqueous**	**6.10**	**12.2**
2.	**Hot aqueous**	**4.58**	**9.16**
3.	**Cold ethanolic**	**3.58**	**7.16**
4.	**Hot ethanolic**	**4.44**	**8.88**
5.	**Flavonoids**	**3.0**	**6.0**

Taple (2): Phytochemical qualitative analysis of all prepared extract of *Phoenix dactylifera*(Gintar) leaflets .

Reagent	Extract type				Test result	Test result
	Cold Aqueous	Hot aqueous	Cold Ethanolic	Hot ethanolic		
Molisch	+	+	+	+	Violet ring	Presence of carbohydrates
Benedict (before hydrolysis)	+	+	+	+	Red precipitate	Presence of reducing sugars
Benedict (after hydrolysis)	_	+	_	_	More red precipitate	Presence of glycosides
Dragendroff		+	+	+	Orange precipitate	Presence of alkaloids
Ferric chloride(1%)	+	+	+	+	Bluish-green precipitate	Presence of phenols
Ethanolic KOH(5N)	+	+	+	+	Yellow precipitate	Presence of flavonoids
$HgCl_2$(5%)	+	+	_	+	White precipitate	Presence of saponins
Lead acetate(1%)	_	_	_	_	No light brown precipitate	Absence of tannins
Libeman-Burchard	_	_	_	_	No two layers	Absence of terpenes and steroids

Table (3) Thin layer chromatography results of flavonoidic compounds separated from *Phoenix dactylifera*leaflets .

Eluent system	Reagent	Spot numbers	Result	R_f Values	Conclusion
Butanol – HAc-H_2O (2 : 2 : 6)	Eyes	2	Light yellow	0.35 , 0.40	Pure compounds
	UV-Lamp	2	Light violet	0.35 , 0.40	Presences of double bond coniugation system
	I_2- Vapor	2	Dark yellow	0.35 , 0.40	Presences of orangic compounds
	$FeCI_3$(1%)	2	Bluish- green	0.35 , 0.40	Presences of flavonoidic compounds

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

Flavonodic compound number	Developer	Rate of flow(R_f) value	Status
1	Ethanolic KOH (5N)	0.35	Pure flavonoidic compound
2	Ethanolic KOH (5N)	0.35	Pure flavonoidic compound

Table(5): Blood glucose concentrations recorded in fasted normalglycemic rabbits by using phenol,2,2'–methylenebis[6-(1,1-dimethyl)-4- methyl]

Flavonoidic compound dose (gm/ml /kg)	Rabbits No.	Blood glucose conc. (mg/100 ml)				
		0 hrs	2 hrs	4 hrs	6 hrs	24 hrs
Control 3ml normalsaline	6	132.7± 2.04	128.83± 1.16	125.66± 1.75	121.50± 1.37	117.5± 2.07
0. 3g/3ml	6	133.5± 4.03	129.66* ± 2.58	121.83 ± 1.94	118.16 ± 1.94	101.66*± 4.96

Table (6): Blood glucose concentrations rescorded in fasted alloxan- induced diabetic rabbits by using phenol,2,2'-methylene bis [6-(1,1-dimethyl)-4- methyl]

Flavonoidic compound dose (gm/ml /kg)	Rabbits No.	Blood glucose conc. (mg/100 ml)				
		0 hrs	2 hrs	4 hrs	6 hrs	24 hrs
Control 3ml normalsaline	6	337.50± 3.08	333.50± 4.08	330.83± 4.57	323.33± 5.42	317.00± 4.85
0. 3g/3ml	6	7.66± 2.50	316.12* ± 4.83	278.00* ± 12.45	208.16** ± 12.62	167.00***± 13.09

Table(7): Blood glucose concentrations recorded in fastednormalglycemic rabbits by using phenol,2,2'-methylenebis-[6-methoxy-3-(2-propenyl)]

Flovonoidic compound dose(gm/ml /kg)	N	Blood glucose conc. (mg/100 ml)				
		0 hrs	2 hrs	4 hrs	6 hrs	24 hrs
Control 3ml normalsaline	6	133.66± 2.06	131.83± 1.72	128.16± 1.94	124.00± 2.60	119.16± 2.63
0. 3g/3ml	6	131.5± 3.88	119.66* ± 3.14	112.66** ± 2.73	92.50*** ± 3.27	87.00***± 2.36

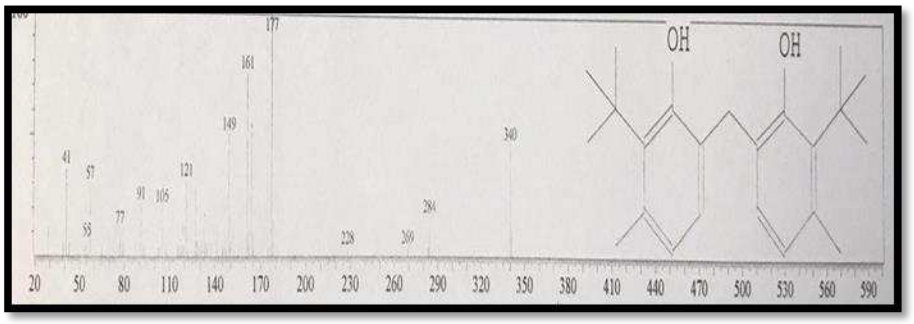
Table (8): Blood glucose concentrations measured in fasted alloxan- induced hyperglycemic rabbits by using phenol,2,2'-methylenebis-[6-methoxy-3-(2-propenyl)] compound

Flovonoidiccompound dose(gm/ml /kg)	N	Blood glucose conc. (mg/100 ml)				
		0 hrs	2 hrs	4 hrs	6 hrs	24 hrs
Control 3ml normalsaline	6	339.50± 7.13	337.50± 6.15	333.33± 7.65	327.00± 5.83	323.00± 3.74
0. 3g/3ml	6	339.82± 5.67	318.00 ± 6.84	224.83* ± 3.55	191.00** ± 5.44	153.16**± 4.07

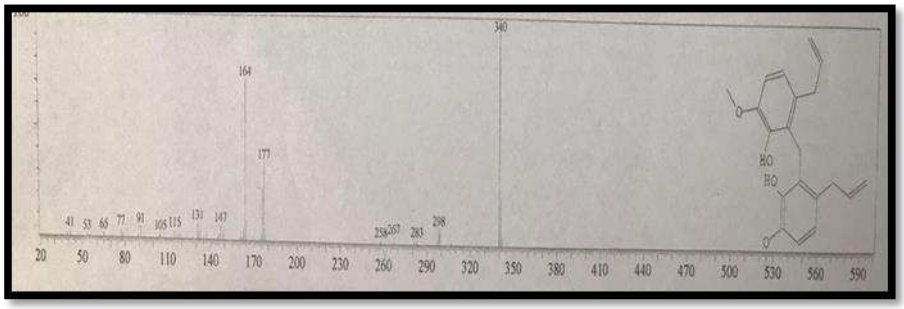
Table(9): Cytoxicity results of flavonoidic compounds mixture isolated from *Phoenix dactylifera* (Gintar) leaflets.

Flavonoidic compounds(A+B) mixture (gm/ml)	Hemolysis result
1:1	-NT
1:10	-NT
1:100	-NT
1:1000	-NT
Conttol negative (Blood+Ringer solution)	-NT
Control positive (tap water +blood)	T+++

A= phenol,2,2'– methylene bis[6-(1,1-dimethyl)-4- methyl]
B= phenol,2,2'-methylenebis-[6-methoxy-3-(2-propenyl)]
T= Toxic , NT= Non toxic



Figure(1): Mass spectrum ofphenol,2,2' – methylene bis[6-(1,1-dimethyl)-4- methyl and its chemical structure



Figure(2): Mass spectrum of phenol, 2,2' - methylene bis [6- methoxy-3-(2-propenyl)] and its chemical structure

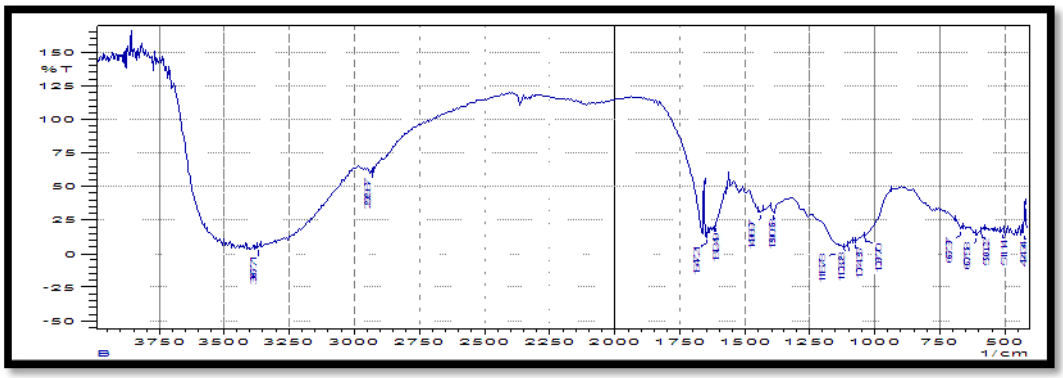
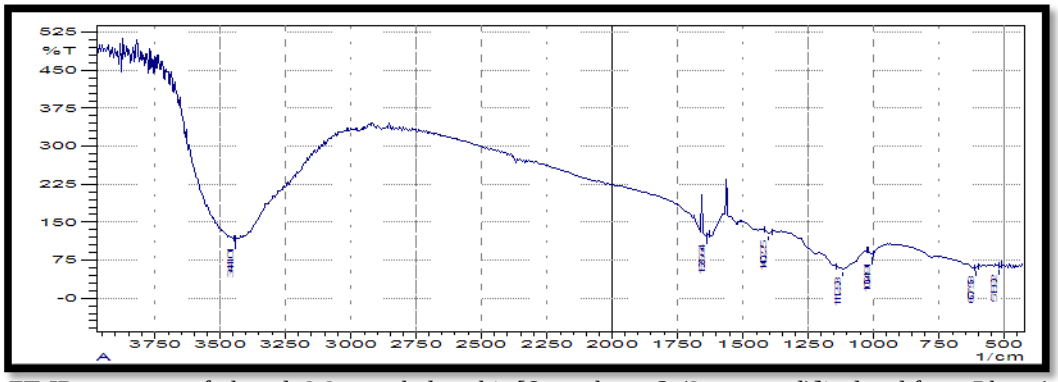


Fig (3):FT-IR-spectrum of phenol, 2,2' - methylene bis [6-(1,1-dimethyl) -4-methyl] isolated from *Phoenix dactylifera*(Gintar) leaflets .



Fig(4):FT-IR-spectrum of phenol, 2,2' - methylene bis [6- methoxy-3-(2-propenyl)]isolated from *Phoenix dactylifera*(Gintar) leaflets .

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