IMPACT OF GRAPE SEEDS EXTRACT AGAINST ALLOXAN INDUCED DIABETES IN MICE

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ABSTRACT

The current study was aimed to evaluate the antidiabetic effect of grape seed extract (GSE) on Alloxan (120mg/kg of body weight) induced diabetes in mice as well as characterize the chemical composition and phytochemical content of grape seeds from three grape cultivars (Ahmer, Halawani, and Kamali) grown in Iraq as well as pomace. Ahmer gave the highest values for crude fat14.84±0.2 and phytochemicals (tannins, saponin, alkaloids, flavonoids, phenol, and anthocyanin) as compared to other cultivars. phytochemical analysis using High-performance liquid chromatography (HPLC) revealed that thehighest concentration of proanthocyanidins polymers (catechine, procyanidin, and epicatechine) was recorded in Ahmer seed extract which were (796, 170, 244) µg/g, respectively, while the lowest amounts were in pomace 489, 99, and 143 µg/g, respectively using HPLC. Oral administration of grape seed natural extract (600 mg/kg/day) reduced the level of glucose in mice which was highly statistically significant (*p* <0.01) compared with the diabetic control mice (untreated), which was 206.83±6.7 and 349±27.50 mg/dl, respectively.

***Key words: DM, fasting glucose level, antioxidant, proanthocyanidins, chemical composition,**
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تأثير مستخلص بذور العنب على مستوى السكر في فئران مصابة بالسكري المستحث بالألوكسان

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المستخلص

هدفت الدراسة الحالية إلى تقييم تأثير مستخلص بذور العنب (GSE) على مرض السكري المستحث عن طريق الألوكسان 120ملغم/كغم من وزن الجسم في الفئران بالإضافة الى توصيف التركيب الكيميائي لثلاث أصناف من بذور العنب المزروعة في العراق وبقايا مخلفات صناعة عصير العنب. أظهر الصنف الأحمر أعلى نسبة من دهون خام 14,84 ± 0,2 والمواد الفينولية النباتية (التانينات والصابونيات والقلويدات والفلافونويدات والفينولات والانثوسيانديدنات) بالمقارنة مع الاصناف الاخرى. التحليل الكيميائي الكمي باستخدام تقنية الكروماتوكرافي السائل الفائق الاداء (HPLC) بين ان أعلى تركيز لبوليمرات البروانثوسيانيدينات (الكاتشين، البروسياندين، الإيبيكاتشين) كانت في مستخلص بذور أحمر (796، 170، 244) ميكروغرام / غرام، على التوالي، بينما كانت أقل الكميات في المخلفات 489، 99، 143 ميكروغرام / غرام، على التوالي. أدى تجريع الفئران بمستخلص بذور العنب الأحمر عن طريق الفم (600 ملغم /كغم/يوم) إلى خفض مستوى الكلوكوز وبشكل معنوي كبير (*p* <0.01) مقارنة بالفئران المصابة بالسكري (غير المعالجة) ، والتي كانت 206,83 ±6,70 و 349 ± 27,50 ملغم / ديسيلتر على التوالي.

*الكلمات المفتاحية: داء السكري، مستوى سكر الصائم، مضادات الأكسدة، البروانثوسياندين، التركيب الكيميائي
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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that continues to present as a main worldwide health problem (27). Grape (*Vitis vinifera* L.) is a fruit widely grown and eaten in the world, which is mainly used for juice and wine production (36). Pomace is a solid waste product remaining from juice and wine processing and generally consists of skins and seeds. Commonly, grape seeds are typically obtained from the waste of processed fruit products such as wine or grape juice as by-products (4). Grape seeds have been traditionally sold to the oil extraction and pharmaceutical field as a good source of antioxidants as well as contain several valuable components, such as tannins and phytochemicals which have been attributed to improving health and nutrition (3). Polyphenols from grape-derived products have been helped prevent of several diseases including neurodegenerative diseases, cardiovascular diseases, and cancer as well as antibacterial (16,23). Grape seeds comprise about 5% of the fruit weight (10), and more than three million tons of seeds are discarded annually worldwide (13). Grape seeds are a significant part of the pomace, corresponding to about 38 – 52% of dry matter (20). Grape-derived products have included proanthocyanidin monomers and oligomers such as epicatechin, catechin, and proanthocyanidin dimers, which are the major components in extracts of grape seed (18). Proanthocyanidins are oligomeric combinations of any combination of the four isomers (±) catechin and (±)-epicatechin. Two individual classes of Proanthocyanidins can be defined based on chemical structure, known as A-type (not found in Grape Seed Extract-GSE) and B-type (37). In grape seed extract, stereotypically the B-type has been reported that is highest concentrations of catechin and epicatechin as well as proanthocyanidin B2 and its isomers (38). Scientific studies have shown that the antioxidant power of proanthocyanidins is 20 times more powerful than vitamin E and 50 times greater than vitamin C (34). The chemical composition of grape seeds is mainly (w/w) 40% fiber, lipid, 11% protein, 7% phenolic compounds, sugars and minerals (19). Another study has shown that grape seeds have high protein content, 10% to 20% oil, and a high vitamin E content as well as polyphenol and procyanidin compounds (4). Alloxan is a classical diabetogenic usually used to destroy β-cell cells due to its selective cytotoxic effect on pancreatic cells. The mechanism of alloxan action is destroying β-cell function by inhibiting the enzyme glucokinase. According to Zhang *et al* (40) who have been reported that glucose transporter 2 (GLUT2) and glucokinase (GK) as target molecules for alloxan (40). Bagchi *et al* (7) have found that GSP has significantly greater protection against free radicals and lipid peroxidation than vitamins C, E, and β-carotene (7). This study was conducted to investigate the protective role of grape seed extract (GSE) against free radical-mediated damage as selective necrosis of β-cells in mice using alloxan-induced diabetes in mice as well as characterize the chemical composition and phytochemical content of grape seed varieties.

MATERIALS AND METHODS

Preparation of samples: Grapes (Ahmer, Halawani, Kamali, and pomace) were purchased form a local market in Baghdad province (Baghdad, Iraq), and their seeds were manually cleaned to remove all impurities and separated from fresh fruits of white and red grape varieties. The seeds were dried in an oven for 24 h at 50°C. All experiment repetitions were technical replicates three times.

Chemical composition and total energy

AOAC (1) methods were used for the chemical characterization of grape seeds (1): Moisture content (method No.934.01) was determined by drying an appropriate amount of the sample in an air circulation oven at 105 ºC until constant weight. Method No.920.39 was applied for the determination of crude fat content using Soxhlet apparatus. Crude fiber content was measured with method No.978.10. Crude protein was determined on the seeds of grape from Kjeldahl nitrogen using a 6.25 conversion factor (method No.990.03). Ash content was measured via method No.923.03 by heating samples in a muffle furnace at 550 ºC for 6 h to constant weight as described in the AOAC manual. Carbohydrate content was calculated based on the method defined by

Merril and Watt (21). Energy value was calculated based on the energy nutrient results achieved using the conversion of the Atwater general factor system, as described by Sousa, *et al*., (35) considering 9 kcal/g (37 kJ/g) for fat, 4 kcal/g (17 kJ/g) for carbohydrate, and 4 kcal/g (17 kJ/g) for protein (35).

Extraction of active compounds from grape seeds: The extraction was carried out using acetone water (70:30 v/v) for determination of proanthocyanidins by HPLC. The grape seeds powder (Ahmer, Halawani, Kamali, and pomace were weighed out at 10g and mixed with 50 ml of acetone-water. Extraction was carried out one day in Gerhard Thermo Shaker at 30˚C and 180 rpm for 24 hours. After the extraction, the extract was centrifuged using centrifuge for 5 min. at 1957 XG. Grape seed extract was distilled by rotary evaporator to remove organic solvent at 40˚c for 20 minutes with a 120 rpm rotation under vacuum. Grape seed extract was freeze-dried to obtain a dried extract powder (6). HPLC samples were prepared by filtering each extract of grape seeds through a 0.45 µm nylon syringe filter into an amber HPLC vial (30). All Samples were stored at -20 ^{o}C until analysis.

Qualitative phytochemical analysis of grape seed: An aliquot of the seed extract (1ml) was added to a few drops of 1% lead acetate, and the yellowish precipitate indicated the presence of tannins. The aliquot of the seed extract (1ml) was mixed with 4ml of distilled water and then agitated in a graduated cylinder for 20 minutes. The formation of foam indicates the presence of Saponins. A few drops of Mayer's reagent were added to 1 mL of grape seed extract. The cream-yellowish or white precipitate was formed, indicating the presence of alkaloids. An aliquot of the grape seeds extract (1ml) and a few drops of sodium hydroxide solution were added to a test tube. The formation of an intense yellow color that became colorless on adding a few drops of dilute HCl indicates the presence of flavonoids. 1ml of grape seeds extract, 1ml of distilled water followed by 50µL of 10 % $FeCl_3$ solution was added. The presence of phenol is indicated by the bluish-black color. An aliquot of the grape seed extract (2ml) was added to 2ml of 2 N HCl and ammonia, the appearance of pink to red color which turns to blue-violet indicating the presence of anthocyanins (32).

High performance liquid chromatography (HPLC) conditions: Grape seeds extract (GSE) were analyzed by HPLC model (SYKAM) Germany. Pump model: S 2100 quaternary gradient pump, Auto sampler model: S 5200, Detector: UV (S 2340) and Column oven model: S 4115. The mobile phase was (Methanol : D.W : formic acid) (70 : 25 : 5) , The flow rate was set at 1.0 mL/min with a 1:10 splitter following the output of the UV detector leading 1.0 mL/min on a gradient C18-ODS column (25 cm * 4.6 mm). The UV detector was set at 254, 280, 370and 520 nm (16).

Animals: Four weeks old albino mice, weighing 24±3 g, were given a commercial diet and water *ad libitum* with a controlled environment (12 h light and dark cycle, 21–23°C). Mice were acclimatized to the laboratory conditions. They were obtained from the animal house of the National Center for Drug Control and Research - Ministry of Health, Iraq.

Experimental design: Fifty (50) adult female mice were grouped randomly into five groups, ten mice in each group. Alloxan monohydrate (CDH, India) was administered intraperitoneal with 120 mg/kg body weight freshly dissolved in normal saline (2). After three consecutive days the mice with blood glucose levels greater than 250 mg/dl were considered diabetic and used for the experiment. The treatments lasted for six weeks (45) days. The route of administration of grape seed extract (GSE) was oral.

The groups and doses administered were summarized below:

Group 1: Normal control mice (n= 10) received distilled water.

Group 2: Positive control (diabetic untreated) mice received only distilled water.

Group 3: Diabetic mice received 200 mg/kg body weight/day of GSE in distilled water.

Group 4: Diabetic mice received 400 mg/kg body weight/day of GSE in distilled water.

Group 5: Diabetic mice received 600 mg/kg body weight/day of GSE in distilled water.

After the last treatment, mice were fasted for eight hours and sacrificed by overdosing with chloroform (CDH, India). Blood was collected from the heart. The serum was separated by centrifugation at 3,000 rpm for 15 min and stored at −18°C until use.

Determination of fasting serum glucose level: Glucose level was determined as quinine amine using a test reagent according to the Tinder method using Cobas c111 systems. The absorbance was measured at 510 nm, and the results were expressed as mg/dl.

Statistical analysis

The results were analyzed by a one-way analysis of variance (ANOVA) test followed by Tukey's test. The data is expressed as means ± standard deviation (SD) using the software for Windows. *P*-Values < 0.05 were considered to be statistically significant result.

RESULTS AND DISCUSSION

The chemical composition of the investigated grape seeds and their energy values are presented in Table 1. The results showed that grape seeds (Ahmer, Halawani, Kamali, and pomace) content was: moisture (10.20±0.2,10±0.3,10.20±0.1, and 9.8±0.2), crude protein (9.98±0.2, 10.3±0.1, 10.3±0.3, and 6.94±0.2), crude fat (14.84±0.2, 11.67±0.5, 9.82±0.2, and 13.7±0.3), Crude fiber (37.11±0.1, 37.22±0.4, 33.9±0.3, and 38.8±0.5), carbohydrates (28.87±0.2, 30.83±0.4, 30.83±0.2, and 27.76±0.3) ash (3.39±0.1, 2.94±0.5, 2.52±0.4, and 3.84±0.3), and energy value (288.96±0.1, 269.55±0.2, 252.9±0.1, and 262.1±0.3), respectively. The results in the present study are consistent with the previous observations of Owon (26) who reported that grape seeds contain 2.86% of ash and 12.69% of oil (26). Baydar and Akkurt (8) found that the oil concentration of 18 grape cultivar seeds ranged from 11.6 to 19.6%, while Mouhammad and Ali (23) noticed that Syrian grape seeds contain 1.45–1.65% of ash (7,22). The oil contents of nine grape seed cultivars were ranged from 10.45% to 16.73% (36). In addition, Mironeasa *et al*., (22) noticed that the results obtained from the determination of the grape seeds ash content were ranged from 2.14 to 8.28% according to cultivar (22). The value of ash is closed to those reported by Elagamey *et al.* (12). Also, Sabir *et al.* (29) reported that the grape seed oil concentration of some different cultivars ranged from 7.3 to 22.4% (29). The determination of the grape seeds protein content was ranged from 6.26-9.01% according to cultivar (22). Al-Samraee (5) reported that ash was (2.93, 3.49, and 3.78) and moisture (10.65, 8.04, and 6.47(% for grape seeds Shada Sodda, Bedha, and waste (residue) of grape juice Straight (5).

Table 1. Chemical composition (g/100g) and energy value of grape seeds.

Type of grape seed	Moisture %	Crude protein %	Crude fat %	Crude fiber %	Carbohydrates %	Ash %	Energy value (Kcal)
Ahmer	**10.20±0.2**	**9.98±0.2**	**14.84±0.2**	**37.11±0.1**	**28.87±0.2**	**3.39±0.1**	**288.96±0.1**
Halawani	**10±0.3**	**10.3±0.1**	**11.67±0.5**	**37.22±0.4**	**30.83±0.4**	**2.94±0.5**	**269.55±0.2**
Kamali	**10.20±0.1**	**10.3±0.3**	**9.82±0.2**	**33.9±0.3**	**30.83±0.2**	**2.52±0.4**	**252.9±0.1**
Pomace	**9.8±0.2**	**6.94±0.2**	**13.7±0.3**	**38.8±0.5**	**27.76±0.3**	**3.84±0.3**	**262.1±0.3**

***Means ± SD of duplicates**

The results of this study were referred to the presence of phytochemical such as tannins, saponin, alkaloid, flavonoids compounds, and total phenolic were represented in seeds extract (Table 2). Ahmer (red grape seeds) is shown that has the highest concentration of phytochemicals compared with other seeds in table 2. These results are in agreement with Grace and Narendhirakannan (14) who found that the presence of alkaloids, flavonoids, saponins and tannins were present in grape seed (14). Phytochemicals with biological activity can be important medicinal value; for instance, Phytochemicals such as saponins, tannins, and alkaloids have anti-inflammatory effects; Flavonoids, tannins and alkaloids have hypoglycemic activities (25). Plants produce these chemical substances to protect themselves, and they are also believed to protect humans against certain diseases (11). Grape seeds are found to have tannins, phenols, and flavonoids which can be potent bioactive agents. Bioactive agents are involved in the therapeutic use of grape seeds like flavonoids can be worked as antioxidant, hypoglycemic, antidiabetic, antimicrobial, anticarcinogenic, and anti-inflammatory.

Table 2. Qualitative phytochemical analysis of grape seeds.

Type of grape seeds	Tannins	Saponin	Alkaloids	Flavonoids	Phenol	Anthocyanins
Ahmer	+++	++	++	+++	++	+++
Halawani	+	+	+	+	+	++
Kamali	+	+	+	+	+	++
Pomace	+	+	+	+	+	++

(+++), (++), and (+) refer to high, moderate, low amount, respectively.=====Results of quantitative analysis are shown in Table 3 and Figure 2. Highest concentration of proanthocyanidins polymers (Catechine, procyanidin, and epicatechine) were recorded in Ahmer grape which were (796, 170, 244) µg/g, respectively. These results are compatible with Granato *et al.*, (15) who mentioned that a tendency linked to the red grape variety was observed based on higher total flavonoid content (15). The lowest amounts of catechine, procyanidin, and epicatechine as proanthocyanidins were found in pomace (seeds and skin) which were 489, 99, and 143 µg/g, respectively.

Table 3. Quantitative analysis by HPLC; proanthocyanidins (catechine, epicatechine, and procyanidin) of grape seed extract (µg/g).

Polyphenolics compounds	Ahmer	Halawani	Kamali	Pomace
Catechine	**796**	**497**	**500**	**489**
Vanillic acid	**452**	**221**	**275**	**172**
epicatechine	**244**	**193**	**300**	**143**
procyanidin	**170**	**113**	**91**	**99**
Tannic acid	**452**	**45**	**35**	**28**
Gallic acid	**359**	**306**	**486**	**210**

The total concentrations polyphenolics compounds such as catechine, epicatechine, and procyanidin were higher in seed extracts than the pomace sample. Concentrations of polyphenols in seed are higher than in skin, and most are tannins that have between 50 and 90% proanthocyanidins (9). Extrusion processing can be reduced total anthocyanins in pomace by 18% to 53% compared with seed without any a process as well as other factors such as soil, environment, and type of seeds (9). As shown in Table 4, a single interperitoneal injection i.p. of Alloxan (120 mg/kg) produced an elevation of serum glucose level which was evidenced 72 h after administration as hyperglycemia; diabetic control (untreated) was 349±27.50 mg/dl. Oral treatments of mice with GSE (200 mg/kg/day), (400 mg/kg/day), and (600 mg/kg/day) for 6 weeks significantly decreased the elevated serum glucose level to normal values compared to the normal group (p<0.01) which were 231.66±6.56 218.83±3.97, and 206.83±6.70 mg/dl, respectively. Treatments with the GSE could suppress the oxidative stress caused by hyperglycemia. Oral administration of 600 mg kg−1 (body weight) of GSE for six weeks significantly increased pancreatic glutathione (GSH) levels and inhibited the increase in lipid peroxidation caused by alloxan (p < 0.001). GSE slightly increased body weight compared with other groups but was not significantly. These findings reveal that grape seed extracts (GSE) are a considerably safe and potential therapy for diabetes mellitus, especially type 2, due to their ability to lower blood glucose levels. GSE has beneficial effects on the biochemical changes associated with Alloxan-induced diabetes due to its work as an antioxidant property. Moreover, the extract of grape seeds has an influence as an inhibitor to alpha-glucosidase and alpha-amylase and increases insulin sensitivity (31, 33).

Table 4. Effect of Ahmer (red grape) grape seed extract on body weight and fasting serum glucose in mice after 6 weeks

Parameters	Normal control mice	DM control mice	DM+GSE (200 mg/kg/day)	DM+GSE (400 mg/kg/day)	DM+GSE (600 mg/kg/day)	P-Value
Initial body weight (g)	**23.9±1.66**	**25.2±4.28**	**26.9±2.72***	**22.3±2.79***	**23.8±3.11**	**0.0206***
Final body weight (g)	**29.6±1.83**	**27.4±3.59**	**27.9±2.72**	**26.1±3.84**	**27±2.62**	**0.1348**
Fasting blood glucose (mg/dl)	**100.66±7.52**	**349±27.50**	**231.66±6.56b**	**218.83±3.97**	**206.83±6.70**	**0****

DM, diabetes mellitus; GSE, grape seed extract. Data are expressed as the mean ± standard deviation of 10 mice per group.
***= significant at P<0.05, **= significant at < 0.01**

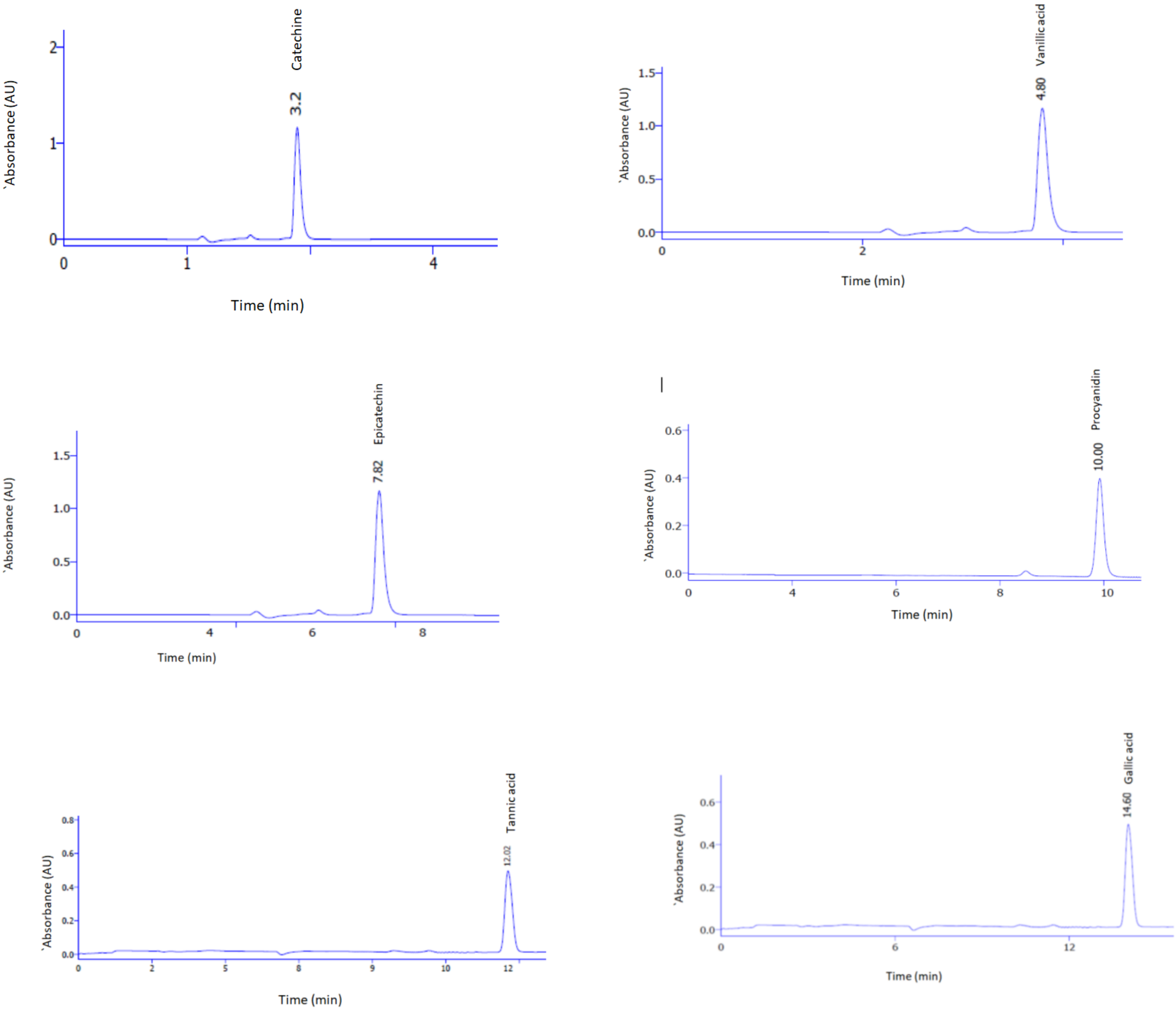

Figure 1. HPLC chromatogram of standards(Catechine, Epicatechine, procyanidin, Vanillic acid, tannic acid and gallic acid).

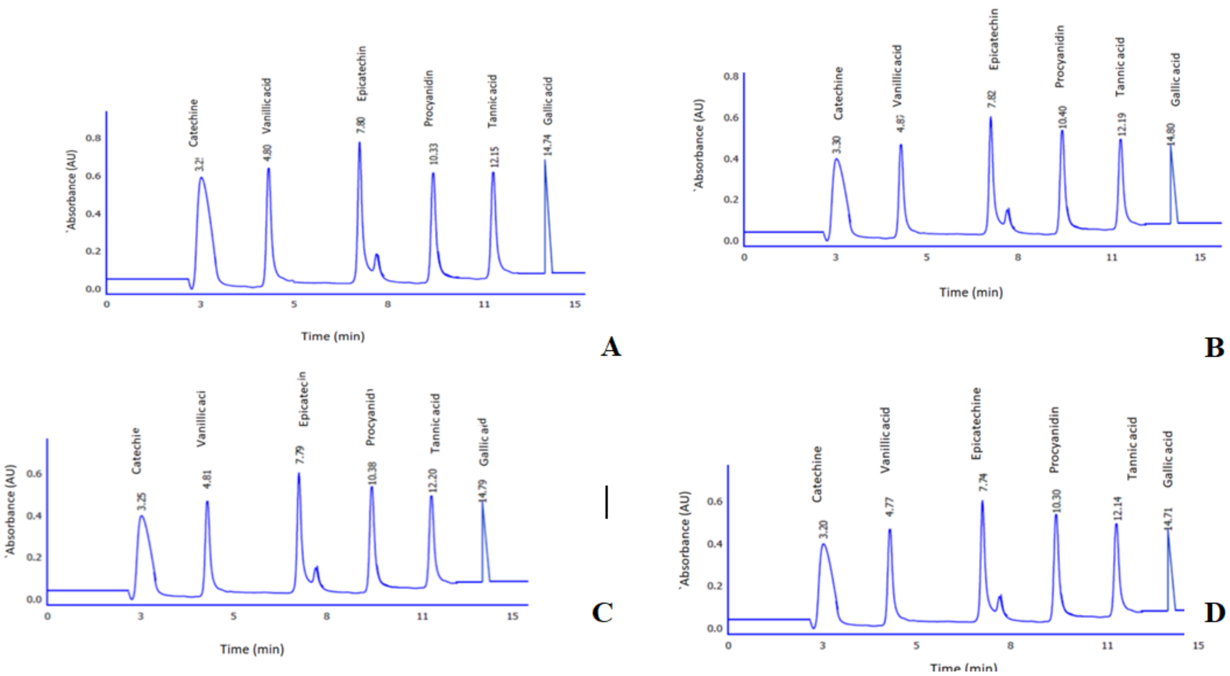

Figure 2. HPLC Chromatogram of grape seeds extracts (GSE); A) Ahmer, B) Halawani, C) Kamali, and D) Pomace

CONCLUSIONS

The results acquired from the detailed investigation of the grape seed composition show that the concentration of the seed components such as proteins, carbohydrates, oil and ash depend on the variety in relatively limited ranges. Grape seeds are a very rich source of phytochemical components that may constitute a good source of healthy compounds that are useful in the prevention of diseases. Ahmer grapes (Red grapes) are rich in phenolic compounds antioxidants which can be useful for pharmaceutical or food industry. Catechine, epicatechine, and procyanidin in pomace were lower than seeds due to extrusion processing. This study encourages the consumption of grape seeds as an herbal may have a significant beneficial effect on the body as natural antioxidants. GSE can be worked as anti-diabetic effects by preserving pancreatic β-cells role. Another suggestion is to use grape seeds as specific additives in the food industry to replace chemical additives or as food supplements.

REFERENCES

1. A.O.A.C., Official method of analysis. Association official analytical chemists. 17th Ed. Virginia, U.S.A. 2000.

2. Aammoud, E. K. and A.C. Saddam. 2024. Improving nutritional and qualitative properties of wheat bread by using mallow (*Malva neglecta* L.) Leaves powder. Iraqi Journal of Agricultural Sciences, 55(1):560-568.
https://doi.org/10.36103/8p73pr77

3. Ahmadi, S., and B. A. Siahsar. 2011. Analogy of physicochemical attributes of two grape seeds cultivars." Ciencia e investigación agraria: revista latinoamericana de ciencias de la Agricultura 38 (2): 291-301.

4. Al Juhaimi, F., Ü. Geçgel, M. Gülcü, M. Hamurcu, and M. M. Özcan. 2017.Bioactive properties, fatty acid composition and mineral contents of grape seed and oils." South African Journal of Enology and Viticulture 38, no. 1: 103-108.
https://doi.org/10.21548/38-1-1042

5. Al-Samraee, A. M. 2011. Extrication of some phenolic compounds from grape seeds Shada soda ,Bedha, and waste of grape juice and stadying antimicrobial and antioxidants activities M.Sc. Thesis. University of Baghdad, Baghdad .

6.Altıok, E. 2003. Production of proanthocyanidins from grape seed M.SC. Thesis. Izmir Institute of Technology, Izmir, Turkey.
http://hdl.handle.net/11147/3732

7. Bagchi, D., A. Garg, R. L. Krohn, M. Bagchi, M. X. Tran, and S. J. Stohs. 1997. Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract in vitro." Research communications in molecular pathology and pharmacology 95 (2): 179-189.

8. Baydar N. G. and M. Akkurt. 2001. Oil content and oil quality properties of some grapes seeds. Turk. J. Agric., 25(3): 163-168.

9. Castro-Lopez, L., G. Castillo-Sanchez, L. Díaz-Rubio, and I. Cordova-Guerrero. .2019. Total content of phenols and antioxidant activity of grape skins and seeds cabernet sauvignon cultivated in Valle de Guadalupe, Baja California, México." In BIO Web of Conferences, vol. 15, p. 04001. EDP Sciences.
https://doi.org/10.1051/bioconf/20191504001

10. Choi, Y., and J. Lee.2009. Antioxidant and antiproliferative properties of a tocotrienol-rich fraction from grape seeds." Food Chemistry 114 (4): 1386-1390.
https://doi.org/10.1016/j.foodchem.2008.11.018

11. Edeoga, H. D. E. Okwu, and B. O. Mbaebie. 2005. Phytochemical constituents of some Nigerian medicinal plants." African Journal of Biotechnology 4 (7): 685-688.
http://dx.doi.org/10.5897/AJB2005.000-3127

12. Elagamey, A. A., M. A. b Abdel-Wahab, M. M. E. Shimaa, and M. Abdel-Mogib. 2013. Comparative study of morphological characteristics and chemical constituents for seeds of some grape table varieties." Journal of American Science 9 (1): 447-454.

13. Fernandes, L., S. Casal, R. Cruz, J. A. Pereira, and E. Ramalhosa.2013. Seed oils of ten traditional Portuguese grape varieties with interesting chemical and antioxidant properties." Food Research International 50 (1): 161-166.
http://hdl.handle.net/10198/8589

14. Grace N. J. and R. T. Narendhirakannan. 2011. Detection and genotyping of high-risk HPV and evaluation of antioxidant status in

cervical carcinoma patients in Tamil Nadu State, India—a case control study. Asian Pac J Cancer Prev 12:2689–2695.

15. Granato, D., M. M. Carrapeiro, V. Fogliano, and S. M. van Ruth. 2016. Effects of geographical origin, varietal and farming system on the chemical composition and functional properties of purple grape juices: A review." Trends in Food Science and Technology 52: 31-48. DOI:10.1016/j.tifs.2016.03.013

16. Gutiérrez, G., Gastón, E. G.Plaza, A. B. Bautista-Ortín, T. Garde-Cerdán, Y. M.-Simunovic, and A. M. Martínez-Gil.2019.Rootstock effects on grape anthocyanins, skin and seed proanthocyanidins and wine color and phenolic compounds from *Vitis vinifera* L. Merlot grapevines." Journal of the Science of Food and Agriculture 99 (6): 2846-2854. https://doi.org/10.1002/jsfa.9496

17. Ho, L., M. G. Ferruzzi, E. M. Janle, J. Wang, B. Gong, T.Y. Chen, and J. Lobo.2013. Identification of brain-targeted bioactive dietary quercetin-3-O-glucuronide as a novel intervention for Alzheimer's disease." The FASEB Journal 27 (2): 769-781. https://doi.org/10.1096/fj.12-212118

18. Hornedo-Ortega, R., M. R. G. Centeno, K. Chira, M. Jourdes, and P. Teissedre. 2020. Phenolic compounds of grapes and wines: Key compounds and implications in sensory perception." Chemistry and biochemistry of winemaking, Wine Stabilization and Aging: 1-27. https://doi.org/10.3390/foods9121785

19. Lachman, J., A. Hejtmánková, K. Hejtmánková, Š. Horníčková, V. Pivec, O. Skala, M. Dědina, and J. Přibyl. 2013. Towards complex utilisation of winemaking residues: Characterisation of grape seeds by total phenols, tocols and essential elements content as a by-product of winemaking." Industrial Crops and Products 49: 445-453. http://dx.doi.org/10.1016/j.indcrop.2013.05.022

20. Maier, T., A. Schieber, D. R. Kammerer, and R. Carle. 2009. Residues of grape *Vitis vinifera L*. seed oil production as a valuable source of phenolic antioxidants." Food Chemistry 112(3): 551-559. DOI:10.1016/j.foodchem.2008.06.005

21. Merrill, A. L., and B. K. Watt. 1973. Energy value of foods: basis and derivation. No. 74. Human Nutrition Research Branch, Agricultural Research Service, US Department of Agriculture.pp:

22. Mironeasa, S., A. Leahu, G. G. Codina, S. G. Stroe, and C. Mironeasa. 2010. Grape Seed: physico-chemical, structural characteristics and oil content." Journal of Agroalimentary Processes and Technologies 16 (1): 1-6.

23. Mouhammad R. and A. Ali. 2008. A study of the main chemical components of the seeds of two syrian grape cultivars and some quality characteristics of their obtained oil. Tishreen University Journal for Research and Scientific Studies Biological Sciences Series, 30(3): 77-94.

24. Nazima, B., V. Manoharan, and S. Miltonprabu. 2015. Grape seed proanthocyanidins ameliorates cadmium-induced renal injury and oxidative stress in experimental rats through the up-regulation of nuclear related factor 2 and antioxidant responsive elements." Biochemistry and Cell Biology 93 (3): 210-226. DOI: 10.1139/bcb-2014-0114

25. Orhan I., E. kupeli, B. Sener and E. Yesilada. 2007. Appraisal of anti-inflammatory potential of the clubmoss, *Lycopodium clavatum* L. j Ethnopharmacol: 109:146- 150. https://doi.org/10.1016/j.jep.2006.07.018

26. Owon M. A. 1999. Untraditional source of edible oil from raw grape *Vitis vinifera* seed. J. Agric. Sci. Mansora Univ., 24(5): 2479 – 2490.

27. Praparatana, R., P. Maliyam, L. R. Barrows, and P. Puttarak. 2022. Flavonoids and Phenols, the Potential Anti-Diabetic Compounds from Bauhinia strychnifolia Craib. Stem." Molecules 27,. (8): 2393. https://doi.org/10.3390/molecules27082393

28. Rahman, M. M., M. S. Rahaman, M. R. Islam, F. Rahman, F. M. Mithi, Taha Alqahtani, M. A. Almikhlafi.2021. Role of phenolic compounds in human disease: current knowledge and future prospec-ts." Molecules 27 (1): 233. DOI: 10.3390/molecules27010233

29. Sabir, A. A., Unver, and Z. Kara. 2012. The fatty acid and tocopherol constituents of

the seed oil extracted from 21 grape varieties (*Vitis spp*.). Journal of the Science of Food and Agriculture, 92(9): 1982- 1987.
DOI: 10.1002/jsfa.5571

30. Saddam, A. C. 2021. Effect of chicory *cichorium intybus* l. leaves extract to protect certain liver enzymes in mice against carbon tetrachloride-induced hepatotoxicity. Iraqi Journal of Agricultural Sciences, 52(5): 1248-1253. https://doi.org/10.36103/ijas.v52i5.1462

31. Saddam, A.C., Foster, H.M., Zhang, M. and Mosby, T.T., 2019. Effect of sex and race on body mass index and percent body fat in young adults. Nutrition, 63, pp:9.
https://doi.org/10.1016/j.nut.2018.10.001

32. Sailaja, V., M. M., and V. Neeraja. 2016.Quantitative phytochemical analysis of some medicinal plant seed by using various organic solvents." Journal of Pharmacognosy and Phytochemistry 5 (2):30.

33. Sekar, V., and H. R. Vasanthi. 2017. Grape Seed Extract and its Effects on Diabetes and its Complications. Current Research in Diabetes and Obesity Journal, 2 (2): 30-33.
DOI: 10.19080/CRDOJ.2017.02.555584

34. Shi, J., J. Yu, J. E. Pohorly, and Y. Kakuda. 2003. Polyphenolics in grape seeds biochemistry and functionality." Journal of Medicinal Food 6. (4): 291-299
DOI:10.1089/109662003772519831

35. Sousa, E. C., A. M. A. Uchôa-Thomaz, J. O. B. Carioca, S. M. de Morais, A. de Lima, C. G. Martins, and C. D. Alexandrino *et al*.2014. Chemical composition and bioactive compounds of grape pomace *Vitis vinifera* L., Benitaka variety, grown in the semiarid region of Northeast Brazil." Food Science and Technology 34: 135-142.
DOI:10.1590/S0101-20612014000100020

36. SS, Osama., 2022. Micropropagation Of Grapevine (*Vitis vinifera* L.) Cvs. Red Globe And Superior. Iraqi Journal of Agricultural Sciences, 53(4), 833-849.
https://doi.org/10.36103/ijas.v53i4.1596

37. Tangolar, S. G., Y. özoğul, S. Tangolar, and A. Torun.2009. Evaluation of fatty acid profiles and mineral content of grape seed oil of some grape genotypes." International Journal of Food Sciences and Nutrition 60 (1): 32-39.

38. Villani, T. S., W. Reichert, M. G. Ferruzzi, G. M. Pasinetti, J. E. Simon, and Q.Wu.2015.Chemical investigation of commercial grape seed derived products to assess quality and detect adulteration." Food Chemistry 170: 271-280.
DOI: 10.1016/j.foodchem.2014.08.084

39. Xu, Y., J. E. Simon, C. Welch, JoLynne D. Wightman, M. G. Ferruzzi, L. Ho, G. M. Passinetti, and Q. Wu.2011.Survey of polyphenol constituents in grapes and grape-derived products." Journal of Agricultural and Food Chemistry 59 (19): 10586-10593.
DOI: 10.1021/jf202438d

40. Zhang, X., W. Liang, Y. Mao, Hui Li, Y. Yang, and H. Tan.2009. Hepatic glucokinase activity is the primary defect in alloxan-induced diabetes of mice." Biomedicine & Pharmacotherapy 63 (3):180-186.13.
DOI: 10.1016/j.biopha.2007.07.006