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Qualitative and quantitative characterization of biologically active compounds of red grape (Vitis vinifera) seeds Extract

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Abstract

Grape seeds are waste products of the winery and grape juice and are good sources of phytochemicals. The present study aimed to investigate the qualitative and quantitative characterization of Egyptian grape seeds by microwave-assisted extraction (MAE), which was determined by high-performance liquid chromatography coupled to electrospray quadrupole-time of flight mass spectrometry (HPLC–ESI-QTOF-MS). According to the data compiled, grape (Vitis vinifera) seeds sample presented the highest levels in flavonoids and other compounds such as Carnosol, rosmaridiphenol, rosmadial, rosmarinic acid, and carnosic acid. On the other hand, higher contents in triterpenes were found in the extracts of rosemary. The current results indicate that the ethanolic extract from Egyptian grape seeds possessed radical-scavenging and antioxidant activities where about 17.442 mg GAE/g of total phenols, 6.687 mg CE/g of total flavonoids and 81.506 mg TE/g DPPH as antioxidants activity in grape seeds. grape (Vitis vinifera) seeds are very rich sources of flavonoids and typical compounds of grape seeds, such as Catachine, Gallic acid, Protochatchuic acid and Syrngic acid which are compounds with many biological properties, especially antioxidant. On the other hand, the Qurecetin were highly represented that indicate the anticancer and anti-inflammatory properties of grape seeds. This aspect should be studied in depth in future research.

Keywords: Grape seeds, red grape, Vitis vinifera, Egyptian grape, seeds extract

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Introduction:

Many plant extracts and their products have been shown to have significant antioxidant activity which may be an important property of medicinal plants associated with the treatment of several ill-fated diseases [1-6]. The grapes (*Vitis vinifera* L.) have been of interest worldwide due to the nutritional properties of the natural product and the pharmaceutical properties of peel and seed extracts derivatives [1,7]. Grape seeds are waste products of the winery and grape juice industry and they contain lipid, protein, carbohydrates, and 5-8% polyphenols depending on the variety [8,9]. Recently, several polyphenolic antioxidants were detected in red grape seeds which contain approximately 89% proanthocyanidins, with dimmers (6.6%), trimers (5.0%), tetramers (2.9%) and oligomers (74.8%) [3, 10-12].

No information about the chemical composition, total phenolic, total flavonoids and antioxidant activity of Egyptian grape seeds. Therefore; the present study aims to characterize the chemical composition and to investigate the qualitative and quantitative characterization of Egyptian grape seeds by microwave-assisted extraction (MAE), which was determined by high-performance liquid chromatography coupled to electrospray quadrupole-time of flight mass spectrometry (HPLC–ESI-QTOF-MS).

Materials and methods

3.1.2. Grape seed extraction

Typically, 150 g of grape seed extraction was added to 30 ml of distilled water.

Analysis of total phenolic content:

The total phenolic content was determined according to the Folin-Ciocalteu procedure. Briefly, the extract (500 µl) was transferred into a test tube and oxidized with the addition of 250 µl of Folin-Ciocalteau reagent. After 5 min, the mixture was neutralized with 1.25 ml of 20% aqueous Na2CO3 solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic content was determined using a calibration curve prepared with gallic acid and expressed as µg of gallic acid equivalent (GAE) per ml of sample.

Analysis of total flavonoid content

The total flavonoid content was determined according to. Briefly, 250 µl of 5% NaNO2 was mixed with 500 µl of extract. After 6 min, 2.5 ml of a 10% AlCl3 solution was added. After 7 min, 1.25 ml of 1 M NaOH was added, and the mixture was centrifuged at 5000 *g* for 10 min. The absorbance of the supernatant was measured at 510 nm against the solvent blank. The total flavonoid content was

expressed as µg of catechin equivalent (CE) per ml of sample.

Determination of radical DPPH scavenging activity

Free radical scavenging capacity was determined using the stable 1,1-Diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$). The final concentration was 50 µM for $DPPH^{\cdot}$ and the final reaction volume was 3.0 mL. The absorbance at 517 nm was measured against a blank of pure methanol at 60 min.

Percent inhibition of the DPPH free radical was calculated by the following equation:

$$\text{Inhibition (\%)} = 100 \times (A_{control} - A_{sample})/A_{control}$$

Where: $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound). A_{sample} is the absorbance of the test compound.

Also, the antioxidant activity was determined using a calibration curve prepared with Trolox and expressed as mg of Trolox equivalent (TE) per unit (volume or weight) of a sample.

Phenolic acids profile

Extraction of phenolic compounds

Sample (1g) was placed in a quick fit conical flask and 20 ml of 2M NaOH was added and the flasks were flushed with N_2 and the stopper was replaced. The samples were shacked for 4 h at room temperature. The pH was adjusted to 2 with 6 M HCl. The samples were centrifuged at 5000 rpm for 10 min and the supernatant was collected. Phenolic compounds were extracted twice with 50 ml ethyl ether and ethyl acetate 1:1. The organic phase was separated and evaporated at 45°C and the samples redissolved in 2ml methanol.

Analysis of phenolic compounds by HPLC

HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an autosampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 70 min and the gradient programme was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.

Results and discussion:

Chromatographic conditions of grape seed:

All the standards were separated within 50 min and showed good resolution between matrix and analyte peaks. Figures (1&2) revealed the chromatogram of mixed standard solution and (Vitis vinifera) samples.

Total phenolic contents:

Table (1) exhibits the total phenols content in Egyptian grape seeds extracts showed a high amount of these compounds. Chyrsin, Cinnamic acid, Coumaric acid, Rutin, Sinapic acid and Gentisic acid were not determined in the total phenols content in Egyptian grape seed extracts. On the other hand; a high amount of catachine and Gallic acid was determined in the total phenols content in Egyptian grape seed extracts. Also; many other phenols content were determined in Egyptian grape seed extracts as Protochatchuic acid, Syrngic acid, Chlorgenic acid, Qurecetin, Vanillic acid, Rosmarinic acid, Kaempferol and Chyrsin.

Total flavonoid contents:

Table (2) exhibits the total flavonoids contents, which is believed to be another determinant of the overall antioxidant activities, were measured, The quantities of flavonoids in the ethanolic extract of rosemary were found to be 6.687 mg CE/g.

Total antioxidant activity:

DPPH

Table (2) exhibits the results indicate that the ethanolic extract from grape seeds possessed radical-scavenging and antioxidant activities which figured out from the result 81,506 mg TE/g.

The current results indicate that the ethanolic extract from Egyptian grape seeds possessed radical-scavenging and antioxidant activities where about 17.442 mg GAE/g of total phenols, 6.687 mg CE/g of total flavonoids and 81.506 mg TE/g DPPH as antioxidants activity in grape seed.

The current results agree with results of grape seeds from another land and indicate that; the antioxidant power of proanthocyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C. Based on numerous proofs, the most important compounds found in grape seed extracts were gallic acid, catechine and epicatechine.2 Considering different literature sources [13-16], the methods of HPLC analysis, the eluents used and the retention time, one may presume that the third compound is epicatechine. Quantitative analysis of this compound will be performed in further studies.

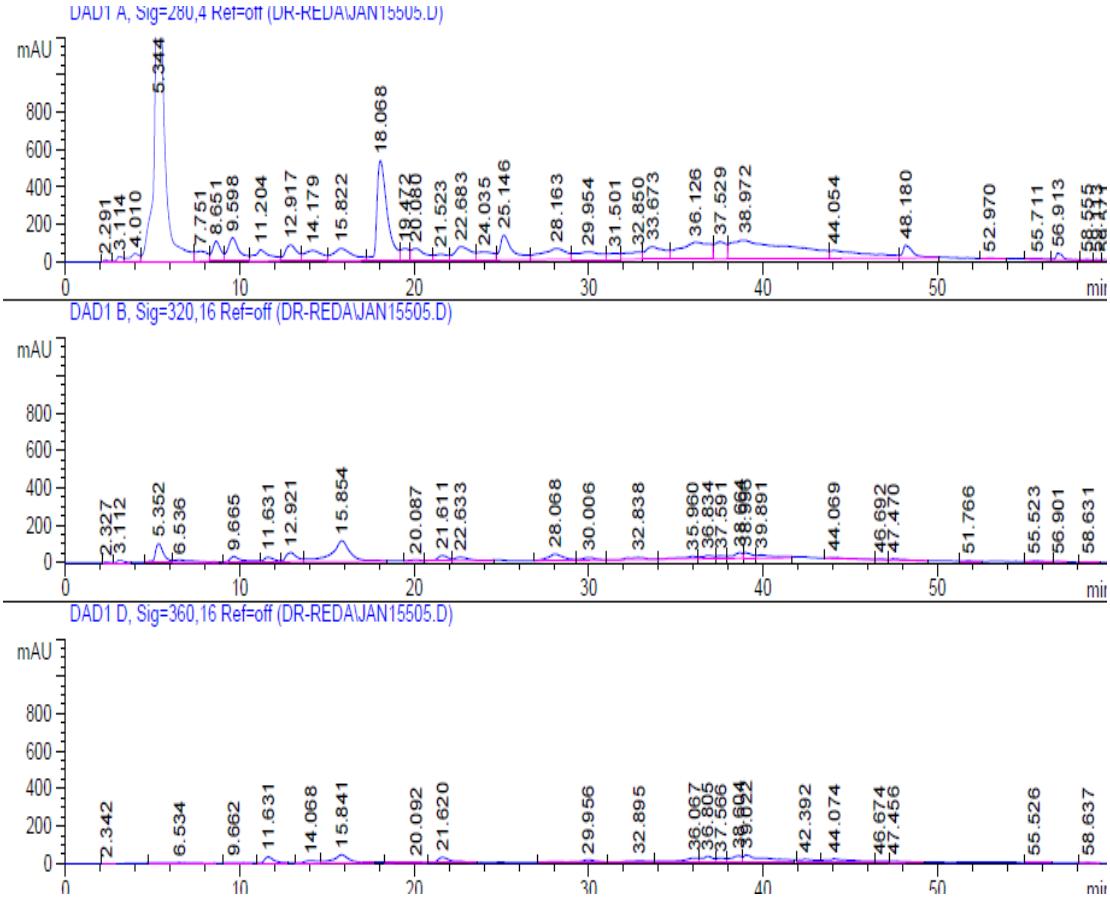

Figure (1): Base Peak Chromatogram (BPC) of grape seed sample.

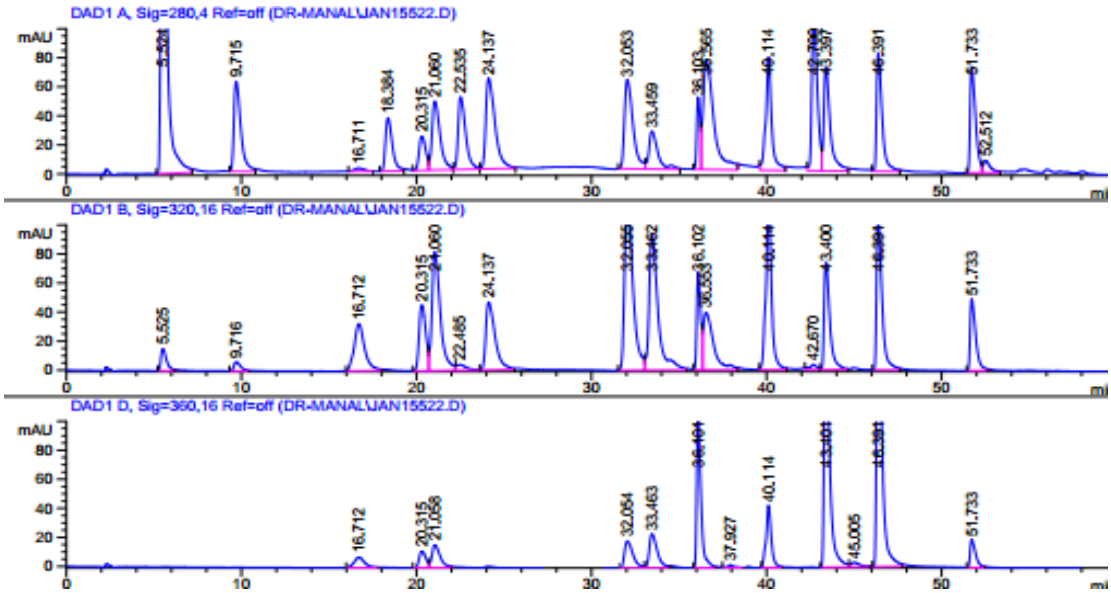

Figure (2): Chromatograms of a mixed standard solution of pyrogallol, gallic, protochatchuic, *p*-hydroxybenzoic, gentisic, chlorgenic, syrngic, vanillic, rutin, *p*-coumaric, hisperdin, apeginin-7-glucoside, myrcetin, Rosmarinic, cinnamic, apegnin and kaempferol.

Table (1): Total phenolic contents: The total phenols content in grape seed extracts showed a high amount of these compounds. Black color = 280 nm Red color = 320nm, Blue color = 360 nm.

	Compound	Tr	Grape seeds
1	Gallic acid	5.6	2750.380
2	Protochatchuic acid	9.7	355.293
3	Gentisic acid	16.7	ND
4	catachine	18.4	4239.911
5	Chlorgenic acid	20.3	21.493
6	Caffeic acid	21	34.435
7	Syrngic acid	22.5	96.805
8	Vanillic acid	24.1	14.663
9	Ferulic acid	32	4.520
10	Sinapic acid	33.5	ND
11	Rutin	36.1	ND
12	Coumaric acid	36.7	ND
13	Rosmarinic acid	40.1	11.270
14	Cinnamic acid	42.7	ND
15	Qurecetin	43.4	19.207
16	Kaempferol	46.4	9.857
17	Chyrsin	51.7	ND

Table (2): The total phenols, total flavonoid content and antioxidants activity in grape seed, (ND, not determined).

Sample	Total phenols (mg GAE/g)	Total flavonoids (mg CE/g)	DPPH (mg TE/g)
content	17.442	6.687	81.506

Conclusion:-

Grape (Vitis vinifera) seeds are very rich sources of flavonoids and typical compounds of grape seeds, such as Catachine, Gallic acid, Protochatchuic acid and Syrngic acid which are compounds with many biological properties, especially antioxidant. On the other hand, Quercetin was highly represented that indicate the anticancer and anti-inflammatory properties of grape seeds. This aspect should be studied in depth in future research.

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