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Biochemical Properties Evaluation of some Libyan dates

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ABSTRACT

The current research targeted to estimate total sugars, fats, proteins, phenols, and antioxidant activity of ethanolic extract of five date varieties (al-Tabouni (TAB), al-Bakrari (BAK), al-Aami (AAM), al-Hamouri (HAM), and al-daqla (DAQ)) available in Libya. Also, phytochemical screening for ethanolic and aqueous extracts were performed. In general, the two extracts were rich in carbohydrates, proteins, phenols, flavonoids, alkaloids, and glycosides. However, steroids didn't exist in aqueous extract and saponins in both extracts. Total sugars were estimated by spectrophotometric methods, the proteins using the Kjeldahl method, and fats by the Soxhlet device, and their percent were ranged between 49 - 66%, 1.43 - 2.25, 0.10 - 0.25% (w/w) for sugars, proteins and fats, respectively. The total phenols were also estimated using the Folin reagent method, where the results are expressed as mg (gallic acid equivalent) per g (extract) and ranged from 13.5 to 20.5 mg/g, and the highest level was in the DAQ variety. The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method was also used to estimate total antioxidants where the two largest levels were found in the DAQ and HAM varieties with concentrations of 10.68 and 10.63 mg (ascorbic equivalent)/g (extract), respectively. DAQ extract has reduced the 50% of DPPH at lower concentration of 0.110 mg/ml (I_{C50}). Furthermore, good positive correlation was found between total phenols and DDPH in ethanolic extract.

1. Introduction

The date palm, Phoenix dactylifera L., is a tree of significant cultural, scientific, agricultural, and economic value in water poor areas such as the North Africa and Middle East [1]. Dates comprise a significant part of the diet in Arab countries [2]. There are over 400 different varieties, but only 50 - 60 are grown over large areas and considered of economic worth [3]. Date palm is local to Arab lands and has abundant varieties, each possessing its particular flavor and nutritional characteristic [4]. Date fruits act a fundamental part in the commercial and social prosperity of populations resident in arid and semi-arid zones of the world [5]. They are an essential main food in many countries throughout the world [6].

Dates are a food of high nutritional value and are an ideal food for humans, because they contain major nutrients such as sugars, proteins, fats and dietary fibers, and they also contain some vitamins such as vitamins (A & C) in addition to a large amount of antioxidants and high proportions of poly phenols [7, 8]. Moreover, it is considered a natural source of minerals; such as calcium, iron, magnesium, sodium and phosphorous, because it contains very essential amounts for building the human body [9, 10]. Lately, number of studies have described such activity of date fruits [11-14]. It was found that consuming 7 dates, i.e. approximately 100 g of dates, supplies the human body with all its daily needs of magnesium, manganese, copper and sulfur, half of its needs of iron and a quarter of its needs of calcium and potassium, and dates contain high amounts of trace elements. Fluorine is estimated at five times what other fruits contain of this element [15]. In addition to the importance of dates as a high source of energy, they are characterized by having high percentages of calories, as they contain 3000 Kcal per kg of dates.

Date fruits retains abundant health helps, involving nephroprotective, antihyperlipidemic, anticarcinogenic, antimutagenic, antiatherogenic, gastroprotective, and

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hepatoprotective activities [16, 17]. All of this refers date fruits a desirable fruit from a medicinal and pharmaceutical viewpoint [6].

Due to the favorable health achieves of date fruits, growing research tries to study their several chemical and biochemical contents, such as: total phenols, antioxidant activity, total sugar, proteins, and minerals [2, 5, 7, 9, 11, 18 – 21].

The major aims of this study were (i) to estimate some nutrients content (sugars, proteins, and fats), (ii) to determine of the total phenols and total antioxidants, and (iii) phytochemical screening of aqueous and ethanolic extracts of five most prevalent dates kinds available in Libyan markets.

2. Results and Discussion

2.1. Phytochemical Screening

Table 1 shows the results of the qualitative detection of the active metabolic compounds of the aqueous and ethanolic extracts. The purple ring appeared in the Molisch test was observed for all date kinds in the two extracts, and this is an indication of the presence of carbohydrates. For steroids test, it gave negative results with all types. With regard to phenols, they were clearly visible in the five kinds and the two extracts, with a strong blue color in the ferric chloride test. Phenols perform a critical role in preserving plants from diseases initiated by bacteria and fungi [33]. The same applies to the test of flavonoids, which gave positive results with all kinds, in terms of appearing the yellow color. Flavonoids are antioxidants that inhibits free radicals and are efficient in decreasing the risk of heart disease [34]. Also with the tests of glycosides and alkaloids, the extracts also gave positive results with all samples in terms of the following colors: red precipitate, brown and creamy precipitates. Glycosides have a function in adjusting osmotic pressure and the carrying of some substances required for plant metabolism [35]. Alkaloids are anti-cancer and bronchodilator. However, for saponins, they gave a thick foam with the aqueous extract for all samples, and a negative result with the alcoholic extract. The saponins distributed richly in the parts of the plant and had a role in reducing cholesterol in the blood. Finally, the results of phytochemical screening agreed with the previous studies [36, 37]

Table 1. Phytochemical Screening of ethanolic and aqueous extracts

Active Compounds	**Ethanolic Extract**					**Aqueous Extract**				
	DAQ	**HAM**	**AAM**	**BAK**	**TAB**	**DAQ**	**HAM**	**AAM**	**BAK**	**TAB**
Carbohydrates	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+
Steroids	–	–	–	–	–	–	–	–	–	–
Phenols	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	+	+	+	+	+

2.2. Total sugar content

Dates are reasonably abounding in kilocalories and hold a considerable percentage of carbohydrates (around 73% of the dry weight), which are largely glucose (~90%), fructose, and sucrose [38]. The fruit also comprises a substantial amount of dietary fibers (estimated at 6.4 to 11.5 percent of the dry weight) including lignin, pectin, resistant starch, hemicellulose, and soluble fiber [39]. As shown in Table 2 and Figure 1, the five date kinds showed similar total sugars contents. The percentage of sugars ranged from 49-66%, where DAQ gave the highest levels of sugars (66%), and this was close to the results of Periyasamy &

Padmanayaki study [40], in which the total sugars percent was (65%), and the lowest levels was in HAM kind (49%), which is similar to the results obtained by Taylor (44 - 48%) [41]. Although, the other two types; TAB and BAK, gave same sugar levels of (57%), which is close to the results of Parvin study (51.8 - 55%) [42], while the percentage of sugars in the AAM kind was (61%).

Table 2. Some biochemical contents of ethanolic date extracts

Date variety	TAB	BAK	AAM	HAM	DAQ
Total Sugar (%)	57±2.3	57±2.9	61±3.1	49±1.0	66±3.3
Total Fat (%)	0.10±0.01	0.25±0.02	0.15±0.01	0.40±.02	0.25±0.01
Total Protein (%)	1.49±0.07	2.25±0.09	1.49±0.05	1.43±0.06	2.13±0.08
Total Phenols mg/g)	17.60±0.72	15.19±0.31	13.51±0.41	17.85±0.99	20.51±1.03
Total Antioxidant mg/g	9.34±0.47	9.41±0.38	9.29±0.28	10.63±0.43	10.68±0.53

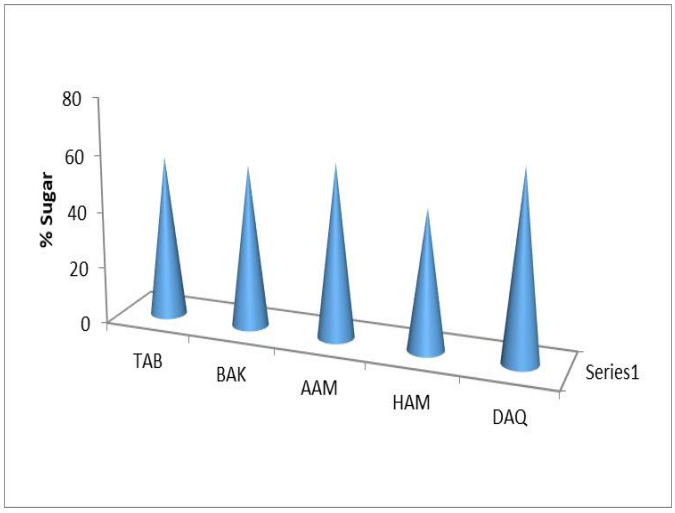

Figure 1. Total sugar contents in date samples

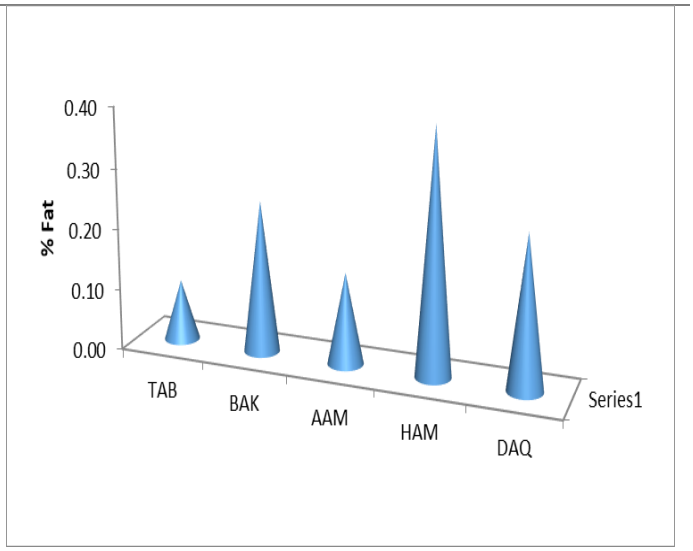

Figure 2. Total fat contents in date samples

2.3. Total fat content

Date fruit contains small fat, which usually varies from 0.5% to 0.1% [41]. Fat is commonly focused in the crust, beside date flesh having about 0.1–0.5% fat. Fat is further essential in the protection of the fruit than in the nutritional value of the date flesh [18]. Eight fatty acids occur in very small levels in the flesh. The main saturated fatty acids in date are myristic, lauric, and palmitic acids, whereas the major unsaturated fatty acid is oleic acid [41]. As displayed in Table 2 and Figure 2, we notice that the fat percent ranged from 0.10 - 0.40%. The levels in BAK and DAQ were the identical and the value was 0.25% for the two kinds. However, HAM had the highest percent of 0.40%, and the lowest was in TAB with 0.10%. These results were confirmed with most studies that the percentage of fat in dates is very low, such as the results of Hasanaoui et al. [44] and others [18, 41, 45].

2.4. Total protein content

Date fruits have protein (nearly 3%) and 23 several amino acids that are not ordinarily found in other fruits [38]. According to Assirey [46], (37–93 mg/100g), alanine (78–105 mg/100 g DW), proline (86–113 mg/100g), glycine (83–102 mg/100g), arginine asparagine (127–225 mg/100g) and glutamate (158–265 mg/100g) were detected as the main fundamental amino acids while tryptophan contained the least (13–46 mg/100g) in ten dates studied. The protein levels of the present study are illustrated in Table 2 and Figure 3. The dates contain a small percentage of proteins which ranged from 1.43% (HAM) to 2.25% (BAK). This study gave similar results with previous researches; such as AL-Aswad [48] (1.84 - 2.38%), and the two studies of Parvin [42] and Assirey [46], which had a protein percent between 2.01 - 3.04% and 1.72 -4.73%, respectively.

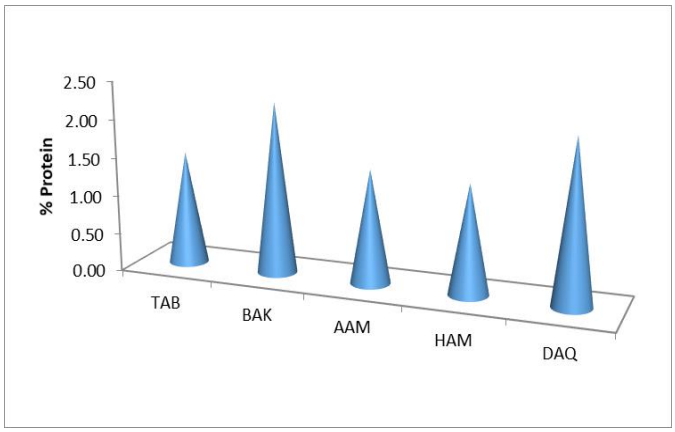

Figure 3. Total protein contents in date samples

2.5. Total phenol content

Polyphenols are additionally one of the ingredients of date, making up 3% (dry basis) of flesh [48]. Variability in the polyphenol level of dates exists depending on the variety and degree of ripeness, as well as geographic site and environmental circumstances [49]. Some of the recognized phenolic compounds in dates are quercetin, luteolin, apigenin, isorhamnetin, malonyl derivatives, chrysoeriol, 3-methyl-isorhamnetin and kaempferol [50]. The occurrence of these phenolic compounds encouraged the antioxidants abilities of dates [38]. The concentrations of total phenols are presented in Figure 4. for the five studied date varieties, there were a similarity in the amount of total phenols, where their concentrations ranged between 13.51 - 20.51 mg/g. The amount of phenols for DAQ Sort was 20.51 mg/g, while AAM sort had the lowest level of 13.51 mg/g. However, the phenolic contents in TAB and HAM sorts were very close to each other with an average of 17,596 and 17.85 mg/g, respectively. The BAK sort had a phenols level of 15.185 mg/g. By comparison with the previous studies; (Ezari, 2013) [51], the results of which showed that the studied samples had low levels in phenolic compounds, with a range of 0.113 - 0.518 mg/g, also the results of (Mansouri et al., 2005) [11] study were minimal in their phenolic contents, which amounted between 2.49 -8.6 mg/g. However, the study of (Matloob et al., 2016) [52] showed higher phenolic contents between 14.5 − 47.5 mg/g, and this indicates that the five studied dates varieties contained a high content of phenols, compared to other varieties studied in other countries.

2.6. Total antioxidant content

Antioxidants plays a fundamental part in food systems, human body cells, and tissues by guarding against oxidative damage of toxic molecules named free radicals [53]. Those free radicals are directly linked with some known diseases such as heart disease, cancer, Parkinson's and Alzheimer's disease. Fresh date is a rich supply of antioxidants [54], phenolic compounds (ferulic acid), free phenolic acids (protocatecoic acid, vanilic acid, syringic acid, and ferulic acid), and bonded phenolic acids (gallic acid, protocatecoic acid, p-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, and o-coumaric acid), Ferulic, vanillic, syringic, (trolox), anthocyanins, (cyanidin 3-glucoside), and carotenoids, [41]. The highest efficient antioxidants are flavonoids and phenolics. Phenolics are considered as effective inhibitors of lipid peroxidation due to their metal-chelating and radical-scavenging properties [55]. In general, date is considered a good source of natural antioxidants, with antiradical activity. It can be benefited as functional food or as one of its ingredients [41].

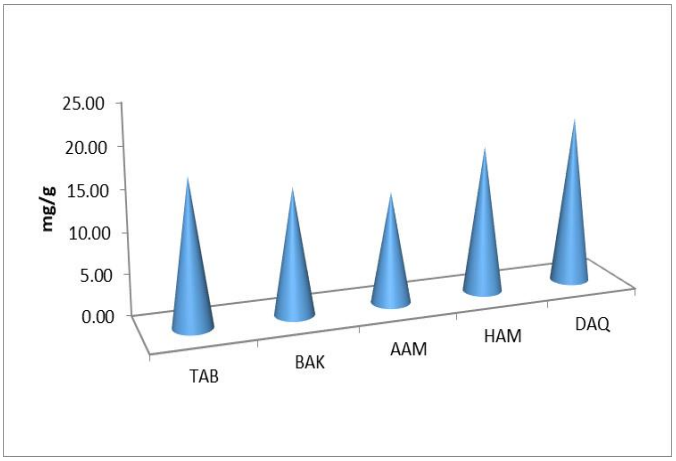

Figure 4. Total phenol contents equivalent to gallic acid in (mg/g) in date samples

Antioxidants were estimated using the DPPH inhibition reagent method. Ascorbic acid was used as a reference material, where the antioxidants content is expressed as the number of milligrams equivalent to ascorbic acid per gram of extract. First, the concentrations of extracts needed to inhibit 50% of free radicals (IC50) were determined, which are shown in Figure 5. These concentrations were ranged between 0.413 - 0.110 mg/ml. As it is known, as the IC50 value decreased, the antioxidant activity of the kind increased. Through the results we obtained, we find that the DAQ variety had a greater antioxidant activity with a value of 0.110 ml/mg, and this results agreed with (Ezari, 2013) [51] study that DAQ had the highest antioxidant activity of 2.662 ml/mg.

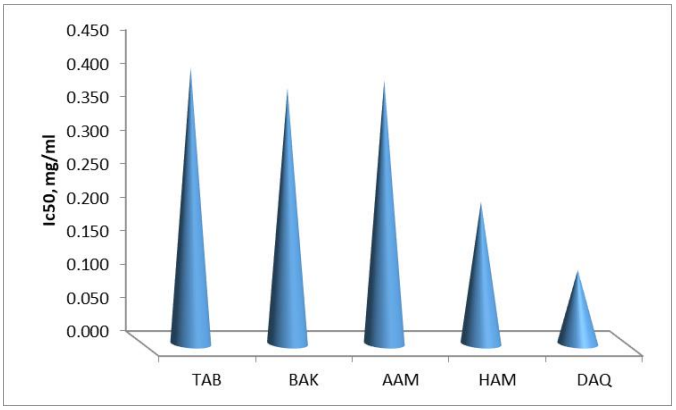

Figure 5. I_{C50} in (mg/ml) for date samples

The amount of antioxidants equivalent to ascorbic acid was also estimated. Figure 6 show the amount of total antioxidants in milligrams equivalent to ascorbic acid per gram of the extract, where the two largest values were recorded for the DAQ and HAM varieties with concentrations of 10.68 and 10.63 mg/g, respectively. While the lowest value was recorded for AAM variety with a concentration of 9.29 mg/g. The results of the study were similar to the study of (Ezari, 2013) [51], where the DAQ variety recorded the highest concentration of antioxidants estimated at 8.05 mg/g. However, it is differed with the study of (Ardekani et al., 2010) [56], which estimated antioxidants in 14 date species, and the results of which showed that Al-Zahdi species had the highest antioxidant activity of 37.42 mg/g. Also, the results of (Mansouri et al., 2005) [11] study were lower than the present study since antioxidants levels were estimated between 0.08 - 0.22 mg/g.

The relationship between the amount of total phenols and total antioxidants has also been clarified, as shown in Figure 7, where we note that there is a good correlation between the amount of total phenols and total antioxidants, as It is known that phenolic compounds have potent antioxidant activity [55].

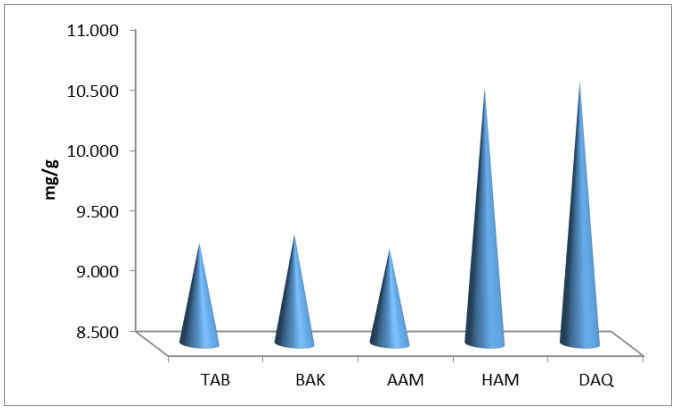

Figure 6. Total antioxidant equivalent to ascorbic acid in (mg/ml) for date samples

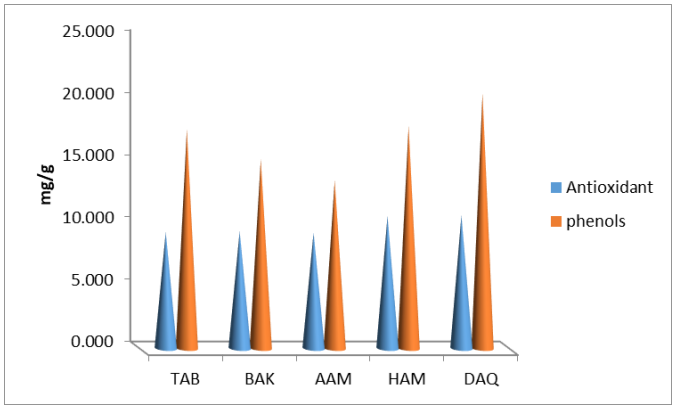

Figure 7. Comparison between total phenols and total antioxidant levels

3. Experimental

3.1. Sample Collection

Five well-known and widespread Libyan dates types were selected from Misurata city and collected during the harvest season (October 2020), which were: al-Tabouni (TAB), al-Bakrari (BAK), al-Aami (AAM), al-Hamouri (HAM), and al-daqla (DAQ). Of each variety, about 2 kg was gathered, sorted, affected fruits excluded, and the fleshy part from the cores separated, and in the freezer kept for later use.

3.2. Drying of dates

The samples were initial oven-dried at 105ºC for 24h. The dried samples were then ground manually in a grinder and were exposed to extraction and then analysis for their total antioxidant, total proteins, total sugar, total fats, total phenols contents, and phytochemical screening.

3.3. Chemicals and reagents

2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, sodium carbonate, glucose, Folin–Ciocalteau's phenol reagent, Ethanol, nitric acid, and ascorbic acid were obtained from Merck (Darmstadt, Germany) and all chemicals were of A. G. grade and were used as received without further purification.

3.4. Extraction Method

The dried date powder (5 g) was extracted with 100 ml Ethanol (or distilled water) at room temperature (25^{oC}) for 72 h using an orbital shaker. The extracts were then filtered through Whatman filter paper (No. 1). The crude extract was then kept in dark glass bottles inside the freezer until use. The storage conditions (time and temperature) were the same for all kinds of dates.

3.5. Total phenolic content

Total phenolics were determined using Folin–Ciocalteau reagent [22]. Date extract (1 and 2 ml of ethanolic) or gallic acid standard were mixed with 1.0 ml of Folin–Ciocalteau reagent (prediluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min, and then 0.8 ml of sodium bicarbonate (7.5%) was added to the mixture. After standing for 30 min at room temperature, absorbance was measured at 765 nm. Results were expressed as mg gallic acid equivalents (GAE)/ g sample [23]. The phenolic content was calculated by using the following linear equation which obtained from the gallic acid calibration curve (from 5 – 100 ppm gallic acid):

$$Y = 0.0046\ X \qquad (1)$$

$$R^2 = 0.9458 \qquad (2)$$

Where Y is the absorbance and X is the concentration as gallic acid equivalents (ppm).

3.6. DPPH radical scavenging activity

The capability of dates extracts to scavenge DPPH radicals was evaluated according to the Blois [24] method. Concisely, 2 ml of a 0.024% (g/ml) methanolic solution of DPPH was mixed with (0.5, 1.0, 2.0, 4.0 ml)

of date ethanolic extract (or ascorbic acid). The mixture was then homogenized energetically and left for 30 min in the dark place (at room temperature). Its absorbance was then measured at 517 nm and expressed as percentage of DPPH scavenging relative to control solution (contains ethanol rather than extract) using the following equation:

$$\text{DPPH scavenging activity I\%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{ X } 100 \quad (3)$$

The concentration of extract required to scavenge 50% of DPPH (IC50) were determined. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity. The total antioxidant concentrations of extracts equivalent to ascorbic acid was also determined for comparison. The total antioxidant concentration equivalent to ascorbic acid was calculated according to the following equation:

$$Y = 12.004\ X \quad (4)$$

$$R^2 = 0.9847 \quad (5)$$

Where Y is the percentage of DPPH scavenging and X is concentration as ascorbic acid equivalents (ppm).

3.7. Total sugar content

Total sugars were determined using the spectrophotometric method proposed by Dubois [25]. To 0.5 ml date extract, 1.0 ml of 5% phenol solution was added and followed by 5.0 ml of concentrated sulfuric acid. After that, the absorption of the mixture was measured at 490 nm. The level of sugars was expressed as mg glucose equivalents per 100 mg date extract. The aqueous extracts were used in this determination. The total sugars concentration equivalent to glucose was calculated according to the following equation:

$$Y = 0.0018\ X \quad (6)$$

$$R^2 = 0.9868 \quad (7)$$

Where Y is the absorbance and X is concentration as glucose equivalents (ppm).

3.8. Total protein content

Kjeldahl method [26] was applied for the evaluation of total proteins. The weight of date sample was 1.0 g and 0.01 N sulfuric acid solution was used as a titrant. The following equation was applied for calculation of % proteins:

$$\%\text{Proteins} = \frac{\text{V X } 0.014 \text{ X } 6.25}{\text{Wt.of sample}} \text{ X } 100 \quad (8)$$

Where: V: volume of titrant in liter, 6.25: protein factor for fruits and vegetables.

3.9. Total fats content

Total fats were determined by Soxhlet Extraction method [26]. 2.0 g of date sample was applied and 125 ml petroleum ether was used for extraction process. Total fats were calculated using the following equation:

$$\%\ \text{Fat} = \frac{\text{Wt.of flask with fat} - \text{Wt.of empty flask}}{\text{Wt.of sample}} \text{ X } 100 \quad (9)$$

3.10. Phytochemical Screening

In this part, aqueous and ethanolic extracts are prepared from dried date samples and then analyzed for the presence of primary and secondary metabolites like carbohydrates, proteins, phenols, alkaloids, terpenes, flavonoids, and others. Standard tests are available in the literature for each class of compounds to be analyzed [27-32]. The color intensity or the precipitate formation was used as analytical responses to these tests.

3.11. Statistical Analysis

The data were analyzed by Microsoft Excel 2010 soft wear. The data were expressed as mean ± standard deviation.

4. Conclusion

The results presented in this study showed the Libyan date is rich in sugars, phenols, and antioxidants. Total sugars for studied date varieties were ranged from 49% to 66%. The ability of date palm fruit to scavenge free radicals is demonstrated by the DPPH methods. Both of DAQ and HAM varieties possessed the highest free radical scavenging activity. Also, it was found that there is a direct relationship between the concentration of phenols and antioxidants. On the basis of our finding, we conclude that date palm fruit constitutes a natural source of potent antioxidants that may prevent many diseases. The presented data in this study confirm that Libyan date fruits can be considered a rich source of many nutrients.

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