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Original Article

Antioxidant-Antibacterial properties and chemical composition of Bekrari and Bronci Libyan date palm fruits.

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Abstract

This study evaluated the cortex, pulp and seed of Libyan Bekrari and Bronci date varieties for antioxidant, antibacterial and nutritional properties using standard methods. For both varieties, tannins, flavonoids, reducing sugars and cardiac glycosides were found in the cortex, pulp, and seed, whereas coumarins were only detected in the cortex and pulp. Saponins were present in seeds and alkaloids were absent in both varieties. The IC_{50} for Bekrari and Bronci seed and pulp extracts were 0.092 - 0.047 mg/ml and 8.81 - 4.98 mg/ml, respectively. The concentration of cortex extracts to reduce DDPH free radicals by 50 % were recorded at 4.25 and 3.69 mg/ml for Bekrari and Bronci, respectively. The seed extract of Bekrari at 100, 50, 25, and 12.5% showed antibacterial activity against *Staphylococcus aureus* with inhibition zones 15.6, 13.3, 13, and 12 mm, respectively; and *MRSA* with inhibition zones 18.3, 16, 14, and 13 mm, respectively. The seed extract of Bronci also inhibited *S. aureus* and *MRSA* giving zones of inhibitions 16, 14.5, 12.5, 11 mm and 16.5, 14.2, 12.5, mm respectively, at the various concentrations. The Bronci and Bekrari seeds respectively contained 6.59 - 6.02 % protein, 5.48-6.60 % fat, 8.36-8.25 % moisture, 1.12-1.16 % ash, 38.45-39.60 % fiber, 0.80-1.24 % fructose, 1.05-1.24 % glucose and 1.32-1.56 % sucrose. The Bronci and Bekrari pulp respectively contained 3.47-2.25 % protein, 0.05-0.04 % fat, 7.67-7.84 % moisture, 1.72-1.85 % ash, 2.26-2.74 % fiber, 32.27-37.46 % fructose, 37.17-40.52 % glucose and 37.17-0.10 % sucrose. The Bronci and Bekrari cortex respectively contained 4.87-3.82 % crude protein, 0.65-0.29 % fat, 7.46-4.66 % moisture, 2.74-1.59 % ash, 17.55-9.79 % fiber 17.95-20.01 % fructose, 17.32-18.41% glucose and 0.01-0.063 % sucrose. These results revealed and highlighted the strong antimicrobial, antioxidant and nutritional potential of Libyan date varieties.

Key words: Antioxidant, Antibacterial, Chemical composition, Date palm.

Introduction

The date palm (*Phoenix dactylifera* L.) belongs to Palmae family (Assirey, 2021). It is cultivated in the Middle East and North Africa countries and plays an essential social, environmental, and economic values

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for many people living in these countries and it is believed to be the most important tree in most of the Arab countries (Jamil *et al.,* 2010). The production of dates has elevated worldwide to reach 7.68 million tons in 2010 from about 4.6 million tons in 1994 (Al-Farsi and Lee, 2008). However, in Libya the average production level of dates in 2008-2012 was about 161.41 thousand tons and it increased to reach 174.04 thousand tons in 2015 (Arab agricultural statistic, 2016) There are five stages of the ripening of dates which include Hababauk, Kimri, Khalal, Rutab, and Tamer (Al-Mssallem *et al.*, 2013). The date palm distributes in three area of Libya. The coastal area characterized by the presence of fleshy-fruited varieties of dates "Alami," "Bronci," "Bekrari," and "Helawi". The central zone contains semi soft varieties "Deglet-Nour," and "Alhamrai." Additionally, the southern oases involve less succulent varieties "Tagiat," "Halima," and "Abel" (Lahmer et al., 2004) . Generally, date fruits are an important source of energy as they contain high carbohydrate content (70–80%) (Maqsood et al., 2020., Yahaya et al., 2015). In addition, date fruits contain protein (2.30–5.60%), fat (0.20–0.50%), dietary fiber (6.40–11.50%), minerals (0.10–916 mg/100 g dry weight), and vitamins C, B1, B2, B3, and A (Mohamed et al., 2014). Date fruits contain important phytochemicals, including phenolics, carotenoids, and flavonoids. Date fruits have many health benefits such as; antioxidant, antimutagenic, and, immune modulatory benefits. Furthermore, they have wide medicinal properties, including antihyperlipidemic, gastroprotective, hepatoprotective, and nephroprotective properties. In addition, it was found that *Phoenix dactylifera* and its constituents play an essential role in the prevention or treatment of bacterial infections. Regarding to date seeds, they are grounded and used to produce caffeine free coffee in many Arab countries (Al-Farsi and Lee, 2008). However, grounded seeds are added to the food of some animal (Al juhaimi *et al.*, 2012). The date fruit consists of four parts: pericarp, mesocarp, endocarp, and one seed (also called kernel, pit).

This study highlights the chemical composition, phytochemical composition, antioxidant activity, and antibacterial properties of date cortex. Considering the nutritional significance of dates, investigation of their phytochemical composition and nutritional quality are increasingly being recognized as a valuable and essential, additionally, published studies are limited on the antioxidant, antibacterial and chemical composition of Libyan dates. Therefore, the aims of the present study are to preliminary phytochemical screening of extracts of date palm fruits, and to evaluate the antioxidant activity of methanolic extracts of date palm fruits and evaluate the antibacterial activity of methanolic extracts of date palm fruits against different bacteria strains and to evaluate the nutrition value of date palm fruits. - and to estimate sugars of each part of date palm fruits separately.

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Materials and Methods

Bacterial strains:

The antibacterial activity of extracts was assessed against five bacterial species: Gram positive, methacillin resistant Staphylococcus aureus (MRSA) ATCC 43300, Staphylococcus aureus ATCC29213; *Klebsiella pneumonia* ATCC13 883, Gram - negative *Escherichia coli* ATCC 25922, *and Pseudomonas aeruginosa* ATCC 9027.

Plant material:

Two varieties of date palm fruits locally known as "Bekrari" and "Bronci" were collected at the end of September, 2018, from date palm orchard in the coastal area (Souq Aljumaa, Tajura).

The samples were identified, authenticated, and deposited in the Libyan National Herbarium under voucher number (D683611), Department of Botany, Faculty of Sciences, and University of Tripoli.

Preparation of plant material:

The ripe fruits of *Pheonix dactylifera* were cut then the seeds and cortex were isolated separately. Thereafter, the flesh (pulp), seeds, and cortex were dried in an oven at 40 °C, the dried pulp, seeds, and cortex were grounded by using an electric grinder to the fine powder and stored in a dry place in airtight container. The extracts of *P. dactylifera* seeds, pulp, and cortex extracts were prepared as described by (Zehra *et al.*, 2015). Each 10 gm of dried seeds, pulp and cortex were extracted separately by adding 90 ml methanol (99%) in a flask, then mixed by a magnetic stirrer for a half an hour and after that filtered with filter paper Whatman's No 41. The remaining materials were re-extracted three times with methanol. The extracts were concentrated by using rotary evaporator at 40 °C and exposed to air to allow evaporation of methanol.

Preliminary phytochemical screening of the extracts:

All extracts (seeds, pulp, and cortex) of *P dactylifera* L were subjected to qualitative chemical screening for identification of various classes of active chemical constituents as following:

-Test for coumarins (fluorescence test):

0.5 g of each extract was dissolved with sodium hydroxide, then spotted on filter paper and examined under UV 366 lamp. The appearance of blue fluorescence spots indicated the presence of coumarins (Suman *et al.*, 2014).

-Test for tannins (Ferric chloride test):

A few drops of 5% ferric chloride solution were added to each extract. The formation of green to blue color was considered as a positive test for tannins (kokate, 2000).

-Test for saponins:

0.5 grams of each extract was shaken vigorously with distilled water for 15 min. The formation of a persistent froth indicated the presence of saponins (kokate, 2000).

-Test for alkaloids:

A few milligrams of each extract (seeds, pulp, and cortex) was separately stirred with 6ml of 1% HCl on a water bath for 5 min and filtered. 1ml of each filtrate was taken individually into 3 test tubes. The first portion was treated with a few drops of Dragedorff's reagent, the second portion was treated with Mayer's reagent and the third portion was

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treated with Wagner's reagent. Turbidity or precipitation with these reagents was considered as evidence for the presence of alkaloids (Abdullahi *et al.*, 2013).

-Test for Flavonoids:

Shinoda test: Ten milligrams of each extract was added to a pinch of magnesium metal, followed by addition of the few drops of concentrated hydrochloric acid. The appearance of a pink color indicated the presence of flavonoids.

Alkaline reagent test: Few drops of sodium hydroxide solution added to the methanolic extract. The appearance of an intense yellow color indicates the presence of flavonoids (Ramos and Bandiola, 2017).

-The standard test for combined reducing sugars:

The solution of the extract was hydrolyzed by boiling with 5 ml of dilute hydrochloric acid (HCl). This was neutralized with sodium hydroxide solution followed by addition of Fehling's solutions. The brick-red precipitate indicates the presence of combined reducing sugars (Ramos and Bandiola, 2017).

-Keller-Killiani test:

One milliliters of glacial acetic acid and 1-2 drops of $FeCl_3$ were added to the methanol extracts, followed by the addition of 1ml of concentrated sulphuric acid at the side of a test tube. A brown ring at the interface confirmed the presence of a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring.

Quantitative DDPH free radical scavenging activity assay:

The free radical scavenging capacity of *P. dactylifera* extracts was determined by using DDPH method (2,2′-diphenyl-1-picrylhydrazyl radical). A solution (0.001% w/v) was prepared in 99% methanol. The stock solution (20-100 mg/ ml) was prepared by mixing all extracts of *P. dactylifera* with solvent. The extracts of *P. dactylifera* were added to cuvettes that contain freshly prepared DDPH solution followed by serial dilutions (1mg to 500 mg) to every cuvette so that the final volume was 1mL and after 30 min, the absorbance was measured at 520 nm using a spectrophotometer. Ascorbic acid was used as a reference standard and was dissolved in methanol to make the stock solution with a concentration of 1 mg/ml. A control sample contained the same volume without extract and reference ascorbic acid. The blank was prepared with 99% methanol (Lara *et al.*,1998).

Percentage Scavenging of the DPPH free radical (2,2-diphenyl-1-picrylhydrazyl)was estimated by using the following equation1:

$$\% \text{ Scavenging Activity} = \{A_0 - A_1\} \times 100 / A_0 \quad (1)$$

where A_0 is the absorbance of the control without a sample. A_1 is the absorbance after adding the sample.

The inhibition curve was plotted for duplicate experiments and represented as % of mean inhibition ± standard deviation. The IC_{50} value was estimated from the graph obtained by using standard ascorbic acid following formula "y=mx+c" from the slope of the graph. The IC_{50} represents the concentration where 50% inhibition of the DPPH radical is obtained.

In vitro antibacterial activity of extracts

Preparation of turbidity standard:

To standardize the inoculum density for a susceptibility test, a BSO_4 turbidity standard equivalent to a 0.5 McFarland standard was prepared

as described by (Cheesbrough, 2009). The antibacterial activity of each extract was conducted by the agar well diffusion method using modified version method of (Daoud *et al.*, 2015). The turbidity of Bacterial suspensions was adjusted to 0.5 McFarland standard, then it was spread on the surface of agar using a sterile cotton swab. Cavities (wells) of 8 mm in diameter were punched in the MH agar plates using a sterilized cork porer. Each extract was dissolved in DMSO 2% and wells were filled with 100 µl of different final concentrations of each extract (100, 50, 25, and 12.5%). DMSO was used as a negative control and Ciprofloxacin (5µ/disc) was used as a positive control. The plate was left for 2 h to facilitate the diffusion of the extracts in the agar (Trigui *et al.*, 2013), and then incubated at 37 °C for 24 h. Antibacterial activity was determined by measuring the diameter of the inhibition zone around the wells in millimeters mm.

Determination of chemical composition:

Crude protein, crude fat, moisture content, total ash, crude fiber, and sugars experiments for pulp, seeds, and cortex were conducted according to Association of Official Analytical Chemists (AOAC, 1992).

Estimation of sugars:

The method used to estimate sugars level was High Performance liquid chromatography (HPLC) coupled with an Evaporative Light Scattering detector (ELSD). The separation was carried out on BEH Amide 1.7 µm3.0×15column. The mobile phase consists of acetonitrile 80% and 20% highly purified type 1 HPLC grade water with triethylamine for better resolution. The flow rate was kept at 0.5 ml /min. The injection volume was 5 microliters and the injector temperature was 50 °C. The pressure was 370 kpa and the oven temperature was, 35℃ and the Gain was 6.

Statistical analysis:

All analytical determinations were performed in triplicate (n = 3). The results were statistically analyzed and was performed by using the Student's t-test (Hill, 1971). Data obtained was analyzed using analysis of variances to determine the significance ($p<0.05$).

Results and Discussion

Extraction yields:

The extraction yield of "Bekrari" cortex, pulp, and, seed was 63.45, 74.95, and 64.36% respectively, table 1. Whereas the extraction yield of "Bronci" cortex, pulp, and, seed was 47.63, 72.61, and 57.48 % respectively.

Phytochemical screening of different parts of two varieties of date palm:

The phytochemical investigation was performed for extracts of different parts (cortex, flesh, and seed) of the date palm and discovered the occurrence of important constituents in this plant. Additionally, several studies have provided evidence which confirms that phytochemicals are bioactive and demonstrate medicinal as well as physiological activities (Masmoudi-Allouche *et al.*, 2016). The extracts of this plant could be a source of promise drugs which contribute to the treatment of disease. Consequently, it is recommended to perform additional investigations to isolate, identify, and

purify these phytochemicals. The results of the phytochemical screening of pulp, seed, and cortex parts of "Bekrari" and "Bronci" dates are shown in table 2. The coumarins were present in the pulp and cortex of both varieties and absent in the seed of the two varieties. The tannins and flavonoids were present in the pulp, seed, and cortex of two varieties. However, the saponins were present only in the seed of both varieties and the alkaloids were absent in all parts of both varieties. Regarding the sugars and glycosides, both of them have existed in all parts of "Bekrari" and "Bronci" date varieties. Our results were consistent with the previous findings of Sadiq *et al.*, (2013), where they searched for the phytochemicals in the water extract of the pulp and the seed of *P. dactylifera*. Their results were similar to ours except for the saponins, where they detected it in the flesh of the date. However, Yahaya *et al.*, (2015), reported that the saponins were absent in the petroleum ether flesh extract of some date varieties and present in the others.

Table 1: Extraction yield of "Bekrari and Bronci" variety samples

	"Bekrari" variety			"Bronci" variety		
Plant	Cortex	Pulp	Seed	Cortex	Pulp	Seed
Sample/gm	10.00	10.00	85.29	10.00	10.01	85.05
Extraction yield %	63.45	74.95	64.36	47.63	72.61	57.48

Table 2: Phytochemical screening of extracts of pulp, seed, and cortex "Bekrari" and "Bronci" varieties.

	"Bekrari"			"Bronci"		
Phytochemicals	Pulp	Seed	Cortex	Pulp	Seed	Cortex
Coumarins	+	-	+	+	-	+
Tannins	+	+	+	+	+	+
Saponins	-	+	-	-	+	-
Alkaloids	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+

Key: *(+) present *(-) absent

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***In vitro* evaluation of antioxidant activity**

Quantitative DPPH free radical scavenging activity assay:

The antioxidant capacity of methanolic extracts of seed, pulp, and cortex was evaluated using DDPH assay. (DPPH) is a dye free radical which has an odd electron. Additionally, DPPH radical is characterized by a strong absorption band at 520 nm. Thereby, the change in absorbance formed by reduced DPPH was used to assess the capacity of fruits, pits, and cortex from "Bekrari" and "Bronci" date palm varieties to work as a free radical scavenger.

The experimental data revealed that the methanolic seed extracts for both varieties showed strong antioxidant activity compared with pulp and cortex extracts, whereas the IC_{50} values for "Bekrari" and "Bronci" seed extracts were 0.09-0.05 mg/ml respectively as shown in table 3. According to a study that was conducted by Masmodi-Alloche et al. (2016) to evaluate antioxidant activity of Tunisian date palm fruits and pits, free radical-scavenging capacities of "Rchidi" and "Fitim" date pits methanolic extracts were in concord to those reported by the current study. On the other hand, the concentrations of pulp extracts which reduced DDPH free radicals about 50% ranged about 8.81mg/ml for "Bekrari" pulp extract and 4.98mg/ml for "Bronci" pulp extract. According to the findings of the same research work by Masmoudi-Allouche *et al.*, (2016), our results have disagreed with their results regarding the IC_{50} of methanolic pulp extracts. Generally, the present study revealed that the antioxidant activity for both cortexes was higher than the pulp, where the IC_{50} value for "Bekrari" cortex was 4.25 mg/ml whereas, for "Bronci" cortex was 3.69 mg/ml. Accordingly, the antiradical activity of seeds for both varieties was the highest compared with the pulp and cortex. The presence of phenolic compounds, Flavonoids and tannins may be responsible for antioxidant properties of date palm fruits. Moreover, other compounds, for instance, vitamin E, vitamin C, carotenoids, and selenium are related to free radical scavenging activity of plants (Fain, 2004). It was found that the phenolic compounds are considered as a reducing agent, due to they have high reactivity as hydrogen donor and also have a metal chelating properties. Thereby, these render them possess antioxidant activity (Keskes *et al.*, 2014), and also have a metal chelating properties.

Table 3: Antioxidant activity of methanolic extracts of both varieties.

	"Bekrari"			"Bronci"			Ref.
IC_{51}	seed	pulp	cortex	seed	pulp	cortex	Ascorbic acid
mg/ml	0.092	8.81	4.25	0.047	4.98	3.69	0.05

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***In Vitro* antibacterial activity of date palm extracts by agar well diffusion method:**

In the present investigation, the antibacterial activities of for cortex, pulp, and seed methanolic extracts of "Bekrari "and "Bronci " date varieties were evaluated against two strains of Gram-positive bacteria (*S. aureus* and *MARSA)* and two strains of Gram-negative bacteria (*E. coli* and *S .poona*, table 4 a,b,c). In addition, the Ciprofloxacin was used as a positive control, whereas the DMSO was used as a negative control. The experiment demonstrated that the methanolic extracts of the cortex and pulp of both varieties did not reveal antibacterial activity against Gram-positive and Gram-negative bacteria. However, the seed extract of "Bekrari" variety exhibited antibacterial activity at different concentrations 100, 50, 25, and 12.5% against Gram-positive bacteria *S. aureus* and inhibition zones were 15.5, 13.3, 13, and 12 mm respectively.

Additionally, it also inhibited the growth of *MARSA* with inhibition zones of 18.3, 16, 14, and 11.5 mm for all concentrations respectively. Based on our result the seed extract of "Bronci" variety also possessed antibacterial activity against both *S. aureus* and *MARSA* and caused inhibition zones ranged between 16-11 mm with *S. aurous* at all concentrations. Whilst, 16-11mm with *MARSA.* Though, both seed extracts did not show any antibacterial activity against tested G-negative bacteria. The resistance of G-negative bacteria toward plant extract is owing to the difference in the cell wall and membrane structure. 2 tailed t-test revealed that the there is a significant difference (p<0.05) between "Bekrari" and "Bronci" extract against *S. aureus* and *MARSA* bacterial strain. Similarly, a study by Zehra *et al.*, (2015) assessed the antibacterial activity of four Omani date varieties "Khalas," "Khasab," and "Fardh." Their findings ravealed that the methanolic extracts of all date varieties did not show any antibacterial activity against Gram-negative *E. coli* and *S. typhii* and other bacterial strains. The antibacterial activity of dates can be different according to date fruit variety. Comparatively, a recent study was conducted on different varieties of dates to evaluate their antibacterial activity. It was observed that the methanol extract of "Safawi" date variety did not exhibit antibacterial activity against Gram-positive *S. aureus.* Conversely, the "Mabroom," "Ajwa," and "Mariami" methanol extracts showed antibacterial activity on *S. aureus.* Additionally, the extracts of "Mabroon" and "Mariami" date varieties did not reveal antibacterial activity against Gram-negative *E. coli.*, whereas, "Safawi" and "Ajwa" date varieties inhibited the growth of *E. coli* (Samad *et al.*, 2016).

In line with our findings regarding the antibacterial activity of seed extract, the study which investigated antibacterial activity for methanolic extract of "Safawy" date seed reported that the methanolic seed extract did not inhibit the growth of Gram-negative *E. coli* (Sufiya and Thigle, 2014). Nevertheless, the same study demonstrated that "Safawy" seed extract did not show antibacterial activity against Gram-positive *bacteria S. aureus* (Sufiya and Thigle, 2014). These findings are

different to those reported by the current study where both seed extracts showed antibacterial activity against *S. aureus*. Moreover, another study revealed that the methanolic extracts of Tunisian date pits "Kentichi," "Deglet-Nour," and "Ruchdi" showed antibacterial activity against *S. aureus* with the zone of inhibition ranged between 14-13-11 mm respectively, whiles "Ftimi" date pit extract did not inhibit the growth of *S. aureus.* moreover, "Kentichi" and "Deglet-Nour" date pit extracts had an inhibitory effect against *E. coli*. though, pits methanol extracts of "Ruchdi" and "Ftimi" date varieties did not show antibacterial activity against *E. coli* (Masmoudi-Allouche *et al.*, 2016). The antibacterial activity of plants methanolic extracts is related to the presence of polyphenols compounds. The ability of phenolic compounds to act as the antibacterial agent is associated with their capability to denaturalize proteins and cause the outflow of cytoplasmic constituents such as minerals, proteins and cross the cells wall. In addition, Polyphenols are able to bind to the peptidoglycan leading to the breaking of the bacterial cell-wall integrity (Dahech, *et al.*, 2013).

The chemical composition of "Bronci" and "Bekrari" different parts varieties:

The chemical composition of the pulp, seed, and, cortex of "Bronci" and "Bekrari "date varieties were determined and reported in table5. The value of crude protein ranged between 3.47-6.5% for the seed, pulp, and cortex of "Bronci" date variety while the value of crude protein ranged between 2.25-6.02 % for the seed, pulp, and cortex of "Bekrari" date variety. There was a significant difference (p<0.05) in the protein percentage of each part of the date between the two varieties. Regarding the percentage of protein in the seed of the two varieties, we reported percentages that were in line with the percentages of protein in the seed of "Fard," "Khalas," and "Lulu" varieties that have been studied by the group of researchers in the United Arab Emirates (Hamad *et al.*, 2002). Regardless of these findings, there is another research work that showed values that are quite different from ours as the percentages of protein in date flesh and seed were 17.15-12.6% respectively (Sadiq *et al.*, 2013). This difference could be attributed to different factors including, differences in harvesting regions, ripening stages, climate, agriculture resources, and nature varieties differences. Our findings of protein percentage in the pulp of the two varieties were similar to the findings of Al-harrasi *et al.* (2014), where they determined the protein percentage in the pulp of 22 date varieties that were harvested in Oman. Regarding the percentage of protein in the cortex, we could not find enough data in the literature. This reason explains the differences in protein percentages in the three parts of our varieties. Relating to the percentage of fat in the seed of the two varieties, the values were 5.48, 6.60% in "Bronci" and "Bekrari" seed respectively. These values were in accordance with the reported

values of (Alfarsi *et al.*, 2007). Concerning the percentages of fat in the flesh of the two varieties, the values ranged between 0.04-0.05 % of "Bekrari" and "Bronci" varieties respectively. Al-harrasi *et al.*, (2014), studied the percentage of fat in 22 varieties of date palm, it was observed that the fat percentages in two date palm varieties were similar to our results. Conversely, Sadiq *et al.*, (2013), reported that the percentages of fat were 1.52-4.50% in flesh and seed respectively. Furthermore, the percentages of fat ranged between 0.65-0.29% for "Bronci" and "Bekrari" cortex respectively. Besides, a significant difference was found (p<0.05) in fat percentage between the seed of both varieties and cortex of two varieties. Whilst, the percentage of fat in the pulp of both varieties was no significantly different (p>0.05). The percentages of moisture content were 8.36-8.25% for "Bronci" and "Bekrari" date seeds respectively. These values that were observed in this study were within the range of values that was presented by a previous study conducted by (Al juhaimi *et al.*, 2012). Nevertheless, according to the study conducted by Metoui *et al.*, (2017) on twelve Tunisian date seed, they reported a high percentage level for moisture content which ranged between 16.06 and 29.47%. Moreover, our data showed that the percentage of moisture in the "Bronci" pulp is 7.67±0.22% whilst, in" Bekrari" pulp is 7.84±0.14%. However, in contrast, these findings are lower than those previously obtained by (Assirey, 2014). There was no significant difference in moisture content between the seed of both varieties and between pulp of both varieties (p>0.05). It was found that the moisture content was significantly different (p<0.05) between cortex of both varieties The moisture content varies according to the date varieties and its origin. The same varieties manifest difference in moisture content due to climate conditions, drying conditions, storage and harvesting period. There is a reduction of moisture content from 85% at the first stage to reach 24% at full ripeness during the development of dates (Metoui *et al.*, 2017). Relating to the percentages of total ash in "Bronci' and 'Bekrari" seeds the values were 1.12-1.16% respectively. Al Juhaimi *et al.,* (2012), investigated the total ash of seven date seed and they reported values in line with our results. Statistically, the total ash percentage in the seed of both varieties was no significantly different (p>0.05). Moreover, the percentages of total ash in "Bronci" and "Bekrari" pulp were 1.72—1.85 % respectively. Our results of total ash percentages were in broad agreement with those findings which were presented by the study of Al-harrasi *et al.*, (2014). Furthermore, there was a significant difference (p<0.05) in total ash percentage between the "Bronci" and "Bekrari" pulp. Our study also determined the percentage of crude fibers in the seed, pulp, and cortex of "Bronci" and "Bekrari" Libyan date varieties and it revealed that the seeds contain a remarkable or a considerable fiber percentage which ranged about 38.45% for "Bronci" seed and 39.60 % for "Bekrari" seed. We recorded values higher than those determined by Al Juhaimi *et al.*, (2012). In addition and according to their study the percentages of fibers for seven seed

varieties ranged between 17.07-23.46%. Additionally, the percentage of crude fibers in Bronci pulp was 2.26% whereas, in "Bekrari" pulp was 2.74%. The similar percentages of fiber were reported for Omani date verities by Al-harrasi *et al.*, (.2014). However, Sadiq *et al.* (2013) presented the percentage of fibers as different from that reported by our study. Furthermore, the percentage of crude fibers also was estimated to be 17.55±1.2 in "Bronci" cortex and 9.79±0.2 in "Bekrari" cortex. There was no significant difference (p>0.05) in fiber percentage between seed of both varieties and between the pulp of both varieties. Whereas, there was a significant difference (p<0.05) between the cortex of both varieties.

Estimation of sugars in three parts (cortex, pulp, and seed) of two varieties:

In the present study, small concentrations of sugars were detected in date seeds compared with date flesh and cortex. It was found that the percentages of fructose of both seed varieties "Bekrari" and "Bronci" were 1.24-0.80% respectively. Besides, the percentages of glucose in the seed of "Bekrari" and "Bronci" were 1.56-1.05% respectively. Furthermore, the percentage of sucrose in the seed of "Bekrari" variety was 1.56% whereas, in the seed of "Bronci" variety was 1.32%. Despite these findings, a study by AL Juhaimi *et al.*, (2012) has shown values higher than that reported by the present study. Statistical analysis revealed that there was no significant difference (P>0.05) in fructose and glucose concentration between both seed date varieties but there was a significant difference (P<0.05) in sucrose concentration between both seed date varieties. The percentage of fructose in "Bekrari" and "Bronci" pulp were 37.46-32.27% respectively. Additionally, the percentage of glucose in the pulp of both varieties were 40.52-37.17% respectively. Borchani *et al.* (2010) estimated sugars concentration of 11 Tunisian date cultivars using HPLC, the fructose percentages of four varieties "Alligh," "Coandi," "Kenta," and "Tranja" were in conformity with the results of the current study. Whereas, "Ilkouat" date variety showed similar glucose concentration with that reported by our study. Moreover, in the present study, the percentage of Sucrose in the pulp of "Bekrai" and "Bronci" varieties were 0.10-0.04%. on the other hand, these values of sucrose concentration are lower than that reported by Borchani *et al.*, (2010) . There was a significant difference (p<0.05) in fructose and glucose concentration between pulp of both varieties. The difference in invertase activity in these varieties leads to the difference in sugar composition, which is mainly responsible for the reduction in sucrose content (Fayadh and Al-showiman, 1990). Whereas, the high concentration of reducing sugars in these varieties is related to the marked invertase activity (Elleuch *et al.*, 2008).

In regards, the percentage of sugars in the cortex of both varieties, our results demonstrated that the percentages of fructose, glucose, and sucrose ranged

between 0.06-20.0% in the cortex of both varieties. There was no significant difference (p>0.05) in fructose, glucose, and sucrose concentration between cortex of both date varieties.

Table 4a: Antibacterial activity of "Bekrari" and "Bronci" pulp

	Inhibition zone mm											
	"Bekrari" pulp						"Bronci" pulp					
Bacteria species	100	50	25	12.5	DMSO	Cipro	100	50	25	12.5	DMSO	Cipro
S. aureus	-	-	-	-	-	23.7±0.57	-	-	-	-	-	24±0.00
MARSA	-	-	-	-	-	20.5±0.70	-	-	-	-	-	24.5±0.70
S. poona	-	-	-	-	-	25±0.57	-	-	-	-	-	27.3±0.57
E. coli	-	-	-	-	-	24.3±1.15	-	-	-	-	-	26±1.41

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Table 4b: Antibacterial activity of "Bekrari" and "Bronci" cortex

	Inhibition zone mm											
	"Bekrari" cortex methanolic extract						"Bronci" cortex methanolic extract					
Bacteria species	100	50	25	12.5	DMSO	Cipro	100	50	25	12.5	DMSO	Cipro
S. aureus	-	-	-	-	-	22.3±0.57	-	-	-	-	-	22±0.00
MARSA	-	-	-	-	-	22.3±0.57	-	-	-	-	-	25±0.00
S. poona	-	-	-	-	-	26.5±0.70	-	-	-	-	-	23±0.00
E. coli	-	-	-	-	-	27.6±0.57	-	-	-	-	-	27±0.00

Table 4c: Antibacterial activity of "Bekrari" and "Bronci" seed

	Inhibition zone mm											
	"Bekrari" seed methanlic extract						"Bronci" seed methanolic extract					
Bacteria species	100	50	25	12.5	DMSO	Cipro	100	50	25	12.5	DMSO	Cipro
S. aureus	15.6±0.94	13.3±1.24	13±0.00	12±0.00	-	23±0.00	16±1	14.5±0.5	12.5±0.5	10±0.00	-	25±0.00
MARSA	18.3±0.47	16±0.81	14±0.81	11.5±0.5	-	23±0.00	16.5±0.5	14.5±0.5	12.5±0.5	11±0.00	-	22.5±0.70
S. poona	-	-	-	-	-	25±0.00	-	-	-	-	-	27.3±0.57
E. coli	-	-	-	-	-	25±0.00	-	-	-	-	-	28±0.00

Table 5: The chemical composition of "Bronci" and" Bekrari" different parts.

	"Bronci"			"Bekrari"		
Components (%)	Seed	Pulp	Cortex	Seed	Pulp	Cortex
Crude protein	6.59±0.02	3.47 ±0.01	4.87 ±0.04	6.02 ±0.01	2.25 ±0.05	3.82 ±0.02
Crud fat	5.48±0.05	0.05 ±0.01	0.65 ±0.03	6.60 ±0.04	0.04 ±0.01	0.29 ±0.03
Total ash	1.12±0.02	1.72 ±0.01	2.74 ±0.06	1.16 ±0.01	1.85 ±0.05	1.59 ±0.02
Total fibers	38.45±0.39	2.26±0.04	17.55±1.2	39.60±0.2	2.74±0.02	9.79±0.2

Results are expressed as mean values of three determinations ± SD.

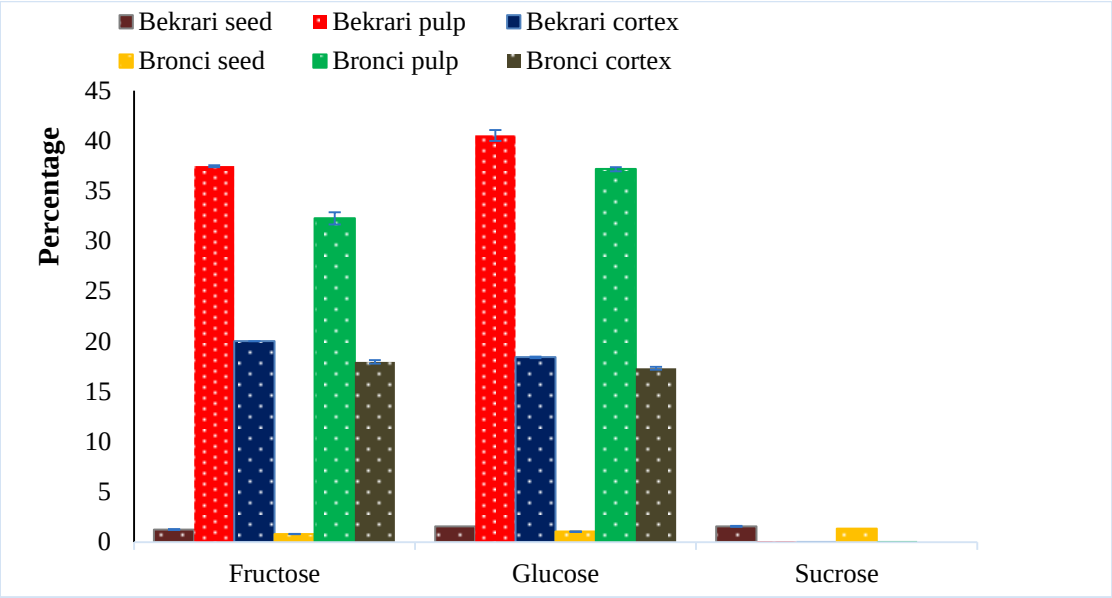

Figure 1:The percentage of sugars in three parts of "Bronci" and "Bekrari" date varieties.

Conclusion

The phytochemical screening of methanolic extracts for cortex, pulp, and seed indicated that all parts of the fruits of dates are the source of important bioactive compounds such as tannins, flavonoids, cardiac glycosides whereas, coumarins were found only in the cortex and pulp of both varieties. Additionally, saponins were detected only on the date seeds of both varieties. These bioactive compounds possess therapeutic properties. Data obtained from antioxidant activity and antibacterial evaluation revealed that the seeds extracts of both date varieties showed a strong antioxidant activity compared to pulp and cortex extracts. Furthermore, the date seeds extracts of both date varieties also possessed antibacterial activity against gram-positive bacteria S. aureus and MARSA. However, they have not shown an inhibitory effect against tested gram-negative bacteria. Furthermore, the pulp and cortex extracts did not show antibacterial activity against both gram-negative and gram-positive bacteria. The chemical composition evaluation and sugars estimation reported that the seeds of both date varieties contain a significant quantity of fibers. Moreover, the percentages of fibers, fat, and protein were higher than that determined in the pulp and cortex in both date seed varieties. However, they contain the low percentage of sugars. In contrary, the pulp of both date varieties contained high levels of sugars and low proteins, fat, and protein thus, it is good source of energy. The percentages of proteins and fat and fibers in both dates cortex were higher than those present in the pulp of both date varieties. Overall, date seed and cortex are considered as waste. This work provides evidence that they are rich in nutritive compounds and minerals and bioactive compounds. In addition, the seed has a potent antioxidant activity. Therefore, we recommend to incorporate it with food and nutraceutical formulation. Further research is required to isolate and identify active constituents which are responsible for antioxidant and antibacterial properties, to study a phenolic profile of date extracts and its relation with antioxidant activity, and to perform a combination including more than extract and evaluate their antibacterial and antioxidant activity

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المجلة الليبية للعلوم الزراعية
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ورقة أصيلة

الخصائص المضادة للأكسدة والمضادة للبكتيريا والتركيب الكيميائي لصنفي البكراري والبرونصي من ثمار النخيل الليبي

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المستخلص

قيمت هذه الدراسة القشرة واللب والنوى من أصناف التمر البكراري والبرونصي الليبي من حيث الخصائص المضادة للأكسدة والمضادة للبكتيريا والتحليل الكيميائي باستخدام الطرق القياسية. تم الكشف على التانينات والفلافونيدات والسكريات المختزلة والجليكوزيدات لكلا الصنفين في القشرة واللب والنوى، بينما تم اكتشاف الكومارين فقط في القشرة واللب. كانت الصابونين موجودة في النوى ولا تحتوي على قلويدات في كلا الصنفين. كان IC50 لمستخلصات نوى بكراري وبرونصي 0.092 - 0.047 مجم / مل و8.81 - 4.98 مجم / مل على التوالي. تم تسجيل تركيز مستخلصات القشرة لتقليل الجذور الحرة DDPH بنسبة 50٪ عند 4.25 و3.69 مجم / مل لكل من بكراري وبرونصي على التوالي. أظهر مستخلص نوى البكراري بتركيز 100 و50 و25 و12.5٪ فعالية مضادة للجراثيم ضد بكتيريا *Staphylococcus aureus* مع مناطق تثبيط 15.6، 13.3، 13، 12 ملم على التوالي. و MRSA مع مناطق تثبيط 18.3 و16 و14 و13 ملم على التوالي. كما أظهر مستخلص بذور برونصي بتثبيط بكتيريا *S. aureus* و MRSA مما أعطى مناطق مثبطات 16، 14.5، 12.5، 11 ملم و16.5، 14.2، 12.5 ملم على التوالي، بتركيزات مختلفة. تحتوي نوى برنصي والبكراري على التوالي على 6.59 - 6.02٪ بروتين، 5.48-6.60٪ دهون، 8.25-8.36٪ رطوبة، 1.12-1.16٪ رماد، 38.45-39.60٪ ألياف، 0.80-1.24٪ فركتوز، 1.05-1.24٪ جلوكوز و1.32 -1.56٪ سكروز. احتوى لب برونصي وبكراري على التوالي على 3.47-2.25٪ بروتين، 0.05-0.04٪ دهون، 7.67-7.84٪ رطوبة، 1.72-1.85٪ رماد، 2.26-2.74٪ ألياف، 32.27-37.46٪ فركتوز، 37.17-40.52٪ جلوكوز و37.17 -0.10٪ سكروز. تحتوي قشرة البرنصي و البكراري على التوالي على 4.87-3.82٪ بروتين خام، 0.65-0.29٪ دهون، 7.46-4.66٪ رطوبة، 2.74-1.59٪ رماد، 17.55-9.79٪ ألياف 17.95-20.01٪ فركتوز، 17.32-18.41٪ جلوكوز و 0.01 -0.063٪ سكروز. كشفت هذه النتائج عن قوة مضادات الميكروبات ومضادات الأكسدة والقيمة الغذائية لهذين الصنفين من التمور الليبية.

الكلمات الدالة : مضادات الأكسدة، مضادات البكتريا، التركيب الكيميائي، نخيل التمر.

للاتصال: ربيعة الأحمر، قسم علوم وتقنية الغذية، كلية الزراعة، جامعة طرابلس، ليبيا.

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