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CHEMICAL STUDIES AND PHYTOCHEMICAL SCREENING OF GRAPE SEEDS (*VITIS VINIFERA L.*)

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ABSTRACT:

Grape seeds (*Vitis Vinifera L.*) are a good sources of phytochemicals and are suitable raw materials for the production of antioxidative dietary supplements. The present study was carried out to assess the proximate composition, phytochemical screening, total phenolic compounds, total flavonoids and antioxidant activities of selected solvent extracts of grape seeds. The results showed that grape seeds content was 38.2% fibers, 15.8% total lipids, 10.7% proteins, 2.58% ash, 22.37% carbohydrates and 10.4% moisture. Phytochemical analysis of grape seed extract revealed the presence of steroids, terpenoids, anthocyanins, emodins, glycosides, flavonoides and phenols in acetone (70%), ethanol (70%) and methanol. Both steroids and terpenoids were absent in water extract. Saponins were absent in methanol and water extracts. *In vitro,* antioxidant activity was estimated as IC_{50} value. Our result revealed that the acetone (70%) is the more efficient solvent to extract the total phenolic compounds and flavonoids from grape seed when compared to the other selected solvents for the study.

Keywords: *Grape seed , Antioxidant activity, Phytochemical* screening, *Phenolic compounds, Flavonoids .*

INTRODUCTION

Grape (*Vitis vinifera L.*) is belong to the *Vitaceae* family. It is one of the fruit crops grown widely in many areas of the world (Anonymous, 1999). Grape is cultivated originally in Asia,

also a minor grows in south Europe, North Africa and Middle East (Chopra *et al.,*1970). In Egypt, grapes occupied the second rank after citrus. (Ministry of Agriculture Statistics, 1999). The kinds of grapes are Thompson *seedless* and Roumy Ahmer grape cultivars which occupies almost two thirds of the total area.

Grape seeds are a complex matrix containing approximately 40% fiber, 10 to 20 % oil (Sabir *et al*., 2012), 11% proteins, 26.43% of total carbohydrates (Owon, 1999) and 7% complex phenols including tannins, in addition to mineral salts, etc. These oils contain nutritionally useful essential fatty acids and tocopherols (El-Mallah and Murui, 1993). *V. vinifera* contains many chemical constituents viz, phenolic acids, flavonoids, anthocyanins, proanthocyanidins, sugars, sterols, amino acids, and minerals (El-Hawary *et al*., 2012).

Grape seed extract (GSE) has different medicinal properties including anti-inflammatory (Terra *et al.,* 2009), anticarcinogenic, platelet aggregation inhibiting, and metal chelating properties, etc (Balu *et al.,* 2005), Also chemoprotective properties against reactive oxygen species (Nandakumar *et al.,* 2008) and anti-bacterial (Mayer *et al.,* 2008), anti-cancer (Kaur *et al*., 2006). The antioxidant effects of grape seed extract has been confirmed in different studies (Hemmati *et al.,* 2008) which seems to have potentials for improving or treating type 2 diabetes and it's associated metabolic disorders. Some human clinical trials investigated various effects of GSE (Saada *et al*., 2009).There are some experimental studies about it's anti diabetic effects (Hwang *et al*., 2009 and Lee *et al*., 2008). Roth (2010) *showed* that grape seed is significantly more effective than a placebo in improving night vision.

The objective of the present study is to prepare an antioxidant rich fractions of grape seed extract, evaluate its *oxidant* activity, and studying qualitative analysis of phytochemicals of grape seed.

MATERIALS AND METHODS*:*

1. Samples:

Grape seeds were Purchased from local garden around Minia University after drying in shade. These seeds were ground into fine powder using an electric grinding machine (Model MX 491N National).

2. Chemical composition:

The chemical composition (moisture, proteins, lipids, ash and fiber) of triplicate samples were determined according to the standard methods of AOAC (1990). The protein content of each sample was calculated by using conversation factor 6.25. The carbohydrates were estimated by differences.

3. Preparation of seeds extract :

The extraction was carried out using four different solvents, separately i.e. ethanol: water (70:30

v/v), acetone: water (70:30 v/v), methanol absolute and water. The seeds powder (0.4 g) was mixed with 20 mL of solvent and stirred for 2 hrs. at 45°C. The extract was centrifuged at 4000 rpm for 10 min and subsequently decanted. The residue was re extracted for 2 hrs and supernatants were combined and sample extract evaporated to dryness (modified of Huali *et al.*, 2008)

4. Quantitative analysis of phytochemicals:

4.1. Determination of Total polyphenols:

Total polyphenols(TP) were determined using the Folin-Ciocalteu reagent, according to Maurya and Singh,2010. The calibration curve was made with standard of solution of gallic acid in the range of 0.01- 0.05 mg ml^{-1} and measures were carried out at 760nm using a UV-Vis spectrophotometer. All analysis were performed in triplicate. Gallic acid was employed as a calibration standard and results were expressed as milligrams of equivalent gallic acid per gram of sample.

4.2. Determination of total flavonoids content:

The flavonoid content of each extract was measured based on methods described by Ebrahimzadeh *et al.* (2008). Briefly, 0.5ml of sample (5g/L) was mixed with 1.5ml of methanol and then 0.1 ml of 10% potassium acetate and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 min. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract). The standard curve was prepared by quercetin in different concentrations (5-50 mg/L).

5. Determination of total antioxidant activity:

The antioxidant activities of the acetone (70%), methanol absolute, ethanol (70%) and water extracts were assessed by measuring free radical scavenging activity via the discoloration of these solvents of the free radical 1,1 diphenyl-2-picrylhydrazyl (DPPH) as described by Brand – Williams *et al.* (1995) as follows: Two ml of acetone (70%), methanol, ethanol (70%) and water solution of either test material at various concentrations (1-64 µg/ml) and methanol solution used as control were added to 2 ml solution of DPPH (25mg/L) in methanol, and the reaction mixture was shaken vigorously and left in darkness for 30 min. Finally, the absorbance of the mixture was measured against pure methanol (blank) at 517 nm T80 UV/Vis spectrophotometer. The percentage of radical scavenging activity was calculated using the following formula:

$$\text{Radical scavenging (\%)} = [(A_0 - A_1 / A_0) \times 100]$$

Where: A_0 is the absorbance of control and A_1 is the absorbance of the sample extracts. The 50% inhibitory concentration value (IC_{50}) is indicated as the effective concentration of the

sample that is required to scavenge 50% of the DPPH free radicals.

6. Preliminary Phytochemical screening:

Qualitative phytochemical analysis.

Steroids: An aliquot of the seed extract (1ml) was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids (Gibbs, 1974).

Terpenoids An aliquot of the seed extract (2ml) was added to 2ml of acetic anhydride and concentrated H_2SO_4. The formations of blue green ring indicate the presence of terpenoids (Ayoola *et al*.,2008).

Tannins: An aliquot of the seed extract (2ml) was added to few drops of 1% lead acetate, and the yellowish precipitate indicated the presence of tannins (Treare and Evans, 1985).

Saponins: An aliquot of the seed extract (5ml) was mixed with 20ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of Saponins (Kumar *et al*.,2009).

***Anthocyanins*:** An aliquot of the seed extract (2ml) was added to 2ml of 2 N HCl and ammonia. The appearance of pink-red which turns to blue-violet indicates the presence of anthocyanins (Farnsworth, 1966).

Glycosides: 2ml. glacial acetic acid, one drop of 5% $FeCl_3$ and conc. H_2SO_4 were added into 5ml extract, the appearance of brown ring indicates the presence of glycosides (Khandewal, 2008).

Emodins: Two ml of NH_4OH and 3 ml of Benzene were added to the extract. Appearance of red colour indicates the presence of emodins (Rizk, 1982).

Alkaloids: a Mayer's test: To the acidic solution, Mayer's regent (Potassium mercuric iodide solution) was added. Cream coloured precipitate indicates the presence of alkaloids (Gibbs, 1974).

Phenol: Half ml of $FeCl_3$ solution was added into 2 ml of test solution, formation of an intense color indicates the presence of phenols (Gibbs, 1974).

Flavonoids: An aliquot of the seed extract (2-3ml) and few drops of sodium hydroxide solution were added into a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids (Khandewal, 2008).

7.Statical analysis:

Experimental results were means ± SD of three parallel measurements. Analysis of variance was performed by ANOVA procedures. GraphPad Prism® was used for statistical calculations GraphPad Software, San Diego, CA, USA (Motulsky, 1999).

RESULT AND DISCUSSION*:*

1. Chemical composition of grape seeds :

The average values for the chemical composition grape seeds are given in Table (1).

Table 1. proximate analysis of grape seeds

Proximate	Grape seeds
Moisture	10.40 ± 0.19
Ash	2.58 ±0.11
Total lipid	15.80 ±1.21
Fiber	38.20 ± 2. 24
Protein	10.70 ± 0.17
Carbohydrate	22.37 ± 2.7

Each value is expressed as the mean± SD (n=3)

The grape seed contented 38.2% fiber, 15.8% total lipid, 10.7% protein, 2.58% ash ,10.4 % moisture and 22.37% carbohydrate.

The moisture content were determined as a function of seed (Razavi and Fathi, 2009) and all physical properties of the grape seeds were significantly affected by the moisture content (Ahmadi and Siahsar, 2011). Also, moisture content of byproduct grape seeds from grape pekmez production was significantly higher than winery by product (Selcuk *et al*., 2011) .

The results in the present study are consistent with the previous observations of Owon (1999) who reported that grape seeds contain 2.86% of ash and 12.69% of oil. Baydar and Akkurt (2001) found that the oil concentration of 18 grape cultivar seeds ranged from 11.6 to 19.6%, while Mouhammad and Ali (2008) noticed that Syrian grape seeds contain 1.45–1.65% of ash. The oil contents of nine grape seed cultivars were ranged from 10.45% to 16.73% (Tangolar *et al.,* 2009).In addition, Mironeasa *et al.,* (2010) 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. The value of ash is closed to those reported by Elagamey *et al.*(2013). Also, Sabir *et al.* (2012) reported that the grape seed oil concentration of some different cultivars ranged from 7.3 to 22.4%. The determination of the grape seeds protein content were ranged from 6.26-9.01% according to cultivar (Mironeasa *et al.,* 2010).

2. Quantitative analysis of phytochemicals:

2.1.Total Phenolics and Flavonoids Content:

In the present study, total phenol and flavonoid content of grape seeds were shown in Table 2. This study have demonstrated that the total phenolic compounds in various extracts of grape seeds ranged from 186 - 528 mg/ g, as GAE. Acetone extract showed the highest total phenolic acids content (528 mg/g), while the water extract had the lowest value (186 mg/g). The phenolic content in various solvents decreases in the order of acetone (70%) > methanol > ethanol (70%) > water.

Phenolic acids are known to act as antioxidants not only because they are able to donate hydrogen or

electrons but also, stable radical intermediates, which prevent oxidation of various food ingredients, particularly fatty acids and oils (Cuvelier *et al.,* 1992).

Table 2. Total phenolic compounds and Total flavonoids of grape seed extra*cts.*

Sample	Total phenolic compounds mg/g)[a] (	Total flavonoids mg/g)[b](
Grape seed extract (Acetone 70%)	528±16.97	14±0. 817
Grape seed extract (Ethanol 70%)	305***±17.32	13.75±0.955
Grape seed extract (Methanol)	372.5***±5.00	11.75**±0.50
Grape seed extract (Water)	186***±8.485	9.75***±0.50

*a:mg GAE /g of dry seed extract; b: mg QE/g of dry seed extract. Each value is expressed as the mean.± SD (n=3). (**and ***) Significant and highly significant respectively at P < 0.05 vs grape seed acetone extract.*

The overall trend was the same as reported by Huali *et al.,* (2008) who found that extraction with acetone (70%) led to the maximum phenolic content, while water gave the lowest phenolic content and indicated that aqueous solution of acetone was better than a single compound solvent system for extraction of total phenolic from plant materials.

Rababah *et al.* (2008) found that the total phenols of different grape seed cultivars extract ranged from 4.66 to 5.12g/100g, also, the amounts and distribution of various phenolic compounds in grape seeds depend directly on the cultivar (Gođevac *et al*., 2010). Grape seeds are richer in phenols than skins or pulp in both red and white grapes (Canals *et al.,* 2008).

In our study, total flavonoids content (Table 2)decreases in the following order acetone (70%) > ethanol (70%) > methanol > water. Acetone extract exhibited the highest value of flavonoids (14 mg/g) while water extract exhibited the lowest value (9.75 mg/g). Ioana *et al*. (2011) reported that polyphenols and total flavonoids content in grape seeds extract were 506.25mg GAE/100g.

Hassan and Nahla (2010) reported that grape seed extract contains a logical amount of phenolic compounds and flavonoids, and ethanol grape seed extract contain high amount of phenolic compounds and flavonoids (66.60 and 11.56mg/g) in comparison with water grape seed extract (31.20 and 6.85mg/g grape seed, respectively).

2.2. Antioxidant activity:

The free radicals (DPPH) was used to find antioxidant (scavenging) activity of various extracts. DPPH is stable free radical at room temperature and accepts an electron/hydrogen radical to become a stable diamagnetic

molecule (David *et al.*, 2004). The reduction capability of DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants. The decrease in absorbance DPPH radical is caused by antioxidants, because of the reaction between antioxidant molecules and radicals, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidant activity (Edamatsu *et al.*, 1989).

Our results showed free radical scavenging activity for all tested grape extracts (Table 3). The highest DPPH scavenging activities were shown by acetone: water (70%) of grape seed extract but there were no significant difference between the scavenging activity of different other solvent extract of grape seed powders.

This results agree with Huali *et al.*(2008) who showed that the highest DPPH scavenging activities by aqueous acetone extract of grape seed powder and the lowest DPPH scavenging activities were shown by water extract of grape seed powder.

Table 3. Antioxidant activity of grape seed extracts.

Sample	%inhibition	IC_{50}*(μg/ml)*
Grape seeds (Acetone 70%)	98.70	36.64
Grape seed(Methanol)	90.70	39.57
Grape seeds (Ethanol70%)	92.13	39.55
Grape seeds(water)	91.80	39.65

The IC_{50} values correspond to the amount of extract required to scavenge 50% of radicals present in the reaction mixture.

3.* Qualitative analysis of phytochemicals*:

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plants produce these chemical substances to protect themselves, and they are also believed to protect humans against certain diseases (Edeoga *et al.*, 2005).

The presence of phytochemicals with biological activity can be valuable medicinal value, for example, Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects (Orhan *et al*., 2007). Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities (Cherian and Augusti, 1995). Steroids and triterpenoids showed the analgesic properties (Rupasinghe, *et al.*, 2003).

The results of phytochemical constituents of grape seeds extract (Table 4) revealed the presence of steroids, terpenoids, anthocyanins, emodins, glycosides, flavonoides and phenols in acetone, methanolic, ethanolic and water extracts but steroid and terpenoids were absent in water

extract, whereas saponins were absent in methanol and water extracts.

These results are in agreement with Grace and Narendhirakannan (2011). They found that the presence of alkaloids, flavonoids, carbohydrates, saponins, tannins triterpenoids, steroids were present in grape seed. Also Emad *et al.,* (2013) reported some of phytochemical screening of grape seed are present such as flavonoids, steroids, tannins, in all grape wastes .

Table 4. Phytochemical screening tests for constituents of grape seed (Acetone 70 % / ethanol 70%/ Methanol and water extract). (+++), (++), (+) and (-) refer to high, moderate, low and absent amount respectively.

Constituent	Aceton70%	Ethanol 70%	Methanol 70%	Water
Steroids	+	++	++	-
Terpenoids	++	+	+++	-
Tannins	+	+++	++	+
Saponins	+	++	-	-
Anthocyanins	+	++	+++	+
Emodins	++	+++	++	+
Alkaloids	+++	++	++	+
Glycosides	+	++	+++	+
Flavonoids	+++	+++	++	+
Phenols	+++	++	++	+

The results of the present study revealed that the acetone (70%) is the more efficient solvent for extract the phytochemical compounds from grape seed. Also Rekha and Bhaskar (2013) revealed the presence of Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids. Cardiac glycosides was absent in grape seed ethanol extract.

CONCLUSION*:*

Among the various solvents used in this study, the acetone extract of grape seed has been found to possess good antioxidant activity , total phenolic compounds and flavonoids content. The extract is also rich in various phytochemical components. So that the grape seed may constitute a good source of healthy compounds, could be useful in the prevention of diseases.

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الدراسات الكيميائية والتحليل الكيميائي النباتي لبذور العنب *(Vitis Vinifera L.)*

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قسم الكيمياء الزراعية – كلية الزراعة- جامعة المنيا

أجريت هذه الدراسة على بذور العنب لتقييم التركيب الكيميائي ، التحليل الكيميائي النباتي، الفينولات الكلية، الفلافونويدات والنشاط المضاد للأكسدة لمستخلص بذور العنب (الأسيتون والإيثانول والميثانول والماء). لوحظ ان بذور العنب تحتوى على 38.2 ٪ ألياف ، 15.8٪ دهون كلية ، 10.7٪ بروتين، 2.58٪ رماد ، 10.4 ٪ رطوبة ، 22.37٪ كربوهيدرات. وفي هذه الدراسة أوضحت التحليلات وجود المواد الكيميائية النباتية مثل الاستيرويدات، التربينات، الانثوسيانين، الايمودين، جليكوسيدات، الفلافونويدات والفينولات الموجودة في مستخلص الأسيتون، والميثانول والإيثانول والماء بينما غياب الاستيرويدات والتربينات في المستخلص المائى ، وغياب الصابونين في مستخلص الميثانول والماء وعند تقدير النشاط المضادة للأكسدة في المعمل وحساب قيمة IC_{50}. ودلت النتائج على أن الأسيتون (70%) هو المذيب الأكثر كفاءة لاستخلاص المركبات الفينولية الكلية والفلافونيدات من بذور العنب عند مقارنتها مع المذيبات المختارة للدراسة. ولذا تعتبر بذور العنب أحد المصادر الجيدة للمواد الكيميائية النباتية والمواد الخام وهي مناسبة لإنتاج المكملات الغذائية المضادة للأكسدة.