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Biodiesel Production and Antioxidant Activity of Different Egyptian Date Palm Seed Cultivars

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ABSTRACT
The *Phoenix dactylifera* (date palm) is one of the member of the genus Phoenix, widely cultivated for its edible fruit. Date seeds, considered as waste product, are either discarded or used as fodder for domestic farm animals and potentiality to use as source of antioxidant and biodiesel production. Methanolic extract of four cultivars of date seeds (Haiany, Ramly, Sewy and Amhat) showed pronounced antioxidant activity against 2, 2 diphenyl-1-picrylhydrazyl (DPPH) method ranged between 93.53% (in Haiany) and 88.37% (in Amhat) compared to the natural antioxidant vitamin C (91.45%) and the same trends were observed with 2,2'- azino-bis (ethylbenzthiazoline-6-sulfonic acid ($ABTS^{\cdot+}$). The antioxidant substances (total phenolic compounds and total carotenoids) as well as oil contents (%) were determined in the date seed cultivars under investigation. Transesterification of the date seed oils (ranged 4.48 and 3.31%) and the characteristics of the produced biodiesel were performed as; color, iodine value, acid value, saponification value, higher heating value and cetane number. The date seed oils were analyzed using gas chromatography for their fatty acid composition. Saturated fatty acids ($C_{8:0}$ to $C_{18:0}$ and $C_{20:0}$) represent the highest percentage in Amhat (79.517%) and Haiany (68.135%), while Ramly has the least saturated fatty acid content (20.986%). Oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids represent the only unsaturated fatty acids in date seed oils. The highest percentage was recorded in Ramly cultivar (20.99% of oleic acid+3.65% of linoleic acid) while the other cultivars have very low relative contents (0.093-3.65%). Infrared spectrum of the methyl ester of date seed oil showed the absence of hydroxyl peak which can be correlated to the transesterification process, in addition to the presence of the ester groups at bands, 1745, 1165, 1107 nm. From the results it was clear that, there are strong potential for date seed oil to be used as a source of biodiesel, as well as in pharmaceutical applications as new sources for antioxidant substances (Phenolic and Carotenoids).

Key words: Date palm seeds, antioxidant activity, biodiesel, physico-chemical characteristics

INTRODUCTION
The date (*Phoenix dactylifera*) has always played an important role in the economy and social life of the people of arid and semiarid regions of the world. Egypt is considered to be one of the vital and known date producing countries. The world production of date fruits was about 6.64 million tons in 2007 (FAO, 2007), Egypt, Iran, Kingdom of Saudi Arabia, UAE and Pakistan being the main producing countries, with a production of 1.13 million ton, 1 million, 983.00, 755.000 and 557.000 ton, respectively. The relative percentage weight of date seeds to the date fruits ranges

from 6.10-11.47% (Habib and Ibrahim, 2008). Date seeds are commonly considered as a waste product that is either discarded or used in animal feed. However, date seeds have been shown to possess extractable high value added components (Habib and Ibrahim, 2011). Date seeds, which are low cost agriculture by-products, are a good precursor for production of activated carbon.

The preventive effects of natural antioxidant in fruits and vegetables are associated to four major groups: polyphenols, vitamins, alkaloids and carotenoids (Rezaire *et al.*, 2014). Date has been used as food for 6000 years (Amer, 1994). It could be used for generations to come due to its remarkable nutritional, health and economic value in addition to its aesthetic and environmental benefits (Khanavi *et al.*, 2009). The fruit of the date palm is well known as a staple food which is composed of a fleshy pericarp and seed (Besbes *et al.*, 2004). The date seeds (pits) constitute approximately 10% of the fruits (Alama and Mahmoud, 1994). A date is a high-energy food being high carbohydrates (70-80%) and low fats and proteinsand a good source of phenolic compounds, carotenoids, vitamin, calcium, magnesium, phosphorous, zink, iron, potassium biodiesel is presently the most widely accepted alternative fuel for diesel engines due to its technical, environmental and strategic advantages. Biodiesel is known as a carbon neutral fuel because the carbon present in the exhaust is originally fixed in the atmosphere. Biodiesel can be produced from vegetable oils like soybean oil (*Glycine max*), jatropha oil (*Jatropha curcas*), rapeseed oil (*Brassica napus*), palm oil (*Elaeis guineensis*), sunflower oil (*Helianthus annuus*), corn oil (*Zea mays*), peanut oil (*Arachis hypogaea*) and cottonseed oil (*Gossypium spp.*) or other sources like animal fats (beef tallow and lard), waste cooking oil, greases (trap grease and float grease) and microalgae (Peterson, 1986; Pearl, 2002). Compared to fossil-based diesel fuels, biodiesel possesses many advantages such as cleaner engine emissions, biodegradable, renewable and superior lubricating property (Hu and Meehl, 2005; Liang *et al.*, 2009; Xue *et al.*, 2011). In spite of its many advantages, biodiesel is not yet commercialized all over the world. The major problem is the cost of the raw material. Biodiesel obtained from neat vegetable oil is costly compared to the petroleum diesel fuels. Biodiesel Free on Board (FOB) costs between 0.65 and 1 U.S. dollars (USD L^{-1}). Its costs are nearly 1.5-2 times to petroleum-based diesel depending upon feedstock oils. It is reported that nearly 70-95% of the total cost of biodiesel production arises from the cost of raw material; i.e., vegetable oil or animal fats (Zhang *et al.*, 2003). The cost of biodiesel can be reduced if we use non-edible oils as frying oils, acid oils and agriculture waste oils instead of edible oils. The *Phoenix dactylifera* (date palm) is one of the member of the genus *Phoenix*, widely cultivated for its edible fruit. Dates have been a staple diet in the Middle East for thousands of years. Date seeds, considered as waste product, are either discarded or used as fodder for domestic farm animals.

The aim of the present study is to evaluate the potential antioxidant activity and record the biodiesel production and its physio-chemical characteristics.

MATERIALS AND METHODS

Chemicals and reagents: Pure hexane, chloroform, ethanol, ether, acetone and methanol were purchased from E.Merch Co. (Germany)and distilled before use. DPPH (2,2 diphenyl-1-picryl hydrazyl radical) and ascorbic acid (Vit.C) were from Sigma, Folin-Ciocalteus phenol reagent were from Merck Co. (Germany).

Sample collection: Four different cultivars of date palm fruits were obtained from Date Institute Research, Giza, Egypt, they are; Amhat, Ramly, Sewy and Haiany. The seeds were soaked in water, washed to get rid of any adhering date flesh, then air dried and each cultivar was separately grinded to powder, kept as -20°C till use.

Total carotenoids content: The amounts of carotenoides were deteminated according to Holden (1965) and Krisnangkura (1986) method. The sample (0.5 g) was grinded in a mortar with methanol in presence of calcium carbonate (0.1 g). The residue was re-extracted for several times with acetone, until the solvents were colorless. The combined extract and washings were made up to a known volume (25 mL) and the absorbance was measured at 663, 645 and 452 nm in 1 cm quartz cell, against blank.

Lipid extraction: Twenty grams of each cultivar of powdered date seeds were used for oil extraction three times with 200 mL petroleum ether (40-60), solvent was evaporated using rotary evaporator (40-50 20°C) and the obtained oil was weighted and kept in deep freezer at -20°C for subsequent processes.

Transesterification of date seed oils: Potassium methoxide (23 g methanol/2 g KOH) was added to the extracted oil from each date seed cultivar and vigorous stirring was performed (400 rpm) for 30 min at 30°C. Separation of glycerol layer using separating funnel was followed by addition of another methanol/KOH mixture to the top ester layer using the same pervious conditions (Meda *et al.*, 2005). Mixture was allowed to stand for the separation of biodiesel and glycerol for at least 12 h. The glycerol was removed by gravity settling (lower layer) whereas the ester layer (biodiesel) was transferred to the flask of a rotary evaporator to remove the rest of methanol in the biodiesel at 65°C. Further purification was carried out to remove the remaining catalyst and glycerol, then the biodiesel was weighted and subjected to different analyses.

Physico-chemical properties of produced biodiesel: Saponification Value (SV), Iodine Value (IV), Acidic Value (AV), Cetane Number (CN) and Higher Heating Value (HHV) were calculated from fatty acid methyl ester compositions of oil with the help of equations (FAO, 2007; Habib and Ibrahim, 2008; Holden, 1965).

Cetane Number (CN) and Higher Heat Value (HHV) of fatty acid methyl esters were calculated from equations (Habib and Ibrahim, 2011; Rezaire *et al.*, 2014; Snedecor and Cochran, 1980).

$$CN = \frac{46.3+5458}{SV-0.225} \times IV$$

$$HHV = 49.43 - [0.041\ (SV) + 0.015\ (IV)]$$

Fourier Transforms Infrared Spectroscopy (FTIR) analysis: FTIR analysis was performed using Perkin Elmer model spectrum (Microanalytical Center, Cairo University) for the detection of transestesification efficiency of date seed oils of Ramly cultivar as example and determination of the active groups produced from this process using bands 400-4000 nm.

Fatty acid composition of the biodiesel using gas chromatography: The esterifies products of the date seed cultivars were analyzed using Gas Chromatography (GC). Analysis was carried out by using Agilent 6890 plus, equipped with a HP-50 capillary column (0.53 nm×30 m, 0.5 μm film) and flame ionization detector. Pure nitrogen was used as a carrier gas. The oven temperature of injector was 250°C and sample size was 1 μL. The identification of the fatty acid esters was established by chromatography of a reference mixture of fatty acid esters of known composition.

Antioxidant activity of date palm seeds:

Methanolic extraction: A known weight (20 g) of each cultivar of powdered date seeds was extracted with methanol (200 mL, w/v). Evaporation of solvents using rotary evaporator at 50°C, weighting of extract residues were followed by preparation of different extract concentrations (50, 100 and 200 μg mL^{-1}).

DPPH radical assay: The 2, 2 diphenyl-1-picrylhydrazyl (DPPH) test was carried out as described by Burits and Bucar (2000). One mililiter of date seed extract at different concentration was mixed with 1 mL DPPH reagent (0.002% (w/v) /methanol water solution). After an incubation period (30 min), the absorbance was measured at 517 nm. Ascorbic acid was used as positive control:

$$\text{Antioxidant activity (\%)} = \frac{Ac - At}{Ac} \times 100$$

where, At was the absorbance of samples and Ac the absorbance of methanolic DPPH solution.

ABTS radical cation scavenging assay: This assay was based on the ability of different substances to scavenge 2,2'- azino-bis (ethylbenzthiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) radical cation. The radical cation was prepared by mixing a 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and leaving the mixture for 4-16 h until the reaction was completed and the absorbance was stable. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.700±0.05 at 734 nm for measurements. The photometric assay was conducted on 0.9 mL of $ABTS^{\bullet+}$ solution and 0.1 mL of tested samples (50, 100 and 200 μg mL^{-1}) or ascorbic acid as standard and mixed for 45 sec, measurements were taken at 734 nm after 1 min. The antioxidative activity of the tested samples was calculated by determined the decrease in absorbance at different concentrations by using the following equation:

$$E = \frac{Ac\text{-}At}{Ac} \times 100$$

where, At and Ac are the respective absorbance of tested samples and $ABTS^{\bullet+}$ (Re *et al*., 1999).

The EC_{50} (The amount of sample necessary to decrease the initial DPPH or ABTS concentration by 50%) for each sample against both radical assay was calculated. The Antiradical Efficiency (AE) is calculated as follows according to Mansouri *et al*. (2005).

$$AE = \frac{1}{EC_{50}}$$

Total phenolic compounds content: Total phenolic contents in the crude methanolic extracts of different date seed cultivars were determined spectrophotometrically according to the method of Kalayasiri *et al.* (1996) using Folin-Ciocalteu's phenol reagent and absorbance was measured at 750 nm. Phenolic contents were expressed as mg/100 g of date seeds and gallic acid was used as standard.

Statistical analysis: The obtained data was analyzed using analysis of variances to determine the significance ($p<0.01$) of differences. Values of different parameters were expressed as the mean±standard deviation according to Snedecor and Cochran (1980), Hsu and Yu (2002).

RESULTS AND DISCUSSION

Egypt is considered the most date productive country in the Arab Word (1.352, 950 tons) and it produces about 17.2% of the total world production (Table 1). Most of the annually produced Egyptian dates were consumed internally and the date seeds which are commonly considered as waste product is either discarded or used in animal feed.

In an attempt to make use of this costless date seeds for biodiesel production, this investigation was carried out. The oil content (extracted with petroleum ether) in the four date seed cultivars ranged between 3.31 and 4.48% as recorded in Table 2.

Ramly showed the highest oil content (4.48%) followed in descending order by Amhat (3.88%) then Sewy (3.34) and Haiany (3.31%).

It is evident from these results and previous investigations that date seeds do not provide high yield of oil (Habib and Ibrahim, 2008) but the oil may serve as a potential source of some important phytochemicals (Habib and Ibrahim, 2011).

Table 1: Date production by different countries and continents (FAO, 2007)

Country	Production (t)	World (%)
Egypt	1,352,950	17.2
Saudi Arabia	1,078,300	13.7
Iran	1,023,130	13.0
U.A.E	775,000	9.8
Pakistan	759,200	9.6
Algeria	710,000	9.0
Iraq	566,829	7.2
Sudan	431,000	5.4
Oman	276,400	3.5
Libya	161,000	2.0
Asia	4,804,126	61.1
Africa	3,011,205	38.3
America	26,003	0.3
Europe	16,121	0.2
World	7,857,455	....

Table 2: Oil contents, biodiesel and glycerol (%) in different date seed cultivars

Species	Oil (%)	Biodiesel (%)	Glycerol (%)
Amhat	3.88	2.05	1.26
Ramly	4.48	2.43	1.43
Sewy	3.34	1.90	1.33
Haiany	3.31	1.84	1.34

Table 3: Some chemical properties of methyl esters of date seed

Species	Acid value (mg KOH g^{-1})	Iodine value (mg I_2 g^{-1})	Saponification value (mg KOH g^{-1})
Amhat	0.92	88.5	185.4
Ramly	0.50	75.3	199.0
Sewy	0.67	90.4	192.5
Haiany	0.72	86.3	202.3

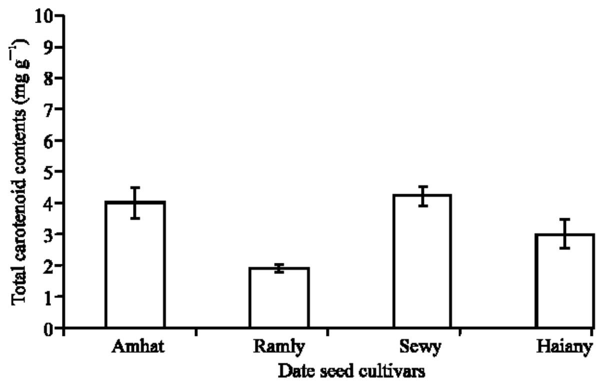

Fig. 1: Total carotenoids (mg g^{-1}) in different date seed cultivars

Transesterification of the extracted date seed oils by methanol/KOH mixture led to the production of fatty acid methyl esters (biodiesel) Table 3.

Physico-chemical properties: Physico-chemical characteristics of the produced date seed oils revealed that it aquire more yellow color than palm oil, soybean oil, sunflower oil and corn oil (Besbes *et al.*, 2005) indicating the presence of a considerable quantity of carotenoids (especially **β**–carotene) ranged between 4.5 mg g and 2 mg g^{-1} as shown in Fig. 1.

These results as well as various published reports suggests the presence of **β**-carotene in different cultivars of date seed oils which have a strong absorbance in the region 418-470 nm (Shalaby and El-Gendy, 2012; Nehdi *et al.*, 2010; Habib *et al.*, 2013).

Iodine Value (IV) is a measure of unsaturation degree. The obtained results in this study revealed that date seed cultivars showed different iodine values (mg I_2 g^{-1}) which ranged between 90.4 mg g^{-1} in Sewy, 88.5 in Amhat, 86.3 in Haiany and 75.3 mg g^{-1} in Ramly (Table 3).

These variable contents may be due to high content of saturated fatty acids in their oils which was confirmed by the GC analysis of the four date seed cultivars.

Shalaby and El-Gendy (2012) reported that waste cooking oil and its methyl esters have low iodine values (~62, 60 mg I_2 100 g^{-1} oil). Also, JUS (JUS EN 14214, 2004) illustrated that methyl esters used as biodiesel fuel must have an iodine value less than 120 mg I_2/100 g of the oil sample (JUS EN 14214, 2004). This means that the iodine values of the produced biodiesel from date seed oils in this study (75.3-90.4 mg I_2/g) as well as from *Phoenix canariensis* seed oil (76.66 mg I_2/g) were due to the high contents of saturated fatty acids leading to low or even no oxidation tendency in these oils (JUS EN 14214, 2004; Predojevic, 2008).

The low Acid Values (AV) recorded in the four cultivars of date seed oils used in this investigation ranged 0.5-0.92 mg KOH/g, indicating that these oils can be stored for longer periods without deterioration which seems in conformity with the results reported by Ojeh (1981).

Table 4: Higher Heating Value (HHV) and Cetane No. (CN) of fatty acid methyl esters of date seed cultivars

Species	HHV (kJ g^{-1})	CN
Amhat	40.50	60.52
Ramly	40.14	55.42
Sewy	40.18	57.03
Haiany	39.84	54.61

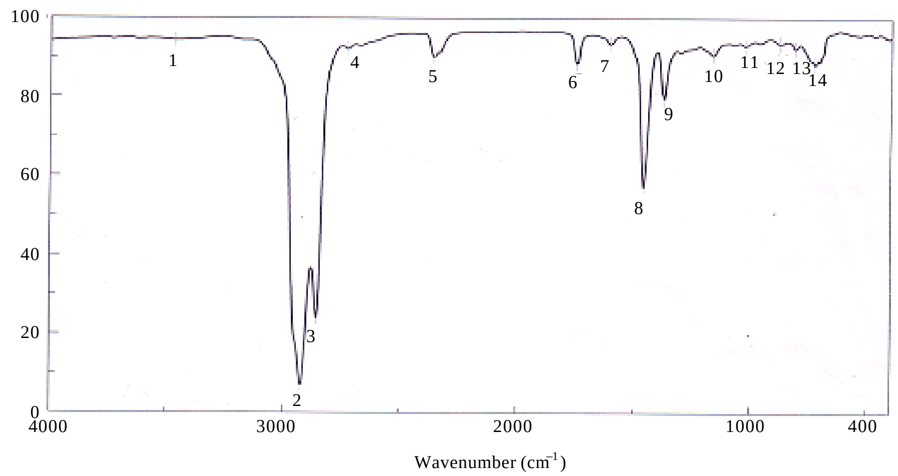

Fig. 2: Infra-red spectrum of Fatty Acid Methyl Ester (FAME) of date palm seed

Acid value of biodiesel was shown to be higher than that of standard petrodiesel but it meets the standard limits of EN 14215 and D-6751, indicating that the free fatty acids content will not cause operational problems such as corrosion and pump plugging caused by corrosion and deposit formation.

Variable Saponification Values (SV) in the studied four date seed cultivars (185.4-202.3 mg KOH g^{-1}) of the *Phoenix canariensis* seed oil (191.28) indicating the presence of low molecular weight triacylglycerols, which are in conformity with those reported by Eskin *et al.* (1996) and Oomah *et al.* (2000) who declared that their saponification values are similar to values of canola oil and raspberry seed oil (Table 3).

More or less similar Higher Heating Values (HHV, Table 4) of the produced fatty acid methyl esters (39.84-40.50 kg g^{-1}) indicated that they have nearly similar stability values due to their low polyunsaturated fatty acids contents in their oils which were clearly observed in the GC analysis of fatty acid methyl esters (Table 4).

Infrared spectrum of the produced oils showed the presence of ester group (-C-O-) at bands 1027, 1165 and 1745, as well as the absence of the hydroxyl peak which can be correlated to the transesterification process of date seed oil (Fig. 2).

These results were in concomitant with those reported by Shalaby and El-Gendy (2012) on waste cooking oil methyl ester.

Fatty acid composition of the esterified products using Gas Chromatography (GC) revealed the presence of eight saturated fatty acids ($C_{8:0}$ -$C_{18:0}$) in the three date seed cultivars; Amhat, Ramly and Haiany. While, cultivar Sewy has only seven saturated fatty acids as recorded in Table 5.

Table 5: Fatty acid contents in different commercial date seed cultivars

		Relative percentage of fatty acids in different date seeds			
Fatty acids	RT	Amhat	Ramly	Sewy	Haiany
C8:0	7.3	50.77	-	-	47.35
C10:0	11.16	28.37	-	57.06	26.88
C11:0	13.36	5.44	1.07	-	8.90
C12:0	15.51	2.77	1.55	9.69	4.82
C13:0	17.51	5.93	22.90	14.9	5.90
C14:0	19.61	2.62	4.52	10.38	4.72
C15:0	21.45	0.97	4.61	3.08	2.70
C16:0	23.27	0.79	7.63	2.48	0.39
C17:0	24.90	-	1.50	1.10	-
C18:0	26.63	-	0.85	-	0.37
C18:1	27.16	2.31	45.90	1.04	1.22
C18:2	28.06	-	7.98	0.19	0.48
C18:3	29.28	-	-	-	-
C20:0	29.76	-	0.96	-	-
SFA		97.69	46.12	98.77	98.3
USFA		2.31	53.88	1.23	1.7
SFA/USFA ratio		42.3/1	0.85/1	80.3/1	57.8/1

*RT: Retention time, SFA: Saturated fatty acids, USFA: Unsaturated fatty acids

Table 6: Antioxidant activity (%) of methanol extract from different cultivars of date palm seeds against DPPH radical

	Concentration (μg mL^{-1})		
Species	50	100	200
Amhat	47.0±2.4^{d}	79.02±5.0^{c}	88.37±4.3^{c}
Ramly	53.61±4.0^{b}	86.27±2.6^{b}	92.74±4.8^{ab}
Sewy	39.85±1.6^{e}	77.36±3.7^{d}	88.54±5.7^{c}
Haiany	55.40±3.5^{a}	88.11±6.2^{a}	93.53±3.6^{a}
Vitamin C	50.45±2.9^{c}	85.64±4.1^{b}	91.45±7.3^{b}

Results are Means±SD (n = 3). Values in the same column, followed by the same letter, are not statistically different (p>0.05) as measured by Duncan's test

Table 7: Antioxidant activity (%) of methanol extract from different cultivars of date palm seeds against ABTS radical

	Concentration (μg mL^{-1})		
Species	50	100	200
Amhat	53.32±1.9^{c}	80.3±5.8^{c}	90.4±6.3^{b}
Ramly	55.4±2.6^{b}	85.4±4.7^{a}	94.9±2.8^{a}
Sewy	42.3±4.5^{d}	78.5±4.0^{d}	89.3±4.7^{b}
Haiany	56.14±3.0^{b}	83.2±2.5^{b}	94.3±6.0^{a}
Vitamin C	58.3±2.7^{a}	85.6±1.9^{a}	93.6±2.8^{a}

Results are Means±SD (n = 3). Values in the same column, followed by the same letter, are not statistically different (p>0.05) as measured by Duncan's test

The highest percentage of saturated fatty acids was shown in Amhat cultivar (97.69%) followed in descending order by those of Haiany (98.13%), Sewy (97.72%) and Ramly (46.12%).

Table 8: Efficient Concentrations (EC_{50}), Antiradical Efficiencies (AE) of different date palm seed varieties

	Against DPPH radical		Against ABTS radical	
Species	EC_{50}	AE	EC_{50}	AE
Amhat	59.4	0.017	48.8	0.02
Ramly	43.8	0.023	44.2	0.022
Sewy	64.7	0.015	62.4	0.016
Haiany	44.3	0.023	45.6	0.021
Vitamin C	48.01	0.020	43.9	0.022

The fatty acids caprylic ($C_{8:0}$) and capric ($C_{10:0}$) constituted the largest percentage of saturated fatty acids in the studied date seeds. Ramly cultivar was the only date seed containing Arachidic fatty acid ($C_{20:0}$) of 0.96%.

The recorded unsaturated fatty acids were oleic ($C_{18:1}$) and Linoleic ($C_{18:2}$) which were present in variable relative percentages in only three of the four date seed cultivars (53.88% in Ramly to 1.23% in Sewy), while linoleic acid was absent in Amhat cultivar (Oleic represented by 1.88%). Ramly recorded the most pronounced content of unsaturated fatty acids (20.99% Oleic+3.65% Linoleic) and the least percentage of saturated fatty acids (46.12%).

Our results go parallel with those published by Nehdi *et al.* (2010) on date palm seeds and seed oils. Recent studies conducted on the characteristics and chemical composition of date palm (*Phoenix canariensis*) seed oil grown in Tunisia suggest that the most important fatty acids were oleic, linoleic, lauric, palmitic, myrisic and stearic which together compose about 98.6% of the total fatty acids (Nehdi *et al.*, 2010). Yong and Salimon (2006) reported that liquid oils with high oleic fatty acid content (as Ramly oil) normally have good flavor and frying stability.

Oleic acid is important in nervous cell construction and can be changed in the organism into a set of compounds close to prostaglandin which have an important role at the vessel level and for blood coagulation (Veeresh Babu *et al.*, 2010). So, Ramly can be of importance in this concern.

Antioxidant activity: Antioxidant activity (%) of date seed methanolic extracts against DPPH and ABTS radical scavenging revealed that, the activity was concentration dependent (Table 6). In DPPH radical assay; Haiany cultivar recorded the highest activity (55.40, 88.11, 93.53% at 50, 100 and 200 μg mL^{-1}, respectively) compared to the other three date seed cultivarsand even more than those of the natural antioxidant standard, Vit. C (50.45, 85.64, 91.45% at the conc. 50, 100 and 200 μg mL^{-1}). Ramly came in the second order (after Haiany) with an antioxidant activity of 53.61, 86.27 and 92.74% at the same used concentrations.

In ABTS radical assay, Ramly cultivar gave the highest value (Table 7), followed by Haiany (85.4, 94.9 and 83.2, 94.3% at 100 and 200 μg mL^{-1}, respectively), while the varieties Amhat and Sewy had the lowest values (80.3, 78.5 and 90.4, 89.3% at 100 and 200 μg mL^{-1}, respectively).

The antioxidant activity was expressed by the parameter Antiradical Efficiency (AE) or antiradical power (ARP), where, the larger the ARP, the more efficient is the antioxidant (Brand-Williams *et al.*, 1995). Ramly and Haiany varieties presented the highest values for AE and EC_{50}. Wherese the varieties Amhat and Siwy had the lowest values (Table 8).

To know to what substance (s) this activity was attributed, the antioxidant active compounds especially total carotenoids (mg g^{-1}) and total phenolics (mg/100 g) were determined in the four date seed cultivars.

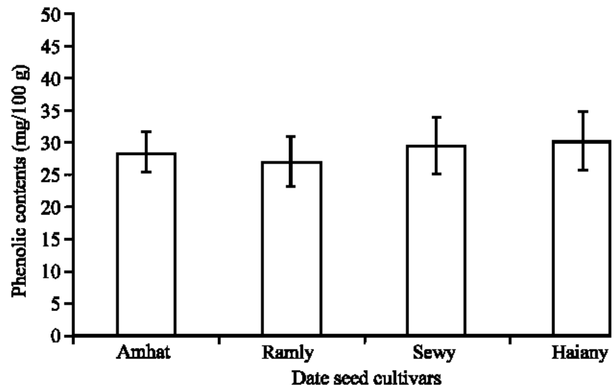

Fig. 3: Phenolic compound (mg/100 g) in different date seed cultivars

Total carotenoid contents showed the order: Sewy>Amhat>Haiany>Ramly (Fig. 1), while total phenolic compounds (Fig. 3) were in the order Haiany>Sewy>Amhat>Ramly.

The highest antioxidant activity of Haiany methanolic extract may be attributed to the pronounced contents of both total carotenoids and phenolic compounds which are known by their potant radical scavenging efficiencies (Liang and Su, 2009; Onofrejova *et al.*, 2012; Caponio *et al.*, 1999).

Ramly showed an activity comparable to that of Haiany inspite of its lower carotenoid content (2 mg g^{-1}), as shown in Fig. 1. This may be attributed to both a relatively important total phenolic contents (27 mg/100 g) as well as to a pronounced unsaturated fatty acids (oleic+linoleic as recorded in Table 5. These substances induced the potent antioxidant activity of date seed cultivar as well as the resistance to oxidative rancidity (Stevenson *et al.*, 1984) and oxidative stability (Salvador *et al.*, 2001).

CONCLUSION

Date seed as an agricultural by-product used in Egypt (The highest country in the date production in Arab countries) can be used as a suitable and cheap feedstock for producing biodiesel. Date seed oil has a considerable amount of low-chain fatty acids which gives special features to biodiesel. The most important advantages of date seed biodiesel are its high cetane number, low viscosity. These factors can increase engine's output and decrease pollution in comparison with other biodiesel fuels. The present experimental results support that methyl ester of date seed oil can be successfully used as biodiesel in the internal combustion engine. In addition to Date seed methanolic extract may serve as a source of natural antioxidant (β-carotene, unsaturated fatty acids and phenolic compounds).

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