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GUAVA SEED (PSIDIUM GUAJAVA) AS A POTENTIAL SOURCE OF BIOACTIVE COMPOUNDS

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ABSTRACT

Fatty acids profile of extracted lipids (9.8 %) from dry guava seed (Psidium guajava) powder showed its high content of C:18 fatty acids (78.7 %), the most common saturated and unsaturated fatty acids. The lipids content are relatively high in unsaturated fatty acids (about 65.8%). Antioxidative efficiency of Guava seed powder (GSP) extracts have been studied by applying nine different solvents varying in their polarity. The concentration of total extractable phenolics in dry GSP using different solvent ranged from 834.83 mg 100 g⁻¹ (water extract of defatted GSP) to 1.10 mg 100 g⁻¹ (chloroform-diethyl ether extract of defatted GSP). Water extracts had the highest concentrations of total extractable phenolic compounds with significant different (P<0.05) compared to other solvents, followed by methanol extracts. The response surface method (contour plot) was used to study the radical scavenging activity of different extracts. Out put data showed that, the effectiveness of water and methanolic extracts at time ranging between 15.8 to 9.2 min with decreasing rate ranging between 46 to 38 %, respectively. An antioxidant activity of water and methanol extracts from GSP and BHT (at concentration 0.02 %) in linoleic acid emulsion system was determined at different temperatures. The results indicated that, there was no significant difference (P>0.05) observed between antioxidant activity values in emulsions prepared using water phenolic extracts

(extracted at 25 °C) compared to BHT at all temperatures. Antioxidant efficiency with different concentrations of extracted phenolic compounds either by water or methanol was measured by monitoring the stability of corn germ oil using the Rancimat induction period (RIP). The concentration of 1500 ppm was the minimal concentration gave RIP value similar to BHT.

INTRODUCTION

Guava (*Psidium guajava*) is an exotic tropical fruit belonging to the Myrtaceae family, very pulpy fruit is often eaten fresh; consumers thus benefit from its high nutritional value based on its vitamins content and soluble-sugar characteristics. It is very suitable for processing into jams, jellies and fruit pastes (**Bourgeois** *et al* 1998).

Guava grown in many areas of Egypt. The total production of guava at year 2004 reached 375000.46 ton (FAO, 2006). The tiny bean-like seeds disseminated throughout guava pulp have so far been considered as waste (Bourgeois *et al* 1998). In terms of weight, guava seeds represent ranging between 15 to 20 % of the fresh fruit.

The fruit have an oily kernel. Oil content in guava seed ranging between 9.1 to 12.4 % (Habib, 1986; Piombo et al 2006). Oil extracted from guava kernels has relatively high unsaturated fatty acids content (~88%), with omega 6 linoleic acid predominating (77-80%). Saturated and unsaturated fatty acids in C:18 are the most common. Other fatty acids are scarce, except for palmitic acid (Bourgeois et al 1998 and Piombo et al

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2006). It's understandable that a big portion of seeds will be difficult to collect because of the direct consumption of fresh fruit by consumers; meanwhile the storage ability of such tender fruit is very poor. Therefore, a large quantity of the fruit is usually oriented to food processing factories reached to 200000 tons annually according to the data, which collected from the chamber of food industries. It could be calculated the amount of guava seeds remainder after processing which is (30000-40000 tons). Therefore, utilization of seeds in industry will help in eliminating or reducing the pollution either inside the factory or in the surrounding area with a possibility of reducing the total production costs. However, in the literature, there are no data about the bioactive effects of phenolic compounds in guava seeds.

There have been increasing efforts in recent years to develop effective natural antioxidants for edible oils in order to retard lipid oxidation, which may lead to off-flavors, the formation of toxic products and the reduction of nutritional quality. Lipid oxidation is a highly deterioration process in foods, as it lead to unacceptable properties for the customer and a loss in nutritional value. In addition, oxidation leads to health disorders such as atherosclerosis and carcinogenesis among others. Hence the presence of antioxidants in foods is essential for their quality, retention and safety (Koleva et al 2003). Oxidative stress can lead to cell injury and death. Therefore, much attention has been focused on the use of antioxidants, especially natural antioxidants, to inhibit lipid peroxidation, or to protect against the damage of free radicals (Vendemiale et al 1999). Current research into free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancers (Kris-Etherton et al 2002) and neurodegenerative diseases (Di Matteo and Esposito, 2003). Toxicological effects of synthetic antioxidants and consumer preference for natural products have resulted in increased interest in the application of natural antioxidants (Castenmiller et al 2002).

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Attmann et al 1986). Thus, due to health protection and economic reasons, many investigations have been undertaken with the aim to enhance the stability of

scid (Bourgeois et al 1998 and Piombo # al

lipids and lipid-containing products (Tomaino et al 2005).

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Zheng and Wang, 2001). Phenolics are able to act as antioxidants in different ways. Hydroxyl phenols are good metal ion chelators. The implication of this is that metal-catalyzed non-enzymatic free radical generation is thus suppressed in the presence of suitable phenolics. Also, phenolic structures often have the potential to interact strongly with protein, mediated both by their hydrophobic benzenoid rings and the hydrogen bonding potential of the phenolic hydroxyl groups (Aruoma et al 1996). Phenolic may also inhibit oxidation by chelating divalent metal ions and thus reducing the formation of free radicals (Robards et al 1999).

Thus the objective of this study was to determine the extractable guava seed oil and identified its fatty acids profile; determine the total phenolic content in guava seeds powder using different solvents. Consequently, improve the oxidation stability of corn germ oil using different concentrations of extracted phenolics. In addition, investigate the radical scavenging activity and antioxidant activity of extracted phenolic compounds using either linoleic acid emulsion system or rancimate instrument.

MATERIALS AND METHODS

Materials

Guava seed (*Psidium guajava*) was donated by Cairo Agricultural Industry Company, Obour City, Egypt. Butylated hydroxytoluene (BHT) was obtained from Sigma- Aldrich Chemical Co. (St. Louis, MO, USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and linoleic acid were obtained from Fluka Chemical Co. (Buchs, Switzerland). Corn germ oil (refined, bleached and deodorized) was donated by Arma Food Industry Company 10th of Ramadan City, Egypt.

Methods

Preparation of guava seeds

The guava seeds were washed by water and dried at 40 $^{\circ}$ C for 12 h. Thereafter, the seeds were grinding, sieving and packed in 100 g size glass jars with screw cap and stored at 5 $^{\circ}$ C.

Determination of Lipid content

Lipid content of guava seed powder was determined using Soxhlet apparatus for 16 h according to the AOAC (2000).

Fatty acid profile

Fatty acid profile of guava seed oil was identified and determined using Agilent 6890 GC according to the method described by **AOAC** (2000).

Extraction of phenolic compounds

Phenolic compounds of GSP were extracted using five different solvents as reported by Azizah et al (1999). A 25 g of ground sample was weighed into 500 ml conical flask and extracted with 250 ml methanol or petroleum ether over night, then stirring for one hour. After air-drying for 3 h, the residues of petroleum ether extract were re-extracted with the solvent combinations as shown in organization Chart (1):

The solvent was then removed using a rotary evaporator.

The guava seed powder aqueous extracts were prepared according to **Rodriguez de Sotillo** *et al* (1994a) as shown in organization Chart (2). A 10 g defatted or non defatted ground sample was weighed into 250 ml conical flask and extracted with 100 ml solvent over night, then stirring for one hour at 25 or 100°C. Consequently, the extracts were drying under vacuum. Al' extracts were kept in plastic vials and stored at room temperature ($20^{\circ}C \pm 2$).

Total phenolics

Total phenolics were analyzed spectrophotometrically using the modified Folin-Ciocalteau colorimetric method (Eberhardt *et al* 2000). A 125 μ l of the extract was mixed with 0.5 ml of distilled water in a test tube; followed by addition of 125 μ l of Folin–Ciocalteau reagent and allowed to stand for 6 min. Then, 1.25 ml of 7% sodium carbonate solution was added and the final volume was made up to 3 ml with distilled water. Each sample was allowed to stand for 90 min at room temperature and the absorbance was measured at 760 nm using spectrophotometer Shimadzu UN-1201 (Shimadzu Co., Ltd., Kyoto, Japan). The total phenolic content was expressed as milligrams gallic acid/100 g GSP equivalents by reference to the gallic acid standard calibration curve.

Determination of antioxidant activity

Antioxidant activity was determined using a diene conjugated formation method according to Lingnert *et al* (1979). The substrate contained 2.86 g of linoleic acid emulsified with an equal amount of Tween 20 in phosphate buffer, pH 7.0 (0.1mol/l). The mixture was then homogenized at high speed for one min with 20 mg of different extracted phenolics. Then, the emulsions were incubated at 30, 40, 50, 70 and 90 °C for 20 h. Absorbance was then measured at 234 nm, using spectrophotometer Shimadzu UN-1201 (Shimadzu Co., Ltd., Kyoto, Japan).

Radical scavenging ability

The radical-scavenging ability of the extracts was tested by the method of **Paiva-Martins and Gordon (2001)**. 10, 30 and 50 μ l of extracts or 50, 100 and 150 μ l methanolic BHT solutions (0.02 %) were added to 1 ml of methanolic DPPH solution (0.128 g/l methanol). The decrease in absorbance was determined at 515 nm after 0.5, 6, 12,



18, 24 and 30 min using spectrophotometer Shimadzu UN-1201 (Shimadzu Co., Ltd., Kyoto, Japan). The scavenged percent of DPPH in the reaction was calculated as a rate of decrease in absorbance.

Rancimat test

Stability of corn germ oil to oxidation using different concentrations of extracted phenolic compounds from GSP (500, 1000, 1500, 2000, 2500 and 3000 ppm) and BHT (200 ppm) was determined according to the method described by **Tsakins** *et al* (1999). The test was performed in five grams of oil on a Rancimat apparatus 679 (Metrohm, Herisau, Switzerland) by measuring the induction period at 100 °C and an airflow rate of 20 l/h. Determination of the induction period was based on the conductometric detection. The determination continued automatically until conductivity reached its maximum value and the induction period was recorded.

Statistical analysis

The obtained data was exposed to analysis of variance. Duncan multiple range at 5 % level of significance was used to compare between means. Results followed by different alphabetical letters were significantly differed. The analysis was carried out using the PROC ANOVA procedure of Statistical Analysis System (SAS, 1996).

Response surface method (contour plot) was used to study the radical scavenging activity as dependent variable with extract volume, and time as independent variables. The response surface method was applied using Harvard ChartXl software version 2.0 to locate the optimum conditions for reaction and identify the best extract as a radical scavenger.

RESULTS AND DISCUSSION

Fatty acids profile

Lipid content of GSP was determined and it was found to be 9.8 %. The extracted guava seed lipid was analyzed for their fatty acids composition. In regard to **Figure (1)**, the major fatty acid of the triacylglycerols was linoleic (34.5 %) followed by oleic (31.3 %) and palmitic (21.3 %). However minor amount, ~12.9 % was detected for stearic acid. Extracted lipid has relatively high content of unsaturated fatty acid (~65.8 %). Saturated and unsaturated fatty acids in C:18 are the most common (~78.7 %).

Total phenolics content

The concentration of total extractable phenolics in dry guava seed powder by using different solvent and temperature ranged from 834.83 mg 100g⁻¹ (P, water extract at 25 °C of defatted GSP) to 1.10 mg 100g⁻¹ (PCD extract of defatted guava seed powder). For the other solvent the total extractable phenolic concentrations fell within this range (Table 1). The water extracts had higher concentration of total phenolic compounds with significant different (P<0.05) compared to other used solvents. The concentration of total phenolic compounds in methanol extracts become in the second order with significant difference (P<0.05) in compared to the phenolic concentration in water extract. Comparatively with other solvent the small phenolic contents were extracted with nonpolar solvent (petroleum ether, diethyl ether and chloroform). Generally, it could be observed that the higher extraction yields of phenolic compounds were obtained with an increase in polarity of the solvent. This finding was agreement with Goli, et al (2005) which reported that the water extracts were found to have high phenolic contents, so the best solvent for extraction of phenolic compounds were water or methanol. The extraction method by water gave the greatest amounts of phenolic compounds (Rodriguez de Sotillo et al 1994b). Yen et al (1996) mentioned that methanol is a widely used and effective colvent for extraction of antioxidant.

Table 1. Total extractable phenolics (mg 100 g⁻¹) in dry guava seed powder using different solvents and temperatures

Extraction solvent	Tota! phenolic [*]		
Water at 100 °C	610.75 °		
Water at 25 °C	829.85 ^a		
P-Water at 100 °C	818.75 ^b		
P-Water at 25 °C	834.83 ^a		
Р	3.25 ^g		
PD	40.45 ^f		
PCD	1.10 ⁸		
М	429.60 ^e		
PM	461.00 ^d		

*, Data expressed as milligrams of gallic acid equivalents per 100 gram seed.

P, petroleum ether; D, diethyl ether; C, chloroform; M, methanol

Means with the same letter are not significantly different (P>0.05).

Guava seed as a source of bioactive compounds



Fig. 1. Fatty acids profile of extracted lipid from guava seed powder

Radical scavenging activity

Response surface study of radical scavenging activity of total phenolic compounds, which extracted using water at different concentrations and times, was performed. The contour plot of this study was show in Figure (2). The extracts were able to scavenge the synthetic nitrogen-centered free radical, DPPH to varying degrees as can be seen from the plot. Radical scavenging activity increased with increasing the volume of extract from 10 to 50 or 50 to 150 µl in water extracts or BHT solution, respectively. BHT demonstrated significantly the highest activity as a radical scavenger on a short holding period for 6 min reached to 40 % compared to water extracts which reached to 36 %. Oppositely, radical scavenging activity of water extracts was raised to the maximal performance after 30 min followed by BHT. This variation was primarily due to differences in potency rather than concentration of total extractable phenolics (Martinez-Valverde et al 2002). The change in radical scavenging activity with time was due to the kinetic behavior of reaction (Gordon et al 2001).

The effect of blanching time on DPPH free radical scavenging activity of the extracted phenolic by M, PM, P, PCD, PD from powder guava seed and BHT was presented in Figure (3). Among the studied extracts, methanolic extracts exhibited the greatest scavenging activity of DPPH free radical. The hydrophilic extracts (P, PCD and PD) showed a weak effect on scavenging of DPPH. Radical scavenging activities of PD, PE and PCD reached to the maximal values of 12, 1.1 and 0.9 %, respectively after blanching for 30 min, except for BHT reached to 30 % at the same blanching time. This result agreed with the data that presented in **Table (1)**, whereas the total extractable phenolics using the non-polar solvent were very low.

Table (2) shows the optimum values of radical scavenging at reaction times; these data were extracted from the response surface study by contour plot of extracted volumes, times and radical scavenging activity. It can be seen that, the water extracts were effective in radical scavenging at time ranging between 27.5 to 15.8 min with decreasing rate ranging between 44 to 46 %. While the methanolic extracts were effective in radical scavenging at 9.2 and 10.5 min for petroleum ethermethanol extract and methanol extracts with values of 38 %, respectively. The P, PCD, PD extracts showed low efficiency in quenching of DPPH free radical. The decrease in efficiency probably due to the lowest phenolic compounds that extracted by its non-polar solvents. Comparable results were observed previously by Martinez-Valverde et al (2002). They reported that, the observed differences in radical scavenging activity are due to the extraction solvents used. The aqueous ethanol used in the DPPH assay would tend to favor the extraction of low quantities of phenolic compounds. The radical scavenging efficiency of various tested extracts was not basically dependent on their concentration alone; but dependent on qualitative/quantitative phenolic compounds. This observation was agreed with Tomaino et al (2005). Sun et al (2004) also reported that freeradical scavenging effects related to its affinity to the radical in the specie site. Wang et al (1996) reported that phenolic compounds from the plant





Fig. 2. Contour plot of radical scavenging (%) of aqueous extracts at different times and volumes

Pedicularis alashanica, such as phenylpropaniod glycosides, may react with superoxide radical by a one-electron transfer mechanism or hydrogen abstraction mechanism to form the semiquinones. Therefore, the scavenging activity of phenylpropaniod glycosides for superoxide radical may be due to their reduction activities, which may be related to the number of phenolic hydroxyl groups and the conjugated system.

Antioxidant activity

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Antioxidant activities of total extractable phenolics using water and methanol from guava seed powder and BHT at concentration 0.02 % in linoleic acid system incubated at 30, 40, 50, 70 and 90° C for 20 h are presented in **Table (3)**. There was an increment in the acceleration of oxidation in linoleic emulsion system with the increment of incubation temperature. The extracts were stable up to 40° C, after which the antioxidant activities

started decrease significantly for all tested systems. The differences of antioxidant activity values were negligible without significant difference (P>0.05) in emulsions containing water extracts

No significant difference (P-0.05) was observed between antioxidant activity values in emulsions prepared using the water phenolic extracts (extracted at 25 °C) compared to BHT at all temperatures. Conversely, methanolic extracts appeared to have significantly (P-0.05) higher values with a lower-antioxidant activity compared to other tested extracts. It may be due to the high content of phenolics which was extracted by water. In addition the bioavailability of this extracts was increase with extraction in cold condition Goli et al (2005) reported that water extracts were found to have high phenolic contents. It could be noticed that phenolic which extracted by cold water is a good emulsion stabilizer, besides its higher activity against oil autoxidation in food emulsions. Extracts incubating at high temperature resulted in Guava seed as a source of bioactive compounds



Fig. 3. Contour plot of radical scavenging (%) of solvent extracts at different times and volumes

Table	2.	Optimu	m value	s of	time	and	radical	scav-
		enging	decreas	sing	rate	of a	different	phe-
		nolic ex	tracts					

Extraction solvent	Time (min)	Rate of decrease (%)		
BHT	5.2	20		
Water at 100 °C	15.8	44		
Water at 25 °C	19.5	46		
P-Water at 100 °C	14.8	44		
P-Water at 25 °C	27.5	45		
P	30.0	I. Farmer		
PD	30.0	12		
PCD	30.0	0.9		
М	10.5	38		
PM M	9.2	38		

BHT, Butylated hydroxytoluene; P, petroleum; D, diethyl ether; C, chloroform; M, methanol

a significant decrease in antioxidant activity. Heat processing may have resulted in degredation of antioxidants present (Gazzani *et al* 1998; Castenmiller *et al* 2002).

Rancimat test

Rancimat test is a test known to determine the oxidative stability by considering the Rancimat induction period (RIP). The RIP values represent the stability of the corn germ oil with different extracts. The greater the RIP value, the sample is less susceptible towards oxidation. RIP was determined using total phenolics of water and methanolic extracts with ignored other extracts, which appear low phenolic content. The concentrations used were (500, 1000, 1500, 2000, 2500 and 3000 ppm) and BHT as a synthetic antioxidant at 200 ppm.

Total phenolic extracts of water or methanol retarded the oxidation of corn germ oil and increased the induction time **Figure (4)**. Longer

Table 3.	Effect of incubation temperatures on antioxidant activity of aqueous ex-
	tracts and methanolic extracts in linoleic acid emulsion system at pH 7
	and concentration of 20 mg 100 g ⁻¹ emulsion

Extraction solvent	incubation temperature (°C)					
Extraction solvent	30	40	50	70	90	
ВНТ	0.186 ^d	0.228 ^{cd}	0.356ª	0.360 ^a	0.440 ^b	
Water 100 °C	0.220 ^{bc}	0.252 ^{bcd}	0.342 ^b	0.352 ^{ab}	0.452 ^{ab}	
Water 25 °C	0.210 ^{cd}	0.223 ^d	0.335 ^b	0.338 ^{bc}	0.442 ^b	
P-Water 100 °C	0.241^{ab}	0.259 ^{bc}	0.329 ^b	0.352 ^{ab}	0.451 ^{ab}	
P-Water 25 °C	0.223 ^{abc}	0.219 ^d	0.297 ^c	0.325°	0.440 ^b	
Μ	0.242 ^{ab}	0.285 ^{ab}	0.367 ^a	0.369 ^a	0.465ª	
РМ	0.250 ^a	0.308 ^a	0.358ª	0.362 ^a	0.469ª	

BHI. Butylated hydroxytoluene; P, petroleum ether; M, methanol.

Means in the same incubation temperature have the same letter are not significantly different ($P \ge 0.05$)



Fig. 4. Rancimat induction periods at different concentrations of extracted phenolics from guava seed powder using different solvents

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induction time indicated higher antioxidant activity. RIP of the different extracts decreased in the following order: cold water>P-cold water>hot water>BHT>PM>M>P-hot water at most concentrations. P-cold water and cold water extracts at 3000 ppm increased the induction time by 21.4 and 12.3%, respectively compared to BHT. While, 1500 ppm was the minimal concentrations gave RIP values similar to BHT.

The other total phenolics which were extracted using other solvent appeared low RIP values compared to the phenolics were extracted by cold water. The total phenol contents in cold-water extracts found in this study ranging from 834.83 to 829.85 mg 100 g⁻¹ could be partially attributed to the high oxidative stability of the corn oil. The antioxidant activity of phenolic extract was affected by the extraction solvent (Moure et al 2000). This finding was agreed with Sun and Ho, (2005) who reported that natural antioxidants may have the potential to prevent the oxidation of lipid food. The antioxidant activity of buckwheat extract showed effective antioxidant activity when compared to artificial antioxidants. Using the Rancimat method, the induction time for methanol extract and BHT were 7.0 and 10.8 h, respectively.

Finally it be concluded that, guava seed have ~9.8 % fat rich in linoleic and oleic acids (34.5 and 31.3 %, respectively). Water and methanolic extracts were a good retard the autoxidation of linoleic acid in emulsion system. Also, the studying response surface of their radical scavenging activity appeared the efficiency of its extracts against DPPH radical solution. RIP values of cold water and P-cold water extracts at 3000 ppm increased the time to 21.4 and 12.3 %, respectively compared to BHT.

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بذور الجوافه كمصدر هام للمركبات الحيويه

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والكحولية كمصنادات أكسدة مقاربة بالبيوتيل هيدروكسى تولوين عند تركيز ٠,٠٢ % وذلك فـــى مستحلب حامض اللينوليك عند درجات حرارة مختلفة، أوضحت النتائج عدم وجود فرق معنوى بين المستخلصات الفينولية المائية (المستخلصة عند ۲۰م) والبيوتيل هيدروكسي تولـوين عنــد جميــع درجات الحرارة. وقد تم استخدام الـــــ contour plot لدراسة كفاءة المستخلصات المختلفة في اصطياد الشقوق الحرة. ودلت النتائج المتحصل عليها على كفاءة المستخلصات المائية وكذلك الكحولية على زمن يتراوح بين ١٥,٨ – ٩,٢ دقيقة مع معدل انخفاض من ٤٦ – ٣٨ % على التوالي. كما أوضحت دراسة فترة الثبات لزيت جنين الذرة على جهاز الرنـسيمات في وجود الفينولات المستخلصة بالماء وكحول الميثانول٬أن اقل تركيز من الفينولات الكلية والـــذى أعطى ثبات مماثل للبيوتيل هيدروكي تولوين هو ١٥٠٠ جزء في المليون.

تحتوى الليبيدات المستخلصة من بــذور الجوافــة على نسبة عالية من الأحماض الدهنية التي تحتوى على ١٨ ذرة كربون (٧٨,٧ %) والتي تعتبر أكشر الأحماض الدهنية المشبعة وغير المشبعة شيوعا، كما أن نسبة الأحماض الدهنية غير المشبعة تـصل الـ ٦٥,٨ % من أجمالي الأحماض الدهنية المكونة لليبيدات المستخلصة. تم در اسة كفاءة مستخلصات بذور الجوافة الجافة والمنزوعة المدهن كمصادات أكسدة باستخدام تسعة نظم استخلاص مختلفة بمذيبات متابينة القطبيه. وقد تراوح تركيز الفينولات الكلية ما بين ٨٣٤,٨٣ ملجم ١٠٠ جـم^{- (} (فـــى المـستخلص المائی) الی ۱٫۱ ملجم ۱۰۰ جم^{۱۰} (فــی مــستخلص الكلوروفورم-داى ايثايل ايئر لمطحون بذور الجوافه منزوع الدهن) وقد لوحظ احتواء المستخلصات المائية على أعلى تركيز من المركبات الفينولية مقارنة بباقئ المذيبات المستخدمة يليها المستخل صبآت المحصرة باستخدام الميثانول. وبدر اسة المستخلصات المائية

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