



ASSESSMENT OF GRAPE SEEDS AS A SOURCE OF ANTIOXIDANT COMPOUNDS

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ABSTRACT

The grape seeds extracted with various organic solvents (methanol, ethanol, acetone and chloroform) either pure (100%) or mixed with 30, 50 and 50% water (except chloroform) were evaluated for its content of antioxidant compounds; i.e. phenolics and flavonoids (by HPLC technique) and/or antioxidant activities (by DPPH test). The extraction yield was ranged between 6-10% depending on solvent type and significantly increased by mixing with water with various percentages. Total phenolics, total flavonoids and antioxidant activity of grape seed extracts were affected by type of solvent. The highest total phenolic compounds and total flavonoids was recorded in methanol 70% extract, while the lowest one was in water 100% extract.

Keywords: Grape seed extract, Antioxidant compounds, HPLC technique, DPPH test, Antioxidant activity, Extraction

INTRODUCTION

Grape seeds extract (GSE) was recommended for using as antioxidant in confectionery and fishery products; i.e. as a food additive but, in Japan it is also used as a healthy food material and its consumption is about 100000 kg/year. The solid wastes that generated by wine industry reaches 30% of used material mainly grape pomace containing seeds, skin, pulp and stem (Teixeira et al 2014, Dwyer et al 2014). High levels of valuable

compounds such as dietary fibers, seeds oil and phenolic compounds are still remain after processing in grape pomace (González-Centeno et al 2013). Such phenolics have a great potential owing to their antioxidant capacity and health benefits for coronary diseases by inhibiting low-density lipoproteins (Otero-Pereja et al 2015). This study tries to assess and identify the antioxidant compounds that highly found in grape seeds extracted with various organic solvents; i.e. methanol, ethanol, acetone and chloroform either pure (100%) or mixed with 30, 50 and 50% water (except chloroform).

MATERIAL AND METHODS

1. Materials

Grape pomace (*Vitisvinifera*) was obtained from Ganaklis Company for Beverages at Alexandria governorate.

2. Preparation of grape seeds extracts

Extracts were prepared according Rehman (2006). Grape pomace (GP) seeds was washed and dried in a hot air oven at 40°C for 16 hours. The dried grape pomace was separated to skin and seeds. Seeds were ground into a fine powder then sieved through an 80-mesh sieve. 10 gm of ground sample were extracted with 100 ml of different organic solvents (100 and 50% ethanol; 100 and 70%, methanol; 100 and 50% acetone; 100

distilled water or 100% chloroform) overnight in a shaker at room temperature. The grape seed extracts were filtered and the residues were re-extracted under the same conditions. The filtrate was evaporated in a rotary evaporator (BÜCHI Rotavapor R-124 Germany) 40°C. All grape seed extracts were dried in oven drier at 40°C then converted to powder form, except water extract was freeze dried at -40°C, volume flow rate 30 m²/h and ultimate pressure 4×10⁻² Pa. (Snijders Scientific b.v., Holland).

3. Analytical methods

3.1. Proximate composition

Moisture, fat, protein, ash and crude fiber were determined as mentioned in **A.O.A.C. (2012)**.

3.2. Determination of antioxidant compounds for waste extracts

3.2.1. Determination of total phenolic content (TPC)

The total phenolic content was determined according to the method reported by **Julkunen-Titto (1985)**. Aliquots of 50 µl of each diluted extract were mixed with 1950 µl water in a 10 ml test tube. One ml of Folin-Ciocalteu reagent was added and the test tube was vigorously shaken. Immediately, 5 ml of 20% sodium carbonate solution was added, the volume of the mixture was brought up to 10 ml and shaken thoroughly again. After 20 min, the absorbance of mixture was read at 735 nm by spectrophotometer (model: CT2200-s/n: RE1310004 – Germany). Phenolic content of extracts was calculated on the basis of the standard curve for gallic acid. The results were expressed as mg gallic acid equivalents per 100 g dry matter.

3.2.2. Determination of total flavonoids

Flavonoids content was measured according to the AlCl₃ method (**Huang et al 2006**). Each extract (0.5 ml) was mixed with 1.0 ml of 2 % methanolic AlCl₃.6H₂O, and absorbance was measured after 10 min at 430 nm. The content of flavonoids was calculated on the basis of the calibration curve of quercetin and expressed as mg quercetin per 100 g dry matter.

3.3. Antioxidant activity of grape seed extracts

3.3.1. DPPH scavenging assay

The DPPH method was carried out as described by **Brand-Williams et al (1995)**. The decrease in the absorbance of 100 µM DPPH- radicals (2.9 mL) dissolved in 80% methanol was evaluated at 515 nm, 30 min after addition of each extract. Total antioxidant activity (on a dw basis) was also expressed in µMol/g of TEAC.

3.4. Fractionation of phenolic compounds and flavonoids by HPLC

Phenolic compounds and flavonoids were performed by HPLC analysis using the method described by **Dragovic-Uzelac et al (2005)**. Beckman model equipped by double piston pump 126 with Fluorescence detector LC 240 (Perkin Elmer); pump for reaction (Dioxin); Derivatizing tube 10 m× 0.33mm; Data handling system (Software Gold); Column Supelcosil LC-18-DB, 25Cm × 4.6 mm, 5µm; Injector 20µl (Beckman). Injection was carried out at wave lengths 280 nm for separation.

RESULTS AND DISCUSSION

1- Proximate composition of grape seeds

On dry weight basis, **Table (1)** showed grape seeds had 7.68, 10.59, 2.28, 45.61 and 33.84% of protein, ether extract, ash, fibers and carbohydrates, respectively. These findings go in parallel with those of **Ovcharova et al (2016)**.

Table 1. Proximate composition of grape seeds

Parameter (%)	Grape seeds	
	WW*	DW**
Moisture	6.82	--
Protein	7.15	7.68
Ether extract	9.87	10.59
Ash	2.13	2.28
Fibers	42.50	45.61
Carbohydrates***	31.53	33.84

* WW: Wet weight basis

** DW: Dry weight basis

*** Calculated by difference

2. Extraction yield of grape seeds

Extraction yield grape seeds as affected by solvents type presented in **Table (2)** were signifi-

cantly ($P \leq 0.05$) affected by solvents type. Extraction yield for all solvent ranged from 6.0 to 14.50%. These results are in agreement with those obtained by **Vayupharp and Laksanalamai (2012)**. The pure solvents such as water, ethanol, methanol, acetone and chloroform that used for extracting grape seeds gave the extract yield of 8.10, 10.0, 8.0, 6.0 and 8.50%, respectively. While, by using mixture of pure solvent (methanol, ethanol or acetone) with water for extracting, the yield was 10.00, 11.5 and 14.5%, respectively.

Table 2. Extraction yield of grape seeds as affected by solvents type

Solvent type (v:v)	Extraction yield (%)
Water (100%)	8.10 ^d
Ethanol (100%)	10.0 ^c
Ethanol: Water, 50:50	11.50 ^b
Acetone 100%	6.00 ^e
Acetone: Water, 50:50	14.50 ^a
Methanol 100%	8.00 ^d
Methanol: Water, 70: 30	10.00 ^c
Chloroform 100%	8.50 ^{de}
LSD at 0.05 level	0.50

Mean values followed by different letters in the column are significantly different ($P \leq 0.05$).

Percent of yield extract was depended on type of components in grape seeds and polarity of solvent. The extraction with pure ethanol gave the highest yield (10.0%). The lowest one (6.0%) was recorded for pure acetone, this might be due to low capability of acetone to extract polar compounds. When 50% of water was added to acetone, the extraction yield was significantly increased from 6.0 to 4.5%. This could be explained by increasing polarity of an aqueous mixture of acetone for extracting more polar compounds in grape seeds. Also, the extraction yield was also significantly increased from 10 to 11.50% for ethanol and from 8.0 to 10.0% for methanol when 50 and 30% of water were added, respectively. The highest yield was observed with acetone 50% followed by ethanol 50%. These results indicated that 50% acetone or 50% ethanol are the best solvents to extract material from grape seeds.

1. Total phenolic, total flavonoids and antioxidant activity of grape seeds

Total phenolic compounds, total flavonoids and antioxidant activity of grape seeds as affected by type of solvent were given in **Table (3)**. Total phe-

nolic compounds and total flavonoids of grape seed extracts were significantly ($P \leq 0.05$) affected by solvent type. Total phenolic compounds and total flavonoids of all grape seed extracts were ranged from 3.558 to 9.501 mg gallic acid/g and 3.260 to 6.707 mg quercetin/g dry matter, respectively. These results are in agreement with those reported by **Librán et al (2013)** found that total phenolic compounds of grape seed ranged from 4.58 to 28.06 mg GAE/g dry sample, depending on the extraction conditions. Also, **Nageb (2015)** found that total phenolic compounds in grape seeds ranged from 115.28 to 324.75 mg gallic acid/100gm lower than those obtained by **Casazza et al (2010)** who found the highest content in total polyphenol are o-diphenols and flavonoids of grape seeds (108.3, 47.0mg gallic acid equivalent/g and 47.2 mg catchin equivalent/g on dry weight, respectively) and also **Rockenbach et al (2011)** reported that total phenolic compounds of grape pomace was 74.75 mg GAE/g higher than those of **Abdrabba and Hussein (2016)** who found that total phenolic content of pulps, seeds and peels of red grape was 11.65, 73.59 and 13.73 mg GAE/100g, respectively.

Table 3. Total phenolic, total flavonoids and antioxidant activity of grape seeds as affected by type of solvents

Solvent type (v:v)	Total phenolic compounds (mg/g)	Total flavonoids (mg/g)	DPPH $\mu\text{MolTrolo xeq/ gm}$
Water 100%	3.558 ^g	3.260 ^f	198.62 ^g
Ethanol 100%	5.804 ^{ef}	4.189 ^e	504.652 ^e
Ethanol: Water, 50:50	8.949 ^b	6.398 ^b	636.50 ^b
Acetone 100%	4.629 ^f	3.433 ^f	449.82 ^f
Acetone: Water, 50:50	7.657 ^c	6.228 ^b	566.32 ^c
Methanol 100%	6.824 ^d	6.060 ^c	577.19 ^c
Methanol: Water, 70: 30	9.501 ^a	6.707 ^a	702.37 ^a
Chloroform 100%	6.085 ^e	5.126 ^d	526.24 ^d
LSD at 0.05 level	0.271	0.234	12.49

Mean values followed by different letters in the column are significantly different ($P \leq 0.05$).

The content of total phenolic compounds obtained with pure solvents (acetone, ethanol, chloroform and methanol 100%) were significantly smaller ($P \leq 0.05$) values revealed 4.629, 5.804, 6.085 and 6.824 mg gallic acid/g dry matter, respectively.

These values were significantly increased with added water to the solvents. The increment ratio was 65.41, 54.19 and 39.23% for acetone, ethanol and methanol when 50, 50 and 30% of water were added, respectively.

Similar trend was also recorded for total flavonoids with corresponding increment ratios of 81.42, 52.73 and 10.68%. This could be explained by increasing polarity of these solvents for extracting more polar compounds in grape seeds (**Vayupharp and Laksanalamai, 2012**). The highest total phenolic compounds and total flavonoids was recorded in methanol 50% extract followed by ethanol 50% extract as well as acetone 50% extract with significant differences between them ($P \leq 0.05$). The lowest total phenolic compounds and total flavonoids were observed for grape seed extracted with 100% water. These results indicated that the methanol 70% or ethanol 50% are the best solvents to extract responsible compounds for the antioxidant effect of grape seed. Similar results were reported by **Nageb (2015)** who revealed that methanol and ethanol were better than that of acetone and ethyl acetate for tested plant wastes at extraction of phenolic compounds owing to their higher polarity and good solubility of phenolic components from plant materials. Also, many studies showed that methanol was the best extraction solvent to obtain phenolic compounds from different plant materials (**Yu et al 2006, Makris et al 2007, Zulkifli et al 2012 and Mohammedelnour et al 2017**).

The same **Table (3)**, the antioxidant activity of grape seed extracts was significantly ($P \leq 0.05$) affected by solvent type. DPPH radical scavenging activity of different grape seed extracts was ranged from 198.62 to 702.37 $\mu\text{MolTroloxeq/gm}$ dry matter. This range was comparable with the value reported by **Rockenbach et al (2011)** for grape pomace acidified methanol extract. However, much lower value was reported by **Selcuk et al (2011)** for grape seed. The difference might be due to the interspecies variation and/or the used extraction solvents as well as the method of antioxidant activity determination.

The antioxidant activity of grape seeds extracted with pure solvents (acetone, ethanol, chloroform and methanol) were significantly low ($P \leq 0.05$). Values revealed 449.82, 504.652, 526.24 and 577.19 $\mu\text{MolTroloxeq/gm}$ dry matter, respectively. These values were significantly increased by adding water. The increment ratio was 25.90, 26.13 and 21.69 for acetone, ethanol and methanol when 50, 50 and 30% of water were added, respectively,

i.e. grape seed methanol (70%) extract and grape seed ethanol (50%) extract were significantly higher ($P \leq 0.05$) antioxidant activity rather than other grape seed extracts. This effect might be attributed to the solvent polarity index. These results indicate that the abovementioned extracts are the best solvents to extract responsible compounds for the antioxidant effect of grape seed (**Mohammedelnour et al 2017**). The higher antioxidant activity of grape seed methanol (50%) extract and grape seed ethanol (70%) extract might be due to its higher total phenolic and total flavonoids. The correlation between antioxidant activity and total phenolic compounds (TPC) has been widely studied in different fruits and vegetables by using different solvents (**Bartolomé et al 2004, Kedage et al 2007, Jayaprakasha et al 2008 and Radovanovic et al 2009**). They found a significant correlation between total phenolic compounds and DPPH radical-scavenging activity of the samples extracted with methanol, while there was excellent correlation between TPC and DPPH radical scavenging of the same sample extracted with ethanol. The lowest antioxidant activity (198.62 $\mu\text{MolTroloxeq/gm}$, dm) was recorded for grape seeds extracted with water.

2. Identification and quantification of individual phenolic compounds of grape seed extracts by HPLC technique

Twenty one phenolic compounds were identified in each grape seed extract (**Table 4**), but the amount of these compounds were varied according to the type of solvent. Grape seed water extract had the lowest quantity of these compounds. These results agree with that of total phenolic compounds given in **Table (3)**. **Vayupharp and Laksanalamai (2012)** confirmed that water was considered not an appropriate solvent for extraction comparing with pure acetone or ethanol. Pyrogallol, benzoic, Salycillic, ellagic, catechin, vanillic and P-OH-benzoic were the most abundant phenolic compounds in grape seed water extract which represented 82.18% of the total phenolic compounds. Pyrogallol (30.98% of the total phenolic compound) was the highest phenolic compound in water extract. However, P-OH-benzoic (5.91% of the total phenolic compound) was the lowest one among the most abundant phenolic compounds grape seed water extract.

Gallic, protocatechic, chlorogenic, catechol, caffeine, caffeic, P-coumaric and 3,4,5-methoxycinnamic were presented in moderate amount in

Table 4. Identification of phenolic compounds (ppm) of grape seeds as affected by solvent type.

Phenolic compounds	Water Extract	Ethanol 100%	Ethanol 50%	Acetone 100%	Acetone 50%	Methanol 100%	Methanol 70%	Chloroform 100%
Gallic	5.54	353.77	793.06	229.63	1013.41	852.76	159.41	280.52
Pyrogallol	91.7	411.08	1925.3	387.6	1720.99	1311.12	1633.95	736.51
4-Aminobenzoic	1.99	13.42	30.68	24.44	72.68	170.63	82.11	31.66
Protocatechoic	5.95	21.00	101.43	26.42	482.46	250.09	720.05	64.54
Chlorogenic	8.29	24.91	380.15	13.77	92.4	362.54	378.27	120.81
Catechein	21.14	57.03	582.73	145.05	759.96	567.22	826.58	234.7
Catechol	5.72	46.57	85.269	42.1	141.84	169.4	232.74	38.17
Caffeine	4.017	32.2	327.00	69.69	345.07	429.73	543.65	98.9
P-OH-benzoic	17.48	128.91	1780.31	431.51	2006.69	1475.89	2100.33	662.63
Caffeic	4.32	21.35	51.49	7.76	38.39	53.14	206.65	15.32
Vanillic	18.27	116.00	338.4	46.22	184.36	338.45	475.34	137.92
P-Coumaric	3.82	14.89	68.67	3.53	62.75	48.57	38.317	10.0
Ferulic	1.8	14.88	40.211	9.88	65.44	22.88	46.69	18.31
Iso-Ferulic	2.05	16.16	35.73	1.13	34.95	34.45	39.82	7.67
Ellagic	24.4	129.2	430.12	34.62	235.49	54.93	608.48	61.83
Alpha-coumaric	0.379	1.51	4.76	0.59	6.26	5.46	3.94	2.57
Benzoic	36.2	185.06	802.65	38.97	336.55	431.14	598.68	118.2
Salysillic	34.01	201.21	327.94	31.2	368.13	321.51	468.75	145.51
3,4,5- methoxycinnamic	6.72	29.7	66.01	3.87	68.09	47.56	98.9	24.93
Coumarin	1.19	8.54	24.1	4.23	46.17	28.07	31.32	12.85
Cinnamic	0.95	1.73	3.86	0.66	9.75	6.61	7.27	1.43
Total PC	295.94	1829.12	8199.87	1552.87	8091.83	6982.15	9301.25	2824.98

grape seed water extract represented 15% of the total phenolic compounds Iso-ferulic (2.05 ppm), 4-aminobenzoic (1.99 ppm), ferulic (1.8 ppm), coumarin (1.19 ppm), alpha-coumaric (0.379 ppm) and cinnamic (0.95 ppm) were presented in trace amount in grape seed water extract consisting 2.8% of the total phenolic compounds.

Regarding to pure solvents which were used in extraction of phenolic compounds from grape seeds, it could be noticed that grape seed methanol 100% extract had higher amount of phenolic compounds (6982.15 ppm) than those extracted with chloroform 100% (2824.98 ppm), ethanol 100% (1829.12 ppm) and acetone 100% (1552.87 ppm). This effect may be attributed to the solvent polarity index. In this concern **Pinelo et al (2005)** reported that methanol solvent expressed the highest selectivity towards phenolic compounds compared with 96% ethanol and water extracts. P-OH-benzoic, pyrogallol, gallic, catechein, benzoic, caffeine, chlorogenic, vanillic, salicylic, protocatechoic, 4-aminobenzoic and catechol were the most abundant phenolic compounds in grape seed methanol 100% extract with 95.68% of the total phenolic compounds. P-OH-benzoic (21.14% of

the total phenolic compound) was the highest phenolic compound in methanol 100% extract. However, catechol (2.43% of the total phenolic compound) was the lowest one among the most abundant phenolic compounds in grape seed methanol 100% extract. Alpha-coumaric (5.46 ppm) and cinnamic (6.61 ppm) were present in trace amount in grape seed methanol 100% extract.

On the other hand, pyrogallol, P-OH-benzoic, gallic, catechein, salicylic, chlorogenic and benzoic were the most abundant phenolic compounds in grape seed chloroform 100% extract represented 81.38% of the total phenolic compounds. Pyrogallol (26.07% of the total phenolic compound) was the highest phenolic compound in chloroform 100% extract. However, benzoic (4.18% of the total phenolic compound) was the lowest one among the most abundant phenolic compounds in grape seed chloroform 100% extract. Also, alpha-coumaric (2.57 ppm) and cinnamic (1.43 ppm) were presented in trace amount in the same extract. The corresponding abundant phenolic compounds in case of ethanol 100% extract are pyrogallol, gallic, salicylic, benzoic, ellagic and P-OH-benzoic with 77.04% of the total phenolic com-

pounds. Pyrogallol (22.47% of the total phenolic compound) was the highest phenolic compound in ethanol 100% extract. However, P-OH-benzoic (7.05% of the total phenolic compound) was the lowest one among the most abundant phenolic compounds in ethanol 100% extract.

Only four major phenolic compounds, i.e. P-OH-benzoic, pyrogallol, gallic and catechin were detected with 76.88% out of total ones in acetone 100% extract. Another eight of phenolic compounds were detected with moderate level ranged between 24.44 and 69.69 ppm. Meanwhile, the nine lowest phenolic compounds level contained in acetone 100% extract was ranged between 0.66 and 13.77 ppm.

Also, addition of water to pure solvent, i.e. methanol, ethanol and acetone (30, 50 and 50%, respectively) led to improve the amount of phenolic compounds extracted from grape seeds. **Librán et al (2013)** reported that highly pure organic solvents (100%) could dehydrate the vegetable cells, making difficult the diffusion of polyphenols from the plant material to the extracting liquid. **Yilmaz and Toledo (2004)** concluded that aqueous solutions of ethanol, methanol were better than a pure compound solvent system for the extraction of phenolic compounds from Muscadine seed. Grape seed methanol 70% extract had higher phenolic compounds (9301.25 ppm) followed by grape seed ethanol 50% extract (8199.87ppm). This effect might be attributed to the solvent polarity index. These results indicate methanol 70% and ethanol

50% the best solvents to extract for extracting phenolic compounds from grape seeds. **Nageb (2015)** reported similar results; i.e. methanol 80% and ethanol 70% were more effective than acetone 80% and ethyl acetate for extracting phenolic compounds from grape pomace. The predominant phenolic compounds that recorded more than 450 ppm for each (nine phenolic compounds) in methanol 70% extract were 85.75% out of total ones.

The corresponding percent of nine predominant phenolic compounds (more than 325 ppm) in ethanol 50% extract was 89.77%. Meanwhile, 89.83% was recorded as major nine phenolic compounds (more than 235 ppm) in acetone 50% extract.

3. Identification and quantification of individual flavonoid compounds of grape seed extracts by HPLC technique

Flavonoid compounds of grape seed extracts as affected by type of solvent were presented in **Table (5)**. Nine flavonoid compounds were identified in each grape seed extract, but the amount was varied according to the type of solvent. Grape seed water extract had the lowest quantity of compounds (128.19 ppm) when compared with other grape seed extracts. Hesperidine was the major flavonoid compound in all extracts (68.9 to 1456.6 ppm) followed by naringin (25.35 to 867.69 ppm), rutin (23.43 to 406.20 ppm) and quercetrin (3.33 to 449.65 ppm). Methanol 100% extract had higher flavonoid compounds than other 100% solvents.

Table 5. Identification of flavonoid compounds (ppm) of grape seeds as affected by solvent type.

Flavonoid compounds	Water Extract	Ethanol 100%	Ethanol 50%	Acetone 100%	Acetone 50%	Methanol 100%	Methanol 70%	Chloroform 100%
Naringin	25.35	261.84	684.85	77.06	651.93	569.4	867.69	191.86
Rutin	23.43	94.51	275.87	39.91	406.2	286.31	381.47	90.28
Hesperidine	68.9	455.73	909.57	124.22	916.19	629.59	1456.6	493.11
Quercetrin	3.33	52.76	289.68	9.09	449.65	222.46	308.7	82.56
Quercetin	0.61	9.73	27.5	2.00	49.59	26.54	76.95	8.22
Naringenin	0.38	1.98	8.85	0.44	9.42	7.20	4.28	1.23
Hespiritin	1.96	5.82	4.23	2.35	32.76	35.9	56.17	15.34
Kampferol	2.16	9.55	31.82	4.04	18.22	28.28	46.57	12.05
Apigenin	2.07	7.57	19.74	2.53	21.51	19.17	31.55	10.17
Total FC	128.19	899.49	2252.11	261.64	2555.47	1824.85	3229.98	904.82

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تقييم بذور العنب كمصدر للمركبات المضادة للأكسدة

[46]

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الموجز

معنويًا بالخلط بالماء بنسب مختلفة وتأثرت كذلك قيم كلا من الفينولات الكلية والفلافونيدات الكلية والنشاط المضاد للأكسدة لمستخلصات بذور العنب طبقاً لنوع المذيب المستخدم وسجلت أعلى قيم للفينولات الكلية وكذلك الفلافونيدات الكلية في العينة المستخلصة بالميثانول 70%، في حين كان أقل محتوى من هذه المركبات هو المتواجد في العينة المستخلصة بالماء المقطر فقط.

الكلمات الدالة: مستخلص بذور العنب، المركبات المضاد للأكسدة، تقنية HPLC، اختبار DPPH، النشاط المضاد للأكسدة، الإستخلاص

تم إستخلاص بذور العنب بواسطة مذيبات عضوية مختلفة (ميثانول، إيثانول، أسيتون، كلوروفورم) سواء على الصورة النقية (100%) أو بالخلط مع 30، 50، 50% ماء مقطر (فيما عدا الكلوروفورم) وتم تقييم محتوى البذور من المركبات المضادة للأكسدة مثل الفينولات والفلافونيدات (بواسطة تقنية HPLC) وكذلك النشاط المضاد للأكسدة لهذه المستخلصات (بواسطة اختبار DPPH).

وتراوحت الكمية المتحصل عليها بين 6-10% طبقاً لنوع المذيب المستخدم وازداد حاصل الاستخلاص