

## Nutritional Value, Cytotoxicity, Anti-Carcinogenic and Beverage Evaluation of Roasted Date Pits

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**Abstract:** Date palm pit is a waste of date's industry and date consumption. Pit is about 12 -15% of the date (wt/wt). This research was conducted to evaluate the date pit beverage in replacement to cocoa drink. 3, 6 and 9 gm of roasted date pits were mixed with milk and water (1:1 v/v) to obtain cocoa substitute beverage, which have been evaluated for sensory attributes. Roasted date pits were also evaluated for its nutritional value, cytotoxic activity and anti-carcinogenic effect. Seventeen phenol compounds and nine flavonoids were identified by HPLC method. Pyrogallol (57.94mg/100g), Cinnamic acid (37.40mg/100g), benzoic acid (73.05mg/100g) and Elagic (36.82mg/100g) were the major constituents of phenol compounds. However Hesperidin was the major flavonoid (17.27mg/100g). Fatty acids profile showed that oleic acid was the main fatty acid 40% followed by lauric acid (15.95%). Glutamic acid (16.44 gm/100gm protein) was found to be the predominant amino acid, however Leucine (6.10gm/100gm protein) and phenyl alanine (5.93gm/100gm protein) present here in moderate amounts. Date pit at concentration 100µl/ml was effective against human colon cancer cell line (53.65% viability) in vitro. Anti-carcinogenic effect was less against human hepatocellular carcinoma (79.95% viability). Date pit beverages (1:1 milk and water respectively) showed higher sensory attributes compared with control in dose dependent manner. The rate of diffusion from a pits powder bag membrane was determined, it was observed that smaller particles of roasted date pits powder diffused faster through pits powder-bag than larger-sized loose powder. This beverage encloses many active compounds that express anticancer activity.

**Key words:** Palm Date Pit • Phenols • Flavonoids • Anticancer

### INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the most economically important fruit trees grown in Egypt [1]. Date production of Egypt alone represented about 20% of the total world production for 2008 [2]. Although the date palm fruit served as the low cost food for millions of people around the world for several centuries, studies on its well-being benefits are inadequate and it is hardly recognized as a healthy food by the health professionals and the public [3]. About 11-18% of the date fruit weight comes from the seed [4]. A large quantity of date seed could be easily collected from the date processing industries or from the waste products originating [5-7].

The fat content in the date seed obtained from several studies ranged from 5.7 to 12.7%, other than, oil, protein, carbohydrate, moisture and ash. The date seed is also composed of several important minerals, namely potassium, magnesium, calcium, phosphorus, sodium and iron [8, 4]. Also, Al-Farsi and Lee [9] believed that date seed concentrates could potentially be an inexpensive source of natural dietary fiber and antioxidants and possibly used as a functional food ingredient. Dietary fiber has important therapeutic implications for certain conditions such as diabetes, hyperlipidemia and obesity and may exhibit a protective effect against hypertension, coronary heart disease, cholesterol, colorectal and prostate cancers and intestinal disorders [10-12].

Phenolic compounds of date fruit seeds mainly phenolic acids and flavonoids, have been shown to possess such benefits as antioxidant [13], anti-carcinogenic [14], antimicrobial [15], anti-mutagenic [16] anti-inflammatory activities [17], as well as reduction of cardio-vascular diseases [18]. Furthermore, the extract of seeds showed the ability to restore the normal functional status of poisoned liver and to protect it against subsequent intoxication with carbon tetrachloride [19].

Roasting the date pits and making a caffeine-free drink introducing choice to people preferring a non-caffeinated coffee with coffee-related flavor. Paranthaman *et al.* [20] stated that roasted date seeds exhibited the highest overall content of flavonoids and antioxidant capacity than raw date seed. The date by-products are safe for human consumption and may possess high value components that may be used in value-added applications, including their use as functional foods and ingredients in nutraceuticals, pharmaceuticals and medicine [21, 22]. Therefore, this study was carried out to evaluate the nutrition value, toxicity and anti-carcinogenic of roasted date pit and the ability of using it as new innovative healthy drinks from by-product low-priced beverage with high-quality characteristics.

## MATERIALS AND METHODS

**Materials:** The main material for this innovation drink product is taken from the date palm seeds. The date palm pit (*Phoenix dactylifera* L.) variety of Barheewas collected from El-Wahat area, Egypt. Coca, sugar and milk were obtained from local market, Giza governorate, Egypt.

### Methods

**Preparation of Roasted Date Pits:** The Date pits were washed to free it from any adhering date flash, samples were roasted at 125°C for 30 min until the pits color turned to light brown. The pits were crushed using pestle and mortar followed by high speed laboratory blender [CONDUX-WE WOLFGANG BEI HONOU (Adam Baumuller GMBT)] then sieved to obtain finely divided powder, then sieved another time to obtain the fine seed powder (60 mesh).

### Chemical Analysis

**Chemical Composition:** Moisture, crude protein, ash, crude fat and dietary fiber were determined by the standard procedures of the AOAC [23]. Carbohydrates were calculated by the difference. Total carbohydrate = 100 - (%protein + %fat + %fiber + %ash). Total dietary fiber,

soluble and insoluble dietary fiber were determined according to the method described by Asp, *et al.* [24].

**Fatty Acids Composition:** The lipid of date seed (300 g) was extracted using hexane (500 ml) in a soxhlet apparatus for 8 h. The lipids were weighed after the solvent evaporation and then stored at -10°C until analysis.

The fractionation and identification of fatty acids were conducted by the method of AOAC [23] using gas chromatography with FID detector (PE Auto System XL) with auto sampler and Ezchrom integration system. Liquid extraction was carried out as described by Folsch *et al.* [25].

**Amino Acid Composition:** Amino acids were determined according to AOAC [26] using high performance Amino Acid analyzer (Biochrom 30).

**Determination of Minerals:** The minerals were determined using the flame photometer (Galienkamp, FGA, England) and perkin Elmer atomic absorption spectrophotometer (model 80, England) as described in AOAC [27].

### Identification of Phenolics and Flavonoids in Roasted Date Pits:

To determine phenolic acids and flavonoids, samples were prepared according to the method described by Jakopiè *et al.* [28]. The chromatographic conditions (mobile phase, gradient program, temperature of column) were similar to those described by Schieber *et al.* [29]. A high performance liquid chromatography system equipped with a variable wave length detector (Agilent, Germany) 1100, auto sampler, Quaternary pump degasser and column compartment. Analyses were performed on a C18 reverse phase (BDS 5 µm, Labio, Czech Republic) packed stainless-steel column (4×250mm). All chromatograms were plotted at 280nm to estimate phenolic acids and at 330 nm for flavonoids. All components were identified and quantified by comparison of peak areas with external standards.

### Measurement of Cytotoxicity Date Pit by Sulforhodamine B (SRB) Assay:

Potential cytotoxicity of date pit was tested using the method of Skehan *et al.* [30] using three human cells WISH (normal amniotic cells), HCT116 (colon cancer cell line) and HepG2 (Human hepatocellular carcinoma).

**Preparation of Roasted Date Pits Drink:** 3, 6 and 9 gm from date pits powder was respectively, mixed with 1:1 milk and water and 4 gm of sugar for sensory evaluation.

**Preparation of Cocoa Drink:** Control sample was prepared from 10gm of cocoa with 1:1 milk and water and 4 gm sugar respectively.

**Sensory Evaluation:** Sensory evaluation method was conducted according to the method described by Mirghani *et al.* [31]. The samples were served hot to 10 members trained panel (Department Staff of Special Food and Nutrition, FTRI, ARC, Giza, Egypt). The panelists were subjected to sensory evaluation using a 10-point hedonic scale for color, taste, odor, flavor and overall acceptability. A numerical basis as a sort of evaluation from 1 to 10 was used where one represented from “dislike extremely” to “liked extremely” [32].

**Diffusion Through Packaging Material:** The pits powder-bag was made from Nonwoven which was purchased from Pulp Paper. This was cut into equal pieces 6 cm × 3cm which were folded into two, making sure that the heat-seal was on the inside. Two sides were then sealed using a hot iron. Using the remaining side, 3 gm of roasted date pits powder were transferred into the bag and then side sealed.

The experiments were carried out in 200 ml of distilled water, to ensure that the bags were completely immersed in water. The flask with its content was placed inside the thermostat bath, which was set to give a temperature of 80°C inside the flask with an accuracy of ±0.1°C. Experiments were also carried out using loose tea leaves as well [33].

**Statistical Analysis:** The data of the present study were subjected to analysis of variance and the Fisher's least significant difference test, (SAS software version 9.3) in order to compare the mean values of the investigated parameters at significance levels of  $P \geq 0.05$ .

## RESULTS AND DISCUSSION

**Chemical Analysis:** Data in Table 1 showed the chemical composition of date pits used in this study. Moisture, protein, ash, total lipids and dietary fiber content found to be 5.59%, 5.90%, 1.21%, 7.97%, 63.81%, respectively. Carbohydrate was calculated by difference (15.52%). In general, the amounts of protein, total lipids, ash and carbohydrate are within the range of values of date pits powder presented earlier in the literature Abdul Afiq *et al.* [34], Akasha *et al.* [35] and Ammar *et al.* [36]. Meanwhile, our result of total lipids content was lower than results of Nehdi *et al.* [4]. They stated that the difference may occur

Table 1: Chemical Analysis of Date Pits

Constituents	Date pits
Moisture (%)	5.59±0.45
Protein (%)	5.90±0.63
Ash (%)	1.21±0.11
Total lipids (%)	7.97±0.89
Dietary fiber (%)	63.81±1.85
Soluble dietary fiber (%)	55.60 ± 0.84
Insoluble dietary fiber (%)	8.21 ± 0.27
Carbohydrate (%)	15.52±1.33

Values are mean of three replicates ±SD.

Table 2: Fatty Acids Content of Roasted Date Pits Oil (%)

Fatty acids	Fatty acids (%)
Palmitic acid	9.40
Stearic acid	3.16
Oleic acid	40.00
Linoleic acid	8.66
Caproic acid	9.74
Capreic acid	11.00
Lauric acid	15.59
Myristic acid	10.70
Myrisoleic acid	0.95
Arachidic 20:0	0.39

due to different date varieties, different origin, different harvesting time and the use of fertilizer which could affect the nutrient content of the date. The total dietary fiber contents in date pits was 63.81 g/100 g, with insoluble dietary fiber (hemicellulose, cellulose and lignin) is considered to be the major constituent of seed fiber (55.60 gm/100g). This data was lower than Al-Farsi *et al.* [21] who reported that the content of total dietary fiber in three seed varieties ranging between 77.8 and 80.2 g/100g. Also, Hamada *et al.* [8] reported that total dietary fiber was 64.5 to 68.8 g/100 g for three date seed varieties. The high nutritional value of date seeds is based on their dietary fiber content, which makes them suitable for the preparation of fiber-based foods and dietary supplements [12]. The by-product of date processing industries could be regarded as an excellent source of food ingredients with interesting technological functionality that could also be used in food as an important source of dietary fiber [37].

**Fatty Acids Content of Roasted Date Pits:** Fatty acids profile was identified in roasted date pits by gas chromatography apparatus as shown in Table 2. The data showed that unsaturated fatty acid comprised 48.66%, however the saturated fatty acids were 51.34%. This characteristic would add to its stability during storage. Oleic acid the mono unsaturated fatty acid was found to be the main fatty acid present in the date pits (40.00%),

Table 3: Amino Acids Content of Roasted Date Pits(g/100 g protein)

Amino acids	Date pits
Isoleucine	2.88
Leucine	6.10
Lysine	2.37
Cystine	0.84
Methionine	1.02
Total sulfur amino acids	1.86
Phenylalanine	5.93
Tyrosine	5.05
Histidine	2.88
Total aromatic amino acids	13.86
Therionine	2.88
Valline	4.57
Aspartic	7.63
Glutamic	16.44
Glycine	4.74
Alanine	4.24
Serine	2.71
Arginine	8.13
Proline	3.55

Table 4: Mineral Contents of Different Date Pits (mg/kg)

Constituents	Concentration (mg/kg samples)
Calcium (Ca)	189.66
Magnesium(Mg)	770
Potassium (K)	3790
Iron (Fe)	36.4
Zinc(Zn)	10.76
Manganies (Mn)	6.80
Sodium (Na)	155
Phosphorus(p)	1225.30
Copper(Cu)	5.30

linoleic acid the poly unsaturated fatty acid, amounted in 8.66%. This finding was in agreement with Biglar *et al.* [38] who found that the oleic acid made up nearly half of the date pits oil ranged from 33.38% to 51.40%. Green [39] stated that the quality of oil and its use depends on the proportion of oleic and linoleic acid.

Lauric acid (15.59%), myristic acid (10.70%) and palmitic acid (9.40%) were the major saturated fatty acids in the pits oil. De Roos *et al.* [40] investigated that substitution of hydrogenate unsaturated fatty acid in margarine preparation using lauric acid generates a favorable lipid profile and decrease the risk of cardiovascular diseases. So, the date pits oil may introduce a new valorization in this direction.

**Amino Acids Content of Roasted Date Pits:** Amino acids of roasted date pits were recorded in Table 3. Glutamic acid was the major amino acid (16.44 g/100g protein) followed by arginine, aspartic and leucine (8.13, 7.63 and

6.10 g/100g protein). The present data is in agreement with findings of Bouaziz *et al.* [41] who detected 17 amino acids from Tunisian date palm fruit pits and found that glutamic acid was the major one. Date pits were rich in total aromatic amino acids (phenylalanine tyrosine and histidine) which recorded (13.86 g/100g protein), as compared with the FAO/WHO [42] which recorded (6.0 g/100g protein). Therefore, date pits could be a very well complement those protein sources that are low in aromatic amino acids.

**Mineral Content of Roasted Date Pits:** Minerals have many health benefits. They are generally important as constituents of bones, teeth, soft tissues, muscle, blood and nerve cells. Minerals are also vital to overall mental and physical well-being [43]. Macro- and microelement concentrations of different date pit are presented in Table 4. Ca, Mg, K, P and Na contents of seeds were found at the high concentrations. Among the minerals, K was found at high levels, its concentration was 3790 mg/kg. Potassium is an important electrolyte in the body which is intimately associated with sodium metabolism. Potassium is essential for the transport of nutrients into each cell and waste products out of the cell; it also helps to normalize the heartbeat. Deficiency of potassium might lead to nervous disorders, insomnia and other serious complications [44].

Phosphorus values were established 1225.30 mg/kg, followed by magnesium, calcium and sodium 770, 189.66 and 155 mg/kg, respectively. These data agreed with those reported regarding Ca, Mg, K and P contents of date seeds which found at the high concentrations [45]. Among the microelements, Fe was found at the high concentration of 36.4 mg/kg. In addition to zinc, found in pits powder, determined to be 10.76 mg/kg followed by Mn and Cu.

**Identification of Phenols in Roasted Date Pits:** Seventeen phenolic compounds of roasted date pits were identified and presented in Table 5. Roasted date pit was found to contain different classes of phenolic compounds that ordered from the largest to smallest amount as follows; Pyrogallol, Cinnamic acid, benzoic acid, Elagic acid, Catechol, Protocatechoic acid, Syringic acid, Caffeine, Vanillic acid, epicatechins, Chlorogenic acid, P-OH-Benzoic, Ferulic, Catechin, gallic acid, caffeic acid, 4-amino-benzoic. Pyrogallol is often used to investigate the role of O<sub>2</sub> in biological systems. It is much more efficient in scavenging O<sub>2</sub> than catechol [46].

Table 5: Identification of Phenols in Roasted Date Pits

Phenols	Amount (mg/100g)
Pyrogallol	57.9449
Benzoic	37.0526
Cinnamic	37.402
Elagic	36.823
Catechol	22.5869
Protocatechoic,	17.2504
Syringic	15.5487
Caffeine	6.6616
Vanillic	6.2343
Epicatechin	4.8443
Chlorogenic	4.7859
P-OH-Benzoic	4.6488
Ferulic	4.4243
Catechin	3.3788
Gallic	0.89456
Caffeic	0.7778
4-amino-benzoic	0.51066

Table 6: Flavonoids Identification of Roasted Date Pits(mg/100g)

Flavonoid	mg/100g
Hesperdin	17.2705
Narengin	7.7034
Rutin	4.3942
Hesperitin	2.1445
Kaempferol	1.3496
Quercetin	1.0571
Rosmarinic	0.7242
7-hydroxy flavon	0.6702
Narenginin	0.4186

Pyrogallol is the major phenolic compounds presented in date pits (57.94mg/100g). The content of Phenolic acids, benzoic and cinnamic acids were 37.05 and 37.4mg/100g respectively. Benzoic acid hydroxylated derivatives such as gallic acid, catechol, protocatechoic acid, p-hydroxybenzoic acid, Syringic and vanillic acid, also cinnamic acid derivatives such as caffeic acid, ferulic acid and coumaric were identified in this study. These compounds were identified in date pits by Al-Farsi and Lee [47] which confirmed the obtained results.

Phenolic compounds of fruit seeds mainly phenolic acids, have been shown to possess such benefits as antioxidant, anticarcinogenic, antimicrobial, antimutagenic and anti-inflammatory activities, as well as reduction of cardiovascular diseases [13]. Thus, it is considered important to increase the antioxidant intake in the human diet and one way of achieving this is by enriching food with phenolics.

#### Identification of Flavonoids in Roasted Date Pits:

Flavonoids are polyphenolic compounds comprising 15 carbons, with two aromatic rings connected by a three

carbon bridge. Nine flavonoids from date palm pits were fractionated and identified as showed in Table 6. Hesperdin that may has potential health benefits, such as the prevention of atherosclerosis progression, lowering cancer risks, bone loss inhibition and positive effects on vaginal symptoms [48, 49], was the major flavonoid component represented in 17.27mg/100g sample followed by narengin (7.70mg/100g), rutin (4.39mg/100g) and hesperitin (2.14mg/100g). However, kaempferol, quercetin, rosmarinic, 7-hydroxyflavon and narenginin were represented in smaller amounts. Our obtained results revealed that seeds of dates contain large amount of flavonoids which confer health benefits as radical scavenging activities, reduction of chronic diseases, cardiovascular disorders and play as antioxidant [50, 21]. Paranthaman *et al.* [20] determined the content of the flavonoids of raw and roasted date pits by HPLC methods and found that the flavonoids content was higher in roasted date pits compared to raw one.

#### The *In vitro* Cytotoxic Activity of Roasted Date Pits:

Table 7 showed the cytotoxic activity of roasted date pits with different concentrations against WISH (normal amniotic cells), HepG2 (Human hepatocellular carcinoma) and HCT116 (colon cancer) cell lines was examined using SRB assay. The increase in the growth inhibition was recorded in dose-dependent manner. Cell viability percentage decreased to 53.65, 79.95 and 85.33% in HCT116, HepG2 and wish cell lines, respectively at concentration of 100µg/ml. These results indicated that the maximum inhibiting effect of date pits was found to be against colon cancer cell lines. This detected cytotoxic effect of roasted date pits may refer to the phenolic compounds content and its antioxidant effects. Evidences have accumulated to suggest that flavonoids have potential role to suppress tumor cell proliferation and may effectively work as chemo-preventive agents against carcinogenesis in humans [51]. The protective effects of a series of phenolic compounds, phenolic acids and flavonoids on the cytotoxicity of CC14 in rat hepatocytes were stated by Anon *et al.* [52]. These results are in parallel with Paranthaman *et al.* [20] who found that roasted date pits exhibited the highest overall content of caffeine and flavonoids and antioxidant capacity than raw date pits which have a pharmacological active substance depending on the dose.

Manosroi *et al.* [53] suggested that samples that had IC50 value less than 125 µg/mL could be a promising candidate for further development to cancer therapeutic agent and samples with IC50 value between 125 and

Table 7: Cytotoxic Activity of Roasted Date Pits (µg/ml)

Concentration (µg/ml)	Viability %		
	WISH (normal amniotic cells)	HCT116 colon cancer cell line	HepG2 (Human hepatocellular carcinoma)
0	100	100	100
0.01	99.62	80.52	83.75
0.1	98.20	78.20	83.18
1	94.48	78.14	82.50
10	91.26	70.73	81.41
100	85.33	53.65	79.95
IC50	114.35	122.18	200.90

Table 8: Sensory Evaluation of Roasted Date Pits Drink

Treatments	Color 10	Taste 10	Odor 10	Flavor 10	Overall acceptability 10
Control (Cocoa drink)	5.9± 0.74d	5.1± 0.74d	5.6± 0.70d	5.8± 0.63d	5.9± 0.75d
Date pits drink (3%)	7.2± 0.42c	7.8± 0.42c	7.3± 0.48c	7.2± 0.42c	7.3± 0.48c
Date pits drink (6%)	8.2± 0.42b	8.7± 0.48b	8.4± 0.52b	8.4± 0.52b	8.5± 0.53b
Date pits drink (9%)	9.7± 0.48a	9.89± 0.32a	9.6± 0.52a	9.7± 0.48a	9.79± 0.43a
L.S.D	0.4828	0.4433	0.5081	0.4708	0.5126

Values are mean of ten replicates ±SD, number in the same column followed by the same letter are not significantly different at 0.05 level

5000 µg/mL was considered to have moderate potential to be developed into a cancer therapeutic agent. In this study, roasted date pits may be considered as a strong potential source for colon cancer (122.18 µg/mL) and moderate potential for hepatic cancer treatment materials (200.9µg/mL). There was no information on cytotoxic properties of date pits.

**Sensory Evaluation of Date Palm Pits Beverage:** Sensory evaluation is an important indicator of potential consumer preferences. Difference in sensory quality attributed of beverage made by date pits results are given in Table 8. Data indicated that date pits beverage was superior in sensory characteristics as compared with the control samples prepared using cocoa.

The studied samples had relatively high scores for color, taste, odor, flavor and overall acceptability compared to control samples. Significant differences ( $P \geq 0.05$ ) were observed in all samples and showed good overall acceptability. In all cases the scores were higher than 7.00. The scores were increased with increasing date pits powder concentration which confirmed the more acceptability to the panelist and the highest scores were recorded to the date pits drink samples with 9% concentration. The cocoa drink scores were lower than 6.00 because of the lower sugar taste and the lower aroma comparing to tested sample. The results of sensory evaluation suggest that the date pits powder can be successfully used as unique natural alternative to coffee or cocoa as well as the sugar amount in date pits drink

was less than cocoa drink which revealed that date pits could be an excellent source of functional food components [54].

**Diffusion Through Packaging Material:** Pits powder bags material had chosen for roasted date pits powder packaging, because of their convenience in handling and disposal. The rate of diffusion from roasted date pits loose form and inside a pits powder bag membrane was determined at 80°C. After many experiments, it was observed that smaller particles of roasted date pits powder diffused faster inside pits powder-bags than larger-sized loose powder. This result was in the same line with Jaganyi and Mdletshe [33] who mentioned that the overall infusion rate of smaller tea leaves inside tea-bags increased over that with larger-sized loose tea leaf.

## CONCLUSION

Considering chemical composition and sensory evaluation of roasted date palm pits it could be concluded that this by-product could be valuable and excellent source of functional food components and low-priced cocoa substitute beverage for the very rapidly price climbed of cocoa. Also, date pits could be regarded as an excellent source of dietary fiber. The results illustrated that roasted date pits have a pharmacological active substance and inhibited colon cancer due to the high content of flavonoids and phenols which increase the antioxidant capacity.

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