Vol. 2, No. 1: 24-29 (2014).
ISSN: 2313-3937

WORLD JOURNAL OF EXPERIMENTAL BIOSCIENCES

Research article

Effect of phenolic and alkaloid compounds extracted from *Brassica oleracea* var. capitata seed on glucose level in blood of alloxan-induced diabetes rabbits

Khalid Abdulkareem Mohammed[1]*, Abbas Dawwas Mattar Al-Maliki[1]

ABSTRACT

Brassica oleracea var. capitata is herbal plant traditionally used in different country to treat diabetes. In present study, the chemical compounds of *B. oleracea var*. capitata seed were specified by GC-Mass technique. Twenty four compounds were found. From these compounds one phenolic compound [2,4-bis(1,1-dimethylethyl)-] and one alkaloid compound (2,3-Dicyano-5,6-diphenylpyrazine) was specified with different concentrations. Alloxan-induced diabetic rabbit was prepared in present study after injecting the experimental rabbit with three doses of alloxan (i.v.). Alloxan-induced diabetic rabbits were divided to two main groups. First group was administrated orally with 0.3 g/kg phenolic compound (test group) while, second group was administrated with 0.3 g/kg alkaloid compounds (test group). The control of both groups was alloxan induced diabetic rabbit administrated orally with normal saline. The lowest level of glucose was observed in alloxan-induced diabetic rabbits that administrated with phenolic compound and the significant reduction was observed after 2 h of administration. While, the moderate reduction of glucose level was observed in alloxan-induced diabetes rabbit that administrated with alkaloid compound and the significant moderate reduction was observed post 24 h. It can be concluded from current study the phenolic compound [2,4-bis(1,1-dimethylethyl)-] that extracted from of *B. oleracea var*. capitata seed is a good treatment for reducing blood glucose in experimental animal and this compound better is better than alkaloid (2,3-Dicyano-5,6-diphenylpyrazine) in reducing blood glucose.

Keywords: Alkaloid (2,3-Dicyano-5,6-diphenylpyrazine), Alloxan, Diabetic rabbits, Phenol [2,4-bis(1,1-dimethylethyl)-].

Citation: Mohammed KA, Al-Maliki, ADM (2014) Effect of phenolic and alkaloid compounds extracted from *Brassica oleracea var.* capitata seed on glucose level in blood of alloxan-induced diabetes rabbits. *World J Exp Biosci* **2***:* 24-29.

Received April 30, 2014; Accepted May 19, 2014; Published May 29, 2014.

*Correspondence: khalid.kreem@yahoo.com
Department Chemistry, College of Education, University of Basrah, Basrah, Iraq
Full list of author information is available at the end of the article

INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder characterized by impaired metabolism of glucose and lipids due to defect in insulin secretion (beta cell dysfunction) or action (insulin resistance [IR]). The characteristic properties of DM are hyperglycemia, microvascular as well as macrovascular, pathologies with more than 17.5 million deaths world-wide [1]. It represents almost two thirds of the world population. A large number of in vivo studies have been conducted on animals to test the claimed activity have demonstrated the hypoglycemic property of many plants, already reported in various literatures [2]. Currently available treatment options in modern medicine have several adverse effects. Therefore, there is a need to develop safe and effective treatment modalities for diabetes. Medical plants play an important role in the management of DM especially in developing countries. More than 400 plants have been incorporated in approximately 700 recipes which are used to treat [2].

Brassica oleracea var. capitata (Brassicaceae) is a member of the food family traditionally known as cruciferous vegetables and is related to kale, broccoli, collards. Important properties of this plant prove the versatility in recent time. Antioxidant property [3, 4] of *B. oleracea* studied over which is also responsible for its anticholinesterase activity [5]. Both analgesic and anti inflammatory [2] properties of the plant were also found. Cruciferous vegetables such as cabbage are among the most important dietary vegetables consumed in Iraq owing to their availability in local markets, cheapness and consumer preference. It is playing a good role in improving the immune system by supporting the host with multi important vitamins that helped to eradicate different infectious diseases resident in studied area (Iraq) such as enter viruses, which resident in the south of Iraq [6-9]. In the recent study of Al-Jawadi showed in the diabetic rats, the protein extracted from *B. oleracea* in a dose of 75mg/kg body weight showed a significant decrease in serum glucose, cholesterol and total lipids levels [10].

In current study, cooled alcohol was used to extract the phenolic and alkaloid compounds from *B. oleracea* var. capitata seeds and checked the affectivity of this extracted against the level of glucose in alloxan induced rabbit was estimated. This kind of work is trying to find the safe treatment of diabetes mellitus.

MATERIALS AND METHODS

Study Plant

Seeds of *B. oleracea* var. capitata were collected from *B. oleracea* var. capitata planted in local market and college of agriculture, University of Basrah, Basrah. The plant was classified in the Herbarium of Basrah, College of Education, University of Basrah. The seeds were dried at 25°C, then ground by a blender (Rotel coffee grinder type 24) and kept in plastic containers at room temperature until they were used.

Preparation of Alcoholic Extracts of *B. oleracea* var. capitata seeds

Fifty grams of dried ground seeds of *B. oleracea* var. capitata were refluxed in 250 ml of 70 % ethanol for 24 hours, the precipitate was removed by filtration, through filter paper no.1, and then filtrate was concentrated under vacuum using freeze drier [11].

Gas chromatography–mass spectrometry (GC-MS) analysis

The GC-MS analysis of the alcoholic extract of seeds was performed using Shimadzu GCMS-QP2010 Ultra system having automatic sampler CTC analysis CombiPAL robotic arm. The inlet temperature was set at 260°C. The specification of the capillary column used was Agilent 19091S-433: 1548, 52849 HP-5MS 5% Phenyl Methyl Silox 30 m × 250 µm x 0.25 µm HP-5MS. The oven temperature was programmed from 50°C to 260°C. The diluted samples (1/100, v/v, in Hexane) of 2 µL were injected.

Isolation of phenols from *B. oleracea* var. *capitata* seeds

Fifty gm of seed powder (defatted powder) was dissolved in 250 ml of (2%) hydrochloric acid and the mixture was put in water bath for 8 h at 60 ^{o}C. The mixture was filtered by Buchner funnel and precipitate was removed. Equal volume of diethyl ether was added to the filtrate. The mixture was in water bath at 32 ^{o}C for 50 min. After that the mixture was concentrated and dried under vacuum rota-evaborator at 70 ^{o}C. The yielded weight of phenolic product was 2.92 gm [11].

Isolation of alkaloids from *B. oleracea* var. capitata seeds

Fifty gm of seed powder (defatted powder) was mixed with 250 ml of (10 %) ethanolic acetic acid and put on magnetic stirrer for 24 h. Then the mixture was filtered and precipitate was removed. The filtrate was concentrated to quarter of previous volume by using vacuum rota-evaborator at 70 ^{o}C and the pH was adjusted to 9 with ammonium hydroxide to precipitate the alkaloids. The mixture was put in separation funnel, 20 ml of chloroform was added and the mixture was mixed well. The organic layer was collected. This step was repeated three times and dried by vacuum rota-evaborator at 70 ^{o}C. The product weight was 1.3 gm [11, 12].

Quantization of Phenols and alkaloids in seeds extracts

The standard method of Al – Maliki, (2012) was followed to estimate the presence and amount of phenols and alkaloids extractions [11].

Animals

Rabbits weighing 2-2.5 kg were procured from central animal house of Basrah University, Basrah, Iraq. Animals were kept in clean polypropylene cages and fed on standard antibiotic free diet. All animals were kept in fast for 24 h before staring the experiments. The study was conducted following approval from the animal ethics committee of Basrah University, Basrah, Iraq.

Diabetes Induction of Rabbits

The diabetes was induced in rabbits using three injections of alloxan monohydrate dissolved in sterile normal saline. Alloxan was used immediately after preparation and administrated intravenously at a period of 48 hrs in a dose of 150 mg/Kg body weight [13], then 20 % of glucose dissolved in drinking water was given to rabbits orally and they were kept in fast for 18 hrs after seven days from last administration. Glucose concentrations were measured in blood of alloxan-induced diabetic rabbits by using glucose oxidase peroxidase enzymatic colorimetric GOD-PAP Method [13].

Effect of phenols and alkaloids on glucose level in alloxan-induced diabetic rabbits

Twelve alloxan-induced diabetes rabbits were divided into two equal groups. The first group was given 3 ml of normal saline and considered as a control group. While the second group was given 0.3 gm/kg body weight of phenolic extract dissolved in 3 ml of normal saline, this group considered as a test group. Blood samples were collected at different time intervals (0, 2, 4, 6, 24 h). The glucose concentrations were measured at each time point [13]. Similar procedure was followed but instead of phenols the alkaloids in same concentration was used.

Statistical Analysis

All values have been taken as mean value and standard error calculated. The differences between test and control were analyzed by using Student's t test employing origin 8 version Software. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Gas chromatography–mass spectrometry (GC-MS) analysis

In the present study GC-MS was used to identify the composition of seed extract of *B. oleracea* var. capitata. The chromatogram of seed extract of *B. oleracea* var. capitata GC-MS is shown in **Fig. 1**.

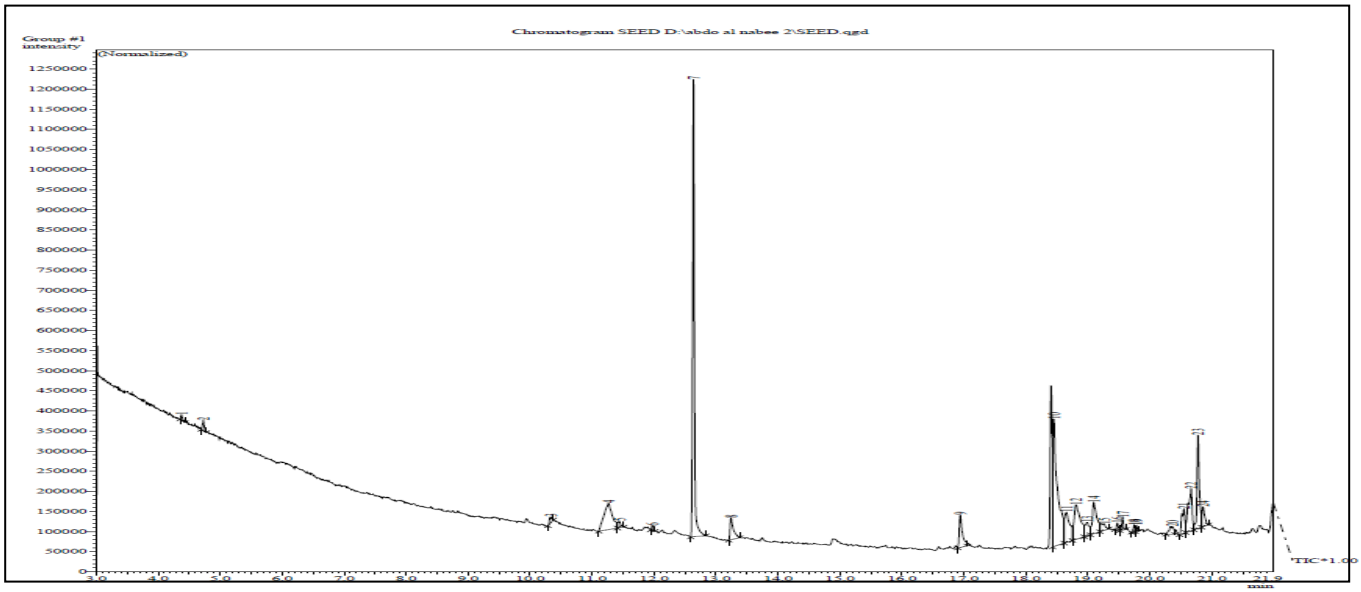

Fig. 1. Seed extract of *B. oleracea* var. capitata shows 24 peaks which indicate that 24 compounds are present in the seed extract.

Experiment

The GC-MS analysis of led to the identification and quantification of 24 components (**Table 1**) which accounted for 100 % of the total extract. 4-Methyl-2,5-dimethoxybenzaldehyde represented the highest component of seed extract followed by 6-

Octadecenoic acid, (Z). The present results showed one phenolic compound [2,4-bis(1,1-dimethylethyl)-] and one alkaloid compound (2,3-Dicyano-5,6-diphenylpyrazine). The last two compounds represented the most important compound as these compounds were used further in current study.

Table 1. Peak report TIC of Chemical compositions of seed extract of *B. oleracea* var. capitata

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	4.374	21768	0.25	12586	0.49	1-Butene, 4-isothiocyanato-
2	4.729	46723	0.55	26630	1.03	Undecane
3	10.330	40975	0.48	15309	0.59	Iberin nitrile
4	11.266	612169	7.16	65571	2.55	Sucrose
5	11.440	50721	0.59	16827	0.65	Benzene, (2-isothiocyanatoethyl)-
6	11.972	19801	0.23	12165	0.47	Phenol, 2,4-bis(1,1-dimethylethyl)-
7	12.637	2225029	26.02	1136925	44.13	4-Methyl-2,5-dimethoxybenzaldehyde
8	13.243	206579	2.42	54487	2.11	Iberin
9	16.941	288631	3.38	80815	3.14	Pentadecanoic acid
10	18.451	1661282	19.43	317065	12.31	6-Octadecenoic acid, (Z)-
11	18.654	422095	4.94	75149	2.92	Octadecanoic acid
12	18.807	615976	7.20	86131	3.34	9,12-Octadecadienoic acid (Z,Z)-
13	18.988	162588	1.90	33654	1.31	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)
14	19.095	391255	4.58	77858	3.02	9,12-Octadecadienoic acid (Z,Z)-
15	19.265	96464	1.13	15735	0.61	N,N-Dimethylheptanamide
16	19.477	33543	0.39	17963	0.70	3-Cyclopentylpropionic acid, 2-dimethylam
17	19.559	53350	0.62	31085	1.21	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-
18	19.746	27778	0.32	16749	0.65	1,E-8,Z-10-Hexadecatriene
19	19.785	24050	0.28	15992	0.62	13-Tetradecenal
20	20.363	107202	1.25	20343	0.79	Oleoyl chloride
21	20.549	219979	2.57	60032	2.33	3-Cyclopentylpropionic acid, 2-dimethylam
22	20.667	461401	5.40	105046	4.08	2,3-Dicyano-5,6-diphenylpyrazine
23	20.777	612384	7.16	232593	9.03	O-Ethyl S-2-dimethylaminoethyl ethylphosp
24	20.858	149505	1.75	49600	1.93	Hexadecanoic acid, 2-hydroxy-1-(hydroxym
		8551248	100.00	2576310	100.00	

Alloxan induces diabetic in rabbits

Diabetes mellitus was induced in the rabbit post injecting with alloxan intravenous three times. The results were appeared as significant increase in the level of glucose in sera of rabbit that injected with alloxan as compared with rabbit injected with normal saline (**Fig. 2**).

Effect of phenols on glucose level in alloxan-induced diabetic rabbits

Administration of extracted phenol from *B. oleracea* var. capitata a dose 300 mg/kg body weight (orally) to diabetic rabbit showed reduction in blood glucose level from 378 mg/dl to 308, 259, 170 and 77 mg/dl at 2nd, 4th, 6th, and 24 h, respectively. Significant decrease ($P<0.05$) in glucose level was observed after 2 h post phenol administration orally. The difference between control and test increased with time thus, maximum difference was observed at time point 24 h (**Fig. 3**).

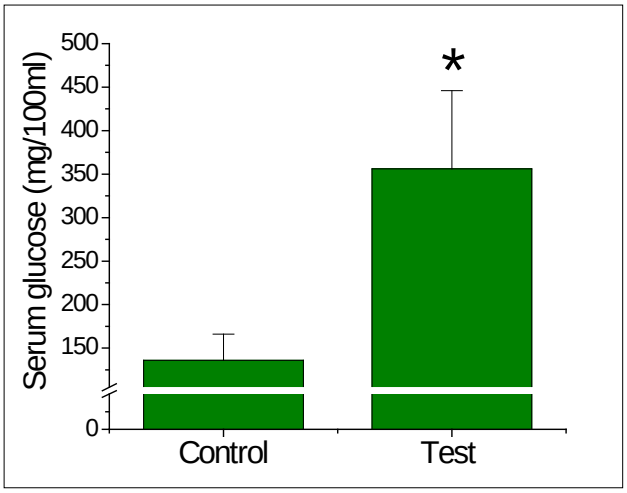

Fig. 2. Effect of three doses of alloxan 150 mg/Kg on level of glucose in sera of experimental rabbits. Contro, rabbit injected with sterile normal saline; Test, rabbit injected with alloxan. Asterisk indicates a significant difference from the control group.

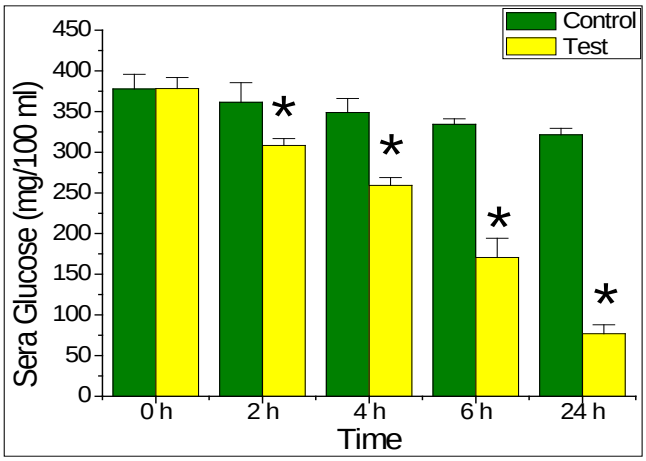

Fig. 3. effect of oral administration of phenolic compounds (0.3 g/kg) on the glucose levels in blood of alloxan-induced diabetic rabbits. Control, rabbits administrated normal saline orally; test, rabbits administrated phenolic compounds (0.3 g/kg) orally. Asterisks indicate a significant difference from the control group.

Effect of alkaloid compounds on glucose level in alloxan-induced diabetic rabbits

Oral administration of alkaloids that extracted from seeds of *B. oleracea* var. capitata in a dose 300 mg/kg body weight to diabetic rabbit showed reduction in blood glucose level from 381 mg/dl to 354, 326, 306 and 247 mg/100 ml at 2nd, 4th, 6th, and 24 h, respectively. Significant decrease ($P < 0.05$) in glucose level was observed after 24 h post alkaloids administration orally. In current study, the little decrease in glucose levels in sera of diabetic rabbit was observed post administration with alkaloid compounds (**Fig. 4**).

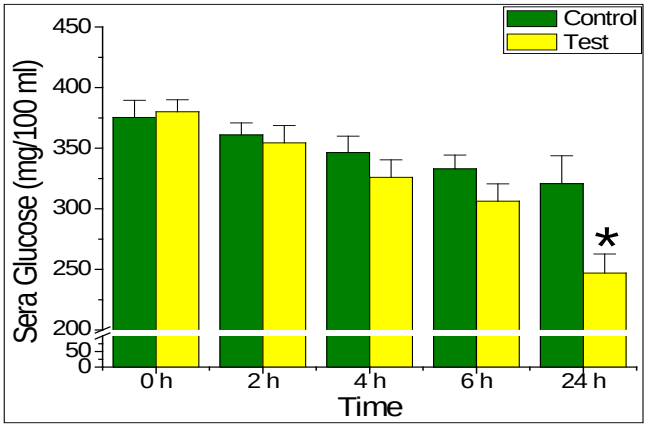

Fig. 4. Effect of oral administration of alkaloid compounds (0.3 g/kg) on the glucose levels in blood of alloxan-induced diabetic rabbits. Control, rabbits administrated normal saline orally; test, rabbits administrated alkaloid compounds (0.3 g/kg) orally. Asterisk indicates a significant difference from the control group.

The present study depicted clearly that the effect of phenolic compounds that extracted from *B. oleracea* seeds was more effective to reduce glucose in blood of diabetic rabbits as compared with alkaloid compounds that extracted from the same plant.

Discussion

Medicinal herbs have been widely used for more than 2000 years to treat diabetes mellitus. In the late 1970's, clinical investigations were reported on the use of herbal medicines as a means of treating diabetes and its complications [14]. It is difficult to check the effect of drug understudy on the diabetes in human that is why most study usually uses the experimental animals in this case. To induce the diabetes in experimental animal, many chemical were used, one of the widely used is alloxan. Alloxan and streptozotocin (STZ) are toxic glucose analogues that preferentially accumulate in pancreatic beta cells via the GLUT2 glucose transporter [15].

In present study, the chemical compounds of *B. oleracea* var. capitata seeds were recognized by GC-mass technique. Twenty four compounds were specified. Only two compounds were used in further experiments, phenol (2,4-bis(1,1-dimethylethyl)- and alkaloid (2,3-Dicyano-5,6-diphenylpyrazine) as most previous study specified the anti-diabetes effects of these compounds (phenols and alkaloids) [16,17]. Alloxan-induced diabetes rabbits were divided into two groups first group was administrated with 0.3 g/kg of phenolic compounds that extracted from *B. oleracea* var. capitata seeds (test). The second group also was administrated orally with 0.3 g/kg alkaloid compounds that extracted from *B. oleracea* var. capitata seeds (test). The best result of reduction in glucose level was found in the subgroups of alloxan-induced diabetes rabbits that administrated with phenols.

STZ-induced experimental diabetes is a valuable model for induction of type I diabetes. During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. Alloxan has two distinct pathological effects: it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it causes a state of insulin-dependent diabetes through its ability to induce Reactive oxygen species (ROS) formation, resulting in the selective necrosis of beta cells. These two effects can be assigned to the specific chemical properties of alloxan, the common denominator being selective cellular uptake and accumulation of alloxan by the beta cell [15].

The mechanism of cruciferous vegetables to reduce glucose levels in diabetes patients still not fully clarified; these vegetables are rich in the antioxidant vitamins C, E and carotene and are good sources of dietary fiber. They also contain sulforaphane and other isothiocyanates, which are believed to stimulate the production of protective enzymes in the body [18, 19]. Sathya and Siddhuraju, (2012) reported the

antioxidant activity of phenols that extracted from *Acacia auriculiformis* [16]. Tiong et al., (2013) reported the antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus* (L.) G. Don [17]. Thus these compounds may be affected on the activity of ROS to damage tissue specially in pancreatic cells (beta cells) that is why; these compounds may have anti-diabetic effects. *Brassica oleracea* var*. capitata* (Brassicaceae) has similar composition as other Brassica vegetables. Other plants also have anti-diabetic activity such as Knol-Khol [20].

From the present study we found that the phenolic compound (2,4-bis(1,1-dimethylethyl)- and alkaloid (2,3-Dicyano-5,6-diphenylpyrazine) have antidiabetic effect in alloxan induced diabetes rabbit but phenolic compound (2,4-bis(1,1-dimethylethyl)- showed the highest antidiabetic activity. Before using 2,4-bis(1,1-dimethylethyl)- for treating the diabetes, several experiments should be carried out such as the toxicity of this compound. This work is going on in our laboratory.

Conflict of interest

The authors declare that they have no conflict of interests.

REFERENCES

[1] **Moore DJ, Gregory JM, Kumah-Crystal YA, Simmons JH.** (2009) Mitigating micro- and macro-vascular complications of diabetes beginning in adolescence. *Vasc Health Risk Manag*. **5**:1015–31.

[2] **Chauhan A, Sharma PK, Srivastava P, Kumar PN, Dudhe R.** (2010) Plants Having Potential Antidiabetic Activity: A Review. *Der Pharmacia Lettre* **2**: 369-387.

[3] **Eberhardt MV, Kobira K, Keck A, Juvik JA, Jeffery EH.** (2005) Correlation analyses of phytochemical composition, chemical and cellular measures of antioxidant activity of Broccoli (*Brassica oleracea* L. var. italic). *J Agricult Food Chem* **53**: 7421- 7431.

[4] **Bidchol AM, Wilfred A, Abhijna P, Harish R.** (2011) Free radical scavenging activity of aqueous and ethanolic extract of *Brassica oleracea* L. var. italic. *Food bioprocess technol* **4**: 1137-1143.

[5] **Boga M, Hacibekiroglu I, Kolak U.** (2011) Antioxidant and anticholinesterase activities of eleven edible plants. *Pharm Biol.* **49**: 290-295.

[6] **Kurilich AC, Tsau GJ, Brown A, Howard L, Klein BP, et al.** (1999). Carotene, Tocopherol, and Ascorbate Contents in Subspecies of *Brassica oleracea*. *J Agricult Food Chem* **47**: 1576–1581.

[7] **Eissa HR.** (2013) Breakdown of Echoviruses in middle and south Iraqi provinces. *World J Exp Biosci* **1**: 1-4.

[8] **Eissa RH, Gupta SK.** (2013) Isolation and Identification of Sabin poliovirus in middle and southern Iraqi provinces. *World J Exp Biosci* **1**: 22-25.

[9] **Abd alwahed WN, Hassan SH.** (2013) T-Lymphocytes Subsets in inactive carrier state of HBV. *World J Exp Biosci* **1**: 29-32.

[10] **Al-Jawadi Zena AM.** (2013) Isolation and Studying the Effect of Protein Fractions of White Cabbage *(Brassica oleracea* var. capitata) on Some Biochemical Parameters in Experimental Diabetic Rats. *Int Res J Medical Sci* **1**:17-22.

[11] **Al – Maliki ADM.** (2012) Isolation and Identification of Phenols and an Alkaloidic Compound from Matricaria chamomilla Plant Flowers and Study of Their Medicinal Activity Against the Pathogenic Bacteria of Skin Infections*. J Univesity Thi-Qar* **7**: 1-17.

[12] **Alarcon-Aquilar FJ, Roman-Ramos R, Jimenz-Estrada M, Reyes-Chipa R, Gonzalez Paredes B, Floressanez JL.** (1997) Effect of three Mexican medicinal plants Astercea on blood glucose level in healthy mice and rabbits. *J. Ethnopharmacol* **55**:171-177.

[13] **Wasfi IA, Bashir AK, Amiri MH, Abdullah AA.** (1994) The effect of Rhazya stricta on glucose hemeostasis in normal and streptozotcion diabetic rats. *J Ethnopharmacol* **43**:141-147.

[14] **Zhou AF**. (1980) Literature review on traditional chinese medicines for treatment of diabetes mellitus in Chinese. *Hubei J Traditional Chinese Med* **2**: 45–8.

[15] **Lenzen S.** (2008) The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* **51**:216-26.

[16] **Sathya A, Siddhuraju P.** (2012) Role of phenolics as antioxidants, biomolecule protectors and as anti-diabetic factors--evaluation on bark and empty pods of Acacia auriculiformis. *Asian Pac J Trop Med* **5**:757-65.

[17] **Tiong SH, Looi CY, Hazni H, Arya A, Paydar M, et al.** (2013) Antidiabetic and Antioxidant Properties of Alkaloids from Catharanthus roseus (L.) G. Don. *Molecules* **18**:9770-9784.

[18] **Kurilich AC, Jeffery EH, Juvik JA, Wallig MA, Klein PL.** (2002) Antioxidant capacity of different broccoli) *Brassica oleracea*) genotypes using the oxygen radical absorbance capacity (ORAC) assay. *J Agricult Food Chem* **50**: 5053-5057.

[19] **Jagdish S, Upadhyay AK, Bahadur A, Singh B, Singh KP, Mathura R.** (2006) Antioxidant phytochemicals in cabbage) *Brassica oleracea* L. var. capitata), *Scientia Horticulturae* **108**:233–237.

[20] **Kusznierewicz B, Bartoszekb A, Wolskaa L, Drzewieckic J, Gorinsteind Sh, Namiesnika J.** (2008) Partial characterization of white cabbages (*Brassica oleracea* var . capitata f. alba) from different regions by glucosinolates bioactive compounds, total antioxidant activities and proteins, *LWT Food Sci Technol* **41**: 1–9.

Author affiliation:

1. Department of Chemistry, College of Education, University of Basrah, Basrah, Iraq.