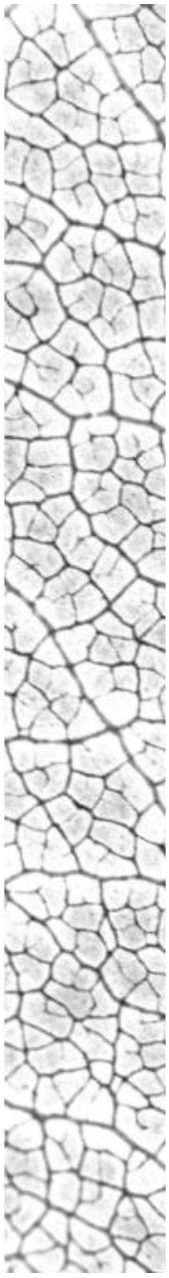

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Antioxidant properties flavonoids and phenolic content of grape seed extracts in Jordan

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Abstract

Background: Grape seeds extract (GSE) is one of recommended natural antioxidants that could be used in various food products. it is used in jordan as healthy food material recording about 10000 kg consumption per year. **Materials and Methods:** The aim of this study is to quantify the antioxidant compounds that found in grape seeds which extracted using organic solvents (acetone 60%, ethanol 60% and methanol 75%) comparing with their 100% extracts. Grape pomace was collected from Jordan market, washed and dried then seeds were removed and powdered into fine particles. Ten g were extracted either by 100% of suggested solvents or by their corresponded dilute concentrations. **Results:** Grape seeds contain 6.79% (moisture), 7.00% (protein), 9.80% (fat), 2.10 (ash), 42.33% (fibers) and 31.98% (calculated carbohydrates). Yield of grape seeds extract was significantly affected by type of solvent either diluted with water or not. Such yield ranged between 5.5 to 10.4% when 100% organic solvents was used, meanwhile the corresponding extraction yield ranged between 10.7 to 14.0 % when such organic solvents were diluted. It could be descending ordered the efficiency of solvents for yielding extract as: Acetone: water (60: 40), Ethanol: water (60: 40) and Methanol: water (75: 25).
A significant difference in total flavonoids content of grape seed extracts was detected owing to solvent type. Grape seeds treated by diluted methanol (75: 25) gave an extract possessed the highest total flavonoids content contrary to that of extracted by 100% acetone. It could be reported that diluting of organic solvents Acetone: water (60: 40), Ethanol: water (60: 40) and Methanol: water (75: 25) enhanced corresponding extraction yield of total flavonoids by 1.8, 1.5 and 1.1 times higher. Similar pattern that found earlier was also detected when the content of total phenolics was considered showing higher efficiency for extracting more phenolics by diluting methanol with 25% of water to be in the first order. **In conclusion**, higher efficiency of extracting flavonoids and phenolics from grape seeds was seen when using 75% methanol for extraction.

Keywords: grape seed extracts, antioxidant properties, flavonoids, phenolic content, ethanolic and methanolic extract

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INTRODUCTION

Grape (Vitis sp.) is a regular source of phenolic compounds connected to vital health benefits. Polyphenols have been linked to the bioactive activity of grapes due to their antibacterial, anti-inflammatory, antioxidant, and anticarcinogenic activities (Daglia, 2011; Rockenbach, et al., 2011). Grape seeds extract (GSE) is one of recommended natural antioxidants that could be used in various food products like fishery or confectionery products. Also, it used in Japan as healthy food material recording about 10000 kg consumption per year. It is of interest to report that wine industry produces 30% as solid wastes containing pulp, skin, seeds or stem (Dwyer *et al.,* 2014 and Teixeira *et al.,* 2014). Phenolic compounds that found in grape seeds are still remain after processing as given by Centeno *et al.* (2013) and have a great potential because of the high antioxidant capacity and wide health benefits for coronary diseases by lowering low-density lipoproteins as reported by Pereja *et al*. (2015).

Many studies display that the antioxidant properties of plant seed extracts were credited to their phenolic contents (Ricardo da Silva et al 1990). Phenolics are defined as organic metabolites comprising benzene ring and belong to a large and complex family. Phenolic compounds are correlated to some characteristics of quality such as odour, taste and colour, of vegetables and fruit**.** They possess also antioxidant and antiradical characteristics. The antioxidant capacity of phenolics are decided by the degree of hydroxylation of the ring structure and its position (Hagr and Adam, 2020; Hayat, et al. 2019; Larki, et al, 2020).

Grape seeds comprise nearly 70 mg/g of complex phenolic compounds like tannins, 160 mg/g of essential oil, 110 mg/g of protein and 400 mg/g of fiber, (Gibis &Weiss, 2012; Perumalla and Hettiarachchy, 2011). The polyphenol structure of grape seed in methanolic or ethanolic extracts is evaluated in multiple world markets but not Jordan, and there is a lack of information regarding Jordanian grape constituents as a rich source of polyphenols. In this context,

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Table 1. Proximate composition of grape seeds on wet weight basis

Component	%
Moisture	6.79
Protein	7.00
Fat	9.80
Ash	2.10
Fibers	42.33
Carbohydrates*	31.98

* Calculated by difference

the aim of this study is to find out the phenolic and flavonoid content in Jordanian grape and to investigate the quality of the antioxidant compounds that found in grape seeds which extracted using different organic solvents (acetone 60%, ethanol 60% and methanol 75%) comparing with their 100% extracts.

MATERIAL AND METHODS

Material and preparation of extracts

Grape pomace named *Vitis vinifera* was collected from Jordan market, washed and dried in hot air oven at 45°C then seeds were removed and powdered into fine particles and sieved throughout 80-mesh siever. Ten g were extracted either by 100% of suggested solvents (acetone, ethanol and methanol) or by their corresponded dilute concentrations; i.e. 60 %, 60 % and 75 % by shaking overnight at room temperature. Extracts were filtered and solvent was evaporated using Büchi HB-140 Rotavapor M (Rehman, 2006).

Analytical methods

Proximate composition

Proximate composition of grape seeds (moisture, protein, fat, ash and fibers) was carried out according to AOAC (2012).

Total phenolic content

Total phenolic content was determined using the method of Titto (1985) by mixing 50 µl of diluted extract with 1950 µl water and adding 1 ml of Folin-Ciocalteu reagent then the tube was vigorously shaken. After that, 5 ml of sodium carbonate 20% was added and volume was brought to be 10 ml and shaked again. The absorbance at 735 nm was measured after 20 min using spectrophotometer. Phenolic content was calculated using standard curve of gallic acid (results given as mg gallic acid equivalents/g dry matter).

DPPH assay

The DPPH method that described by Williams et al. (1995) was applied and antioxidant activity as µ mol Trolox eq. /g was expressed.

Total flavonoids content

The total flavonoids content was determined according to Huang et al. (2006), results were calculated using calibration curve of quercetin and were given as mg quercetin/g dry matter.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to differentiate between means ($p<0.05$) as reported by Snedecor and Cochran (1994).

Table 2. Extraction percentage (yield) % against solvent type used

Solvent (V:V)	Extraction yield %
Acetone 100%	5.51^{e}
Ethanol 100%	10.41^{c}
Methanol 100%	8.43^{d}
Diluted acetone 60: 40	14.00^{a}
Diluted ethanol 60: 40	11.41^{b}
Diluted methanol 75: 25	10.74^{c}

Mean values by various small letters in column are significant differed ($P\leq0.05$)

Table 3. Total flavonoids and total phenolics content of grape seeds extracted by various solvents

Solvent (V:V)*	Total flavonoids (mg/g)	Total phenolics (mg/g)
Acetone 100%	3.441^{f}	4.633^{f}
Ethanol 100%	4.196^{e}	5.810^{e}
Methanol 100%	6.071^{c}	6.830^{d}
Diluted acetone 60 : 40	6.234^{b}	7.662^{c}
Diluted ethanol 60: 40	6.402^{b}	8.954^{b}
Diluted methanol 75: 25	6.710^{a}	9.506^{a}

* Volume of solvent: volume of water

Mean values followed by various small letters in column are significantly differed ($P\leq0.05$)

RESULTS AND DISCUSSION

Proximate composition

Table 1 shows proximate composition of grape seeds. It could be seen that such seeds contain 6.8% (moisture), 7.0% (protein), 9.8% (fat), 2.1 (ash), 42.3% (fibers) and 31.9% (calculated carbohydrates). These results are in agreement with those of Ovcharava et al. (2016).

Solvent extraction percentage

The extraction percentages (yield) of various suggested solvents were given in **Table 2**. From this Table it could be seen that yield of grape seeds extraction was significantly affected by type of solvent either diluted with water or not. Such yield ranged between 5.51 to 10.4% when 100% organic solvents was used, meanwhile the corresponding extraction yield ranged between 10.7 to 14.0 when such organic solvents were diluted with water with different ratios for extracting. The extraction yield increased by 2.5, 1.1 and 1.3 fold when acetone, ethanol and methanol were diluted to be 60: 40, 60: 40 and 75: 25 (v/v), respectively.

The extraction with pure ethanol was the highest one, while pure acetone was the lowest one for extracting grape seeds owing to low capability of acetone to extract polar compounds. Adding 40% water to acetone solvent gave a reversible pattern of extracted yield to be the highest one (14.0%). This is due to increasing polarity of aqueous acetone for extracting more polar compounds found in grape seeds. Similar trend was recorded in other used organic solvents; i.e. ethanol or methanol. These findings are in accordance with those of Vayupharp and Laksanalamai (2012). Therefore, it could be descendingly ordered the efficiency of solvent for yielding extract as: Acetone: water (60: 40), Ethanol: water (60: 40), and Methanol: water (75: 25)

Antioxidant compounds of grape seed extracts

The total flavonoids as well as the total phenolic compounds that play an antioxidant role were determined in grape seed extracts and given in **Table 3**.

A significant difference in total flavonoids content of grape seed extracts was detected owing to solvent type.

Type of solvent*					
A	B	C	D	E	F
505.82^{e}	637.30^{b}	650.30^{b}	505.28^{c}	578.13^{e}	701.78^{a}

Mean values followed by different small letters in the row are significantly different (P≤0.05).
A= Ethanol 100%
B = Ethanol 60% + Water 40%
C = Acetone 100%
D = Acetone 60% + Water 40%
E = Methanol 100%
F = Methanol 75% + water 25%

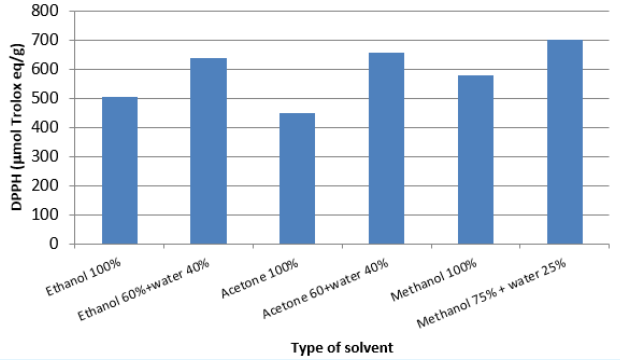

Fig. 1. Antioxidant activity of grape seed extracts affected by solvent's type

Grape seeds treated by diluted methanol (75: 25) gave an extract possessed the highest total flavonoids content (6.7 mg quercetin/ g dry matter) contrary to that of extracted by 100% acetone which contained 3.4 mg quercetin/g dry matter as lowest total flavonoids content. It could be reported that diluting of organic solvents (i.e. acetone, ethanol or methanol) by 25, 40 or 40% water, respectively enhanced corresponding extraction yield of total flavonoids by 1.81, 1.53 and 1.1 times higher. This findings are in agreement with Nageb (2015).

Total phenolics content as mg gallic acid/g dry matter was also determined and given in same Table (3). Similar pattern that found earlier was also detected when the content of total phenolics was considered. It ranged between the highest one (9.5 mg gallic acid/g dry matter) and the lowest (4.6 mg gallic acid/g dry matter). This exhibits higher efficiency for extracting more phenolics by diluting methanol with 25% of water to be in the first order for extracting followed by diluted ethanol (with 40%) then diluted acetone (with 40% too) with high significancy (P≤0.05).

Such above findings are closely to those of Casazza et al. (2010), Rackenback et al. (2011), Vayupharp and Laksanalamai (2012), Zulkifli et al. (2012), Libran et al. (2013), Mohammedelnour et al. (2017) whom reported the following explanations:

- Total phenolic compounds of grape seeds are dependent on extraction conditions.
- The highest content of total polyphenol, O-diphenols, flavonoids and catchin are found in grape seeds.
- Total phenolic compounds of grape pomace is higher than those of pulps, seeds and peels.
- Increased polarity of diluted organic solvents makes these solvents ready for extracting more polar compounds in grape seeds.
- Methanol as well as ethanol are better than that of ethyl acetate or acetone for extracting phenolic compounds from plant wastes owing to their higher polarity as well as the good solubility of such phenolic components from plant sources.

Antioxidant activity of grape seed extracts

The antioxidant activity (as DPPH µmol Trolox eq./g) of grape seed extracts affected by solvent's type was given in **Fig. 1**. The type of solvent used for extracting grape seeds was significantly affected the antioxidant activity expressed as DPPH radical scavenging activity. As a general trend, pure solvent had less antioxidant activity than its corresponding diluted one that showed 1.2 fold of increasing in all of three types of diluted extractors. Such difference is due to the interspecies variation, used extraction solvents as well as to the method of determination (Selcuk, 2011).

The extracts consisted of 75% methanol or 60% ethanol significantly higher antioxidant activity, this is owing to the solvent polarity index which indicates such extracts as the best solvents for extracting antioxidant compounds (Mohammedelnour *et al.,* 2017).

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