Effect of Gamma irradiation on some biochemical properties, antioxidant and antimicrobial activities of Sakouti and Bondoky dry dates fruits genotypes

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ABSTRACT
This work investigated the effect of gamma irradiation with different doses (0.0, 2.5, 5 and 10 kGy) on some biochemical parameters, antioxidant and antimicrobial activities of two Egyptian dry date fruit genotypes. Reducing sugars (%) were significantly increased to 65.96% and 68.09% at 5 kGy for Sakouti and Bondoky cultivars, respectively. The dose level 5 kGy showed the highest total soluble sugars (84.62% and 87.66%) for Sakouti and Bondoky cultivars, respectively. Sakouti cultivar has higher content of glucose and fructose at dose 5 kGy compared with Bondoky cultivar. Significantly sucrose content in both cultivars was decreased by increasing γ-irradiation dose level. Total phenolic and flavonoid contents were significantly increased at 5 kGy. The highest level (82.85%) of scavenging activity (DPPH %) was recorded at 5 kGy by Sakouti cultivar. Gamma irradiation caused a significant increase in antimicrobial activity of both cultivars against bacterial (Gram+ve and Gram–ve) and fungal strains. Antimicrobial activities against Escherichia coli and Enterobacter Cloacae were more pronounced with Sakouti than Bondoky cultivar. Antifungal activity was stronger than the antibacterial for both cultivars. Results concluded that γ-irradiation enhanced phenolic and flavonoids compounds, the antioxidant activity and antibacterial potentials of the studied two Egyptian dry palm fruits.

1. Introduction
Date palm is one of the most valuable and important fruits to human health. Date fruits are eaten worldwide and considered very necessary industrial crops all over the world (Mrabet et al., 2016). Date fruit is a wealthy energy source (80–85% carbohydrate) providing 200 to 300 Kcal./100 g depending on cultivar and the moisture content. As well as rich in riboflavin, thiamine, folic acid and vital minerals such as iron, copper, sulfur, potassium, and manganese but poor in protein (2–3%) and fat (about 2–3%) (Yousif, Benjamin, Kado, Alldin, & Ali, 1982). However, date fruits are a rich source of phenolic compounds which have in vitro antimutagenic and antioxidant properties (Osman, Al-Humaid, Al-Redhaiman, & El-Mergwali, 2012). Dates have excessive antioxidant content that can prevent many illnesses different than being a powerful agent in the food industry towards oxidative stress. Al-Farsi, Alasarver, Morris, Baron, & Shahidi (2005a) stated that date fruits possess a powerful potency to scavenge and inhibit the free radicals with different pathways. Date extracts have strong antioxidant and free-radical-scavenging activities (Yasin, El-Fawal, & Moussa, 2015). Several studies have revealed a direct relationship between the antioxidant activity and phenolic content on several date fruits (Allaith, 2008). Antioxidants in date fruits may save the body from many degenerative disorders by reducing oxidative stress owing to: (a) the phenolic compounds that suppresses nucleic acids, proteins and lipids oxidative damage (El-Beltagi, El-Ansary, Mostafa, Kamel, & Safwat, 2019b; El-Beltagi, Mohamed, Elmelegy, Eldesoky, & Safwat, 2019a; Nasir et al., 2015) and (b) activating of both enzymatic and non-enzymatic antioxidant systems (Boronov-Neori et al., 2015). Dates are classified by their moisture content, more than 30% of the soft date consists of moisture. The semi-dry dates have a humidity content of 20–30% and dry dates have less than 20% moisture (El-Badawy, 2001). Dates own the highest amounts of polyphenols between all the dried fruits as there is a strong relationship between antioxidant activities of date fruits and concentrations of phenols (Vincon, Zubik, Bose, Samman, & Poch, 2005). The highest postharvest losses in date fruits were due to the spoilage of insect-infected dry fruits, pathological issues in the tropical regions and physiological or chemical changes during storage period Mohammadzai et al. (2010). Bacteria and molds like yeast and fungi caused the microbial infection to date fruits (Tafti & Fooladi, 2005). Thus, there are specific quality processes and treatments that should be applied.
to date fruits when they are designated to export. Some of the chemical treatments that are used in commercial packaging processes of date fruits promote the existence of continuous sorts of pests (Kinay, Mansour, Gabler, Margosan, & Smilanick, 2007). Increasingly, other forms of conservation are either too expensive or would require careful attention. Alternative approaches that would be safe and sustainable for food preservation are needed to enable longer storage duration and reduce meal loss all through storage. Gamma irradiation is a wonderful alternative, comparatively cheap, and fine nonchemical treatment to control the postharvest losses of date fruits (Afify, Rashed, Ebtesam, & El-Beltagi, 2013; El-Beltagi, Mohamed, Mohammed, Zaki, & Mogazy, 2013; Hallman, 2011). Recently, the usage of gamma irradiation is the most ideal methods to take control and disinfect the microbial contamination in dates successfully (Goodburn & Wallace, 2013). Thus, gamma irradiation is considered as an excellent antimicrobial tool.

The purpose of the current study is to evaluate the impact of different doses of gamma irradiation on carbohydrates, phenolic content, antioxidant activity and antimicrobial characteristics of two Egyptian dry date genotypes.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu reagent, 2,2-diphenyl-1- picrylhydrazyl (DPPH), 3,5- dinitrosalicylic acid (DNS), sodium carbonate, Aluminum chloride and chemical HPLC-grade standards (purity >95%) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Other reagents utilized in the experiments were of analytical grade.

2.2. Dates materials

Dry date fruits were gathered from local cultivars grown in Egypt; date palm samples (Phoenix dactylifera L.) weighing 1 kg of the two dry date genotypes (Sakouti and Bondoky) were obtained from the local market, Cairo, Egypt in 2018. Mature fruits with uniform size, free of physical harm, insect's damage selected and used for this study.

2.3. Irradiation process

Gamma irradiation of date fruits was performed in the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt by using 60Co (Indian Gamma cell) Ge-4000A. Dates were placed in clean plastic bags and exposed to various dose levels; 0.0, 2.5, 5.0 and 10.0 kGy at the time of the experiment the dose rate was 1.9 kGy/h.

2.4. Preparation of date flesh extract

The seeds have been manually isolated from the flesh, the flesh samples were grounded using a household grinder, and then they had been kept at −20°C in a sterile plastic bag until extraction. Then, at room temperature, 5.0 gm of each powdered pattern to be extracted with 25 ml ethanol 80% for 24 h over the shaker. Through filter paper Whatman No.1, the extracts had been filtered and the manner used to be repeated three times. Extracts collected were filtered and centrifuged (7000 rpm for 15 min); then, at 40°C, the ethanol was evaporated on a vacuum rotary evaporator. The obtained ethanolic extracts were used to analyze sugars, total phenolic content, total content of flavonoid, antioxidant activity, and evaluation of the antimicrobial.

2.5. Total soluble sugars

The phenol-sulfuric acid technique as reported by Dubois, Gilles, Hamilton, Rebers, & Smith (1956) was used to determine total soluble sugars in ethanol extract of date tissue. At 490 nm the optical density (O.D.) was evaluated using spectrophotometer (Jasco V530) in opposition to blank, which performed as the test except the distilled water was added to the sample instead. Three replicates were used for each sample. Glucose standard curve was used to determine total soluble sugar concentration and result values have been expressed as Glu equal (Glu) per 100 g of sample dry weight (g Glu/100 g D.W.).

2.6. Reducing sugars

Reducing sugars were determined by the colorimetry of 3,5- dinitrosalicylic acid (DNS) in opposition to glucose widespread (Miller, 1959). To one of pattern extract 1.0 DNS reagent was added. The mixture was left for 15 min in a boiling bath of water. After cooling to room temperature (25°C) in a cold water bath, 10 ml of distilled water has been added. The absorbance of obtained glucose solutions was determined at 540 mm (Jasco V 530), interpolating the calculated values of glucose solution of regarded known concentration. The blanks were performed by replacing distilled water with a sample solution. Three replicates were used for each sample. Glucose standard curve was used to determine reducing sugar concentration and results values have been expressed as Glu equal (Glu) per 100 g of sample dry weight (g Glu/100 g D.W.).

2.7. Sugars by HPLC

A sample of extract (2.0 g) was homogenized with deionized water (50 ml), authorized to stand at room temperature overnight sonicated at 40°C in an ultrasonic bath for 30 min and then centrifuged (4,000 rpm, 15 min). Before HPLC assessment, the supernatant
adapted to 50 ml with deionized water was filtered by an aliquot (1 ml) of this solution through a 0.2 μm nylon membrane. The HPLC Knauer, Germany, Column used PhenomenexRezex RCM-Monosaccharide, 300 mm x 7.8 mm operated at 80°C. The separation is achieved using Isocratic elution by HPLC grade water with a 0.6 ml/min flow rate. The quantity that was injected 20 μl. Detection: Refractive Index (RI detector), data integration by clarity-chrom software. The sugar standards were obtained from Sigma and consisted of sucrose, glucose, fructose, and melezitose.

2.8. Determination of total phenolic content

The total phenolic content was determined using Singleton, Orthofer, & Lamuela-Raventos (1999)’s suggested technique. An aliquot of ethanol extract (0.5 ml) was combined with Folin-Ciocalteau reagent (0.5 ml) accompanied with the aid of addition 1.0 ml saturated sodium carbonate solution and accomplished to 10 ml of water. The mixture was made to stand at room temperature in the dark for 1 h. The UV-Vis spectrophotometer was estimated to absorb all samples at 765 nm. Results had been displayed as gallic acid equivalent (GAE) milligram per g of samples dry weight.

2.9. Determination of total flavonoid content

The content of total flavonoids was performed according to the procedures described by Marinova, Ribarova, and Atanassova (2005) aluminum chloride method. Ethanolic extract sample (0.5 ml) was combined with 0.3 ml of 5% sodium nitrite. Five minutes later, 0.3 ml of 10% aluminum chloride was added, 2 ml of 1.0 M sodium hydroxide was added after 6 min, and the complete volume with distilled water was made up to 5.0 ml. The absorption of the blend against the reagent blank was recorded at 510 nm. Results were displayed as the samples dry weight of mg quercetin equivalent (QUE)/g.

2.10. Phenolic compound profile using HPLC

The HPLC analysis of successive methanolic extracts was performed by re-dissolving a hundred mg of extract in 1.0 ml of methanol (80%) and filtering via sterilized membrane 0.2 μm filter prior to HPLC assessment. Injection by means of injection with 50 μl fixed loop using Rheodyne injection values (Model 7125) was used. Two portable phases used a steady flow rate of 1 ml/min; (A) 0.5% acetic acid in distilled water at pH 2.65; and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear beginning with (A) to (B) over 50 min, using a 254 nm wavelength UV detector. The concentration of individual measurements of the polyphenolic peak area was recorded using standard phenolic compounds.

2.11. Determination of antioxidant activity using DPPH radical

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity of sample extracts was determined according to the previous method reported by Gulluce et al. (2004), and were conducted at 517 nm. The following formula was used to calculate the radical-scavenging activity.

\[
\text{DPPH scavenging activity (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100
\]

where

- \(A_0\) = The absorbance of control reaction (containing reagents except the test compounds).
- \(A_1\) = The absorbance in the presence of the tested extracts.

2.12. Antimicrobial of the activity of date fleshes

Antimicrobial activity of the two date fleshes Sakouti and Bondoky tested ethanolic extracts were estimated by the agar disc diffusion method. The used bacterial strains Staphylococcus aureus, (Escherichia coli and Enterobacter cloacae) and (Aspergillus niger, Penicillium sp. & Candida albicans) as representatives of (Gram-positive), (Gram-negative) bacteria and fungi, respectively; they were previously isolated and characterized at Microbiology Department, NCRRT. Atomic Energy Authority, Cairo, Egypt. Cultures were grown on nutrient agar (NA) plates (Peptone 5 g/l; yeast extract 3 g/l; NaCl 5 g/l; lab lemco 1 g/l; agar 15 g/l; dist water 1000 ml/L at pH 7.1 ± 0.1) for bacterial strains and Sabouraud-Dextrose agar (SDA) (Glucose 40 g/l; Mycological peptone 10 g/l; Agar 15 g/l; Dist water 1000 ml/L at pH 5.6 ± 0.2) for fungal strains and maintained in the agar slants at 4°C.

2.12.1. Preparation of bacterial suspensions

To prepare the bacterial suspensions, four to five well-isolated colonies were selected and emulsified in a tube containing sterile distilled water and the suspensions were then diluted in tubes containing sterile distilled water to have a final concentration of 10³. An inoculum of 100 μl of each bacterial suspension was plotted in petri dishes, each containing 20 ml of Muller Hintone agar (MHA) medium g/l (Beef extract 2.0; Acid hydrolyzate of casein 17.5; Starch 1.5; Agar 17.0; Dist water 1000 ml at pH 7.3 ± 0.1) and left hanging for 5 min before inserting the discs.

2.12.2. Evaluation of antibacterial activity

The experimental protocol consisted of depositing a sterile Wattman paper disk (6 mm in diameter) impregnated with 20 μl of extract dissolved in 0.05% of DMSO. The dishes were left for 1 h at room temperature and then incubated at 37°C for 18 h. After incubation, the radius of the inhibition zone was measured in millimeters.
2.12.3. Evaluation of antifungal activity

The disc diffusion method (Wistreich, 1997) was used to investigate the antifungal activity of flesh date different extracts of SDA medium was prepared and poured into 90 mm diameter petri plates. The paper disc size was 6 mm (Whatman no. 1). Each fungus was streaked on the surface of the SDA medium using a sterile cotton swab in order to get a uniform fungal growth on both the control and test plates. A 20 µl from different extracts was placed on the discs. A positive control was made by placing antifungal disc (Flucanazole) on the agar plate. Plates were kept in laminar flow for 30 min for pre-diffusion of extract and then incubated at 28°C for 18–48 h. Antifungal activity was evaluated by measuring the diameter of the inhibition zone (mm) on the surface of plates. Solvent controls were used.

2.13. Statistical analysis

All analyses had been conducted in triplicate, and the outcomes were expressed as imply ± S.E.; comparisons of means were done using one-way analysis of variance (ANOVA) accompanied by Duncan’s test. Statistically significant differences had been considered at p < 0.05. The SPSS/PC computer program statistical package version 25 was used for statistical analysis of outcomes.

3. Results and discussion

3.1. Sugars content

The content and quantity of sugars of Sakouti and Bondoky cultivars are presented in (Table 1), the variance analysis recorded a significant difference (p < 0.05) between reducing sugars (glucose and fructose) values and total soluble sugars for two studied dates’ cultivars. Reducing sugars (%) were increased significantly from 59.97% to 62.46% in control samples by rising the irradiation dose level gradually up to 5 kGy to 65.96% and 68.09% for Sakouti and Bondoky cultivars, respectively. Also, total soluble sugar content (%) was the same trend was observed in three Omani date assortments. In the same concern, Bhat, Sridhar, Karim, Young, & Arun (2009) cited that there was an increase in sugars due to the irradiation which can be attributed to the breakdown of complex sugars (polysaccharides) into simple extractable forms. Mikki, Taisann, Al-Tai, & Oraifi (1993) reported a gradual and significant increase in reducing sugars and total sugar contents during the storage process of date palm fruits. The gradual increase in the reducing sugar content was relevant to the hydrolysis. On the contrary, the non-reducing sugar content was decreased in all the treatments during storage periods up to the end storage time, due to the invertase action on sucrose (Al-Kahtani et al., 1998).

| Dose (kGy) | Total soluble sugars (%) | Reducing sugars (%) |
|-----------|-------------------------|--------------------|
|           | Sakouti | Bondoky | Sakouti | Bondoky |
| Control   | 75.85 ± 0.45a | 79.61 ± 0.77a | 59.97 ± 0.20c | 62.46 ± 0.34c |
| 2.5       | 80.17 ± 0.38b | 85.94 ± 0.41b | 62.85 ± 0.27b | 66.88 ± 0.29b |
| 5         | 84.62 ± 0.80a | 87.66 ± 0.19a | 65.96 ± 0.56a | 68.09 ± 0.13a |
| 10        | 78.72 ± 0.26a | 85.59 ± 0.43a | 61.84 ± 0.18a | 66.64 ± 0.30a |

Data are presented as mean ± SE (n = 3). Different superscript letters indicate significant difference at p < 0.05.

3.2. Sugars content using HPLC

The major sugars in two studied date fruit cultivars are glucose, fructose, and sucrose. Reducing sugars (glucose and fructose) were dominant in Bondoky and Sakouti cultivars. This variant in sugar structure showed the presence of comparatively important invertase activity in two analyzed cultivars, which showed an elevated quantity of reducing sugars, that would significantly decrease its sucrose content (Fayadh & Al-Showiman, 1990).

Obtained results as shown in Figures 1 and 2 demonstrated that Bondoky cultivar has the largest concentration of glucose (36.20 g/100 g D.W.) and fructose (37.65 g/100 g D.W.) at dose 5 kGy. Sakouti cultivar exhibited a lower concentration of glucose (30.14 g/100 g D.W.) and fructose (29.92 g/100 g D.W.) at the same dose level (5 kGy). However, Bondoky and Sakouti cultivars contained the highest of sucrose content (7.31 and 4.81 g/100 D.W.) for control samples which was decreased by rising the γ-irradiation dose level to 2.5 and 5 kGy, respectively. Results emphasized that Bondoky cultivar has higher content of glucose and fructose increased by raising the dose of irradiation than Sakouti cultivar. The present study results were confirmed by the previous findings of Al-Farsi, Alasalvar, Morris, Baron, & Shahidi (2005a) and Hasnaoui, Elhoumaizi, Borchani, Attia, & Besbes (2012), who discovered that the total sugar content increased in 14 numerous Moroccan date assortments and the same trend was observed in three Omani date assortments. In the same concern, Bhat, Sridhar, Karim, Young, & Arun (2009) cited that there was an increase in sugars due to the irradiation which can be attributed to the breakdown of complex sugars (polysaccharides) into simple extractable forms.

3.3. Total phenolic and total flavonoid contents

Results of the total phenolic content of un-irradiated and γ-irradiated samples of two date fruit genotypes were given in (Table 2). In irradiated samples, expanding irradiation dose level recorded a tremendous increment in the whole phenol content up to the dose 5 kGy (5.82 and 3.82 mg GAE/g D.W.) for both cultivars Sakouti and Bondoky, respectively. On the other hand, increasing the dose to 10 kGy caused a decrease in the total phenol
content from 4.14 to 2.25 mg GAE/g D.W. in control samples to 4.13 and 2.93 mg GAE/g D.W. for Sakouti and Bondoky cultivars, respectively. There were significant variations in total flavonoid content that had been recognized between the investigated two date cultivars. Sakouti cultivar had the largest total flavonoid content (2.43 mg QUE/g D.W.) in comparison with Bondoky cultivar (1.03 mg QUE/g D.W.) in control samples. The amount of flavonoid contents was significantly increased while the irradiation dose level increased to 5 kGy (4.74 and 1.61 mg QUE/g D.W.) in Sakouti and Bondoky, respectively. On the other hand, by increasing the dose level to 10 kGy flavonoid contents were decreased in both cultivars. These results are in good agreement with those obtained by Lee et al. (2009), Aly, Maraeti, & Ayadi (2018); Aly, Eliwa, & AbdEl-Megid (2019).

Table 2. Effect of γ-irradiation with different dose levels (control, 2.5, 5, and 10 kGy) on total phenols (mg GAE/g D.W.), total flavonoid content (mg QUE/g D.W.), and DPPH (%) of Sakouti and Bondoky date cultivars.

| Dose (kGy) | Parameter            | Sakouti | Bondoky | Sakouti | Bondoky | Sakouti | Bondoky |
|------------|----------------------|---------|---------|---------|---------|---------|---------|
|            | Total phenols (mg GAE/g D.W.) | 4.14 ± 0.19c | 2.25 ± 0.49c | 2.43 ± 0.04a | 1.03 ± 0.03a | 70.48 ± 0.45c | 46.85 ± 0.34d |
| 2.5        | Total flavonoids (mg QUE/g D.W.) | 4.82 ± 0.32b | 2.80 ± 0.08b | 3.44 ± 0.04b | 1.42 ± 0.04b | 79.20 ± 0.66d | 50.37 ± 0.32c |
| 5          |                      | 5.82 ± 0.00a | 3.82 ± 0.05a | 4.74 ± 0.14a | 1.61 ± 0.01a | 82.85 ± 0.60d | 60.77 ± 0.43c |
| 10         |                      | 4.13 ± 0.10c | 2.93 ± 0.11b | 1.49 ± 0.20a | 1.14 ± 0.01a | 61.20 ± 0.02d | 55.61 ± 0.39b |

Data are presented as mean ± SE (n = 3). Different superscript letters indicate significant difference at p < 0.05.
mentioned that the total flavonoid contents were increased as affected by gamma irradiation in some wheat cultivars and eggplant. On the other hand, Aly & Elfaramawy (2010) found that there was a significant decrease in irradiated *Vicia faba* L. seeds at dose levels (2.5, 5.0, 10, and 20 kGy).

### 3.4. Analysis of the phenolic compounds using HPLC

Table 3 shows the phenolic compounds (mg/Kg D.W.) of significant phenolic compounds that had been determined via HPLC assessment as provided in (Table 3). In the explored date fruits, 23 free phenolic acids and flavonoid compounds had been identified and evaluated and compared to the retention times and UV spectra of standards that were analyzed under comparable circumstances. The findings showed that Sakouti and Bondoky cultivars added elevated concentrations quinol, myricetin, naringen, benzoic acid, synergetic acid, P-hydroxy benzoic acid, caffeine, and quer cetin in the most treatments of Sakouti and Bondoky cultivars and they are raised by raising irradiation dose level up to the dose level 5 kGy and decreased for the doe level 10 kGy for both cultivars. Catechol (6.69 and 4.49 mg/kg D.W.) and caffeine (21.81 and 10.52 mg/kg D.W.) appeared only in the control samples of both cultivars Sakouti and Bondoky, respectively, and vanished in the all other treatments. Rutine and ellagic acids were present in 2.5 and 5 kGy only of both cultivars. Otherwise, caffeeic acid appeared only in Sakouti cultivar at a concentration of 0.99, 1.30, and 1.06 mg/kg D.W. responding to the irradiation dose level; control, 2.5 and 5 kGy, respectively. Additionally, Kampherol appeared in the Sakouti cultivar for all the treatments and increased by increasing the dose of irradiation to the maximum (243.19 mg/kg D.W.) at irradiation dose level 10 kGy (Table 3). The obtained results showed that cinnamic acid developed only in Bondoky cultivar and increased as irradiation dose level increased and came to the highest concentration (6.53 mg/kg D.W.) at dose level 10 kGy. Topuz & Ozdemir (2004) published as a consequence of gamma irradiation treatment, that phenols contents had been significantly enhanced in relation to tannin degradation and the modification of molecule structure. Furthermore, phenylalanine ammonia-lyase enzyme recreation, which is accountable for polyphenolic acid synthesis, was increased by increasing the gamma irradiation dose level (Aly, Eliwa, & AbdEl-Megid, 2019; Oufedjikh, Mostafa, Josèphe, Amiot, & Lacroix, 2000).

The contemporary findings have been reported by Harrison & Were (2007), who showed that the rise of date fruits phenolic content should be given to their launch from glycosidic precursors and the degradation of bigger polymeric phenolic compound to lower gadgets through gamma-ray treatments. Furthermore, Naresh, Varakumar, Variyar, Sharma, & Sarathi Reddy (2015) indicated that the degradation of conjugated phenolic compounds should define the higher phenolic content in gamma-irradiated date samples. In the same concern Aly, Maraei, & Ali (2016) found that phenolic and flavonoids were significantly expanded by expanding irradiation dose levels of Egyptian *Moringa oleifera* seed oils. On the other side, Carocho et al. (2012) demonstrated that contents of flavonoid decreased in chestnut

| Phenolic compound | Retention time (min) | Control | 2.5 (kGy) | 5 (kGy) | 10 (kGy) |
|-------------------|---------------------|---------|-----------|---------|----------|
| Pyrogallol        | 3.591               | 147.44  | ND        | ND      | ND       |
| Quinol            | 3.749               | 216.0   | 206.22    | 239.69  | 223.41   | 282.29   | 286.45   | 255.06   | 145.03   |
| Gallic acid       | 4.458               | 2.76    | 2.59      | 6.99    | 5.58     | 8.75     | 7.72     | 4.75     | 3.12     |
| Catechol          | 7.517               | 6.69    | 4.49      | ND      | ND       | ND       | ND       | ND       | ND       |
| p-Hydroxy Benzoic acid | 9.546          | 22.92   | 18.96     | 32.87   | 32.87    | 38.98    | 25.83    | 22.99    | 17.17    |
| Caffeine          | 10.442              | 21.81   | 10.52     | ND      | ND       | ND       | ND       | ND       | ND       |
| Chlorogenic       | 10.789              | 3.30    | 4.39      | 6.66    | 4.96     | 7.40     | 5.40     | 7.72     | 2.74     |
| Vanillic acid     | 10.918              | 4.43    | 6.25      | 6.31    | 10.37    | 8.77     | 14.70    | 6.35     | 3.76     |
| Caffeic acid      | 11.223              | 0.99    | ND        | 1.30    | ND       | 1.06     | ND       | ND       | ND       |
| Syringic acid     | 12.200              | 26.08   | 11.56     | 43.92   | 12.85    | 63.98    | 14.74    | 24.39    | 14.74    |
| Vanillin          | 13.312              | 9.92    | 1.06      | 11.59   | 4.23     | 13.44    | 7.17     | 6.98     | 1.24     |
| p-Coumaric acid   | 15.154              | 0.93    | 0.85      | 0.97    | 2.26     | 1.37     | 2.51     | 1.01     | 1.42     |
| Ferulic acid      | 16.258              | 11.28   | 10.95     | 13.91   | 12.15    | 15.71    | 14.31    | 7.54     | 5.31     |
| Benzoic acid      | 17.842              | 32.37   | 20.85     | 35.44   | 31.17    | 50.38    | 41.26    | 30.55    | ND       |
| Rutin             | 18.100              | ND      | ND        | 2.82    | 2.37     | 5.69     | 4.99     | ND       | ND       |
| Ellagic           | 18.600              | ND      | ND        | 64.95   | 45.35    | 92.18    | 52.87    | ND       | ND       |
| o-Coumaric acid   | 19.176              | 4.77    | 3.95      | 6.39    | 5.01     | 9.32     | 6.96     | 10.95    | 7.87     |
| Salicylic acid    | 20.372              | 3.10    | 1.07      | 4.49    | 3.00     | 5.95     | 4.67     | ND       | ND       |
| Myricetin         | 21.646              | 86.66   | 44.11     | 95.02   | 55.27    | 130.41   | 85.71    | 70.03    | 56.32    |
| Cinnamic          | 24.500              | ND      | 1.78      | ND      | 3.76     | ND       | 4.69     | ND       | 6.53     |
| Quercetin         | 24.899              | 11.67   | 8.63      | 13.89   | 11.71    | 17.89    | 14.70    | ND       | ND       |
| Neringein         | 26.962              | 38.95   | 25.65     | 74.88   | 43.89    | 87.14    | 55.32    | 96.11    | 62.45    |
| Kamphenol         | 27.300              | 6.85    | ND        | 8.89    | ND       | 97.17    | ND       | 243.19   | ND       |

ND: Not detected
as a result of ionizing radiation, revealing that these compounds can also be susceptible to ionizing radiation. However, Khattak, Simpson, & Ihsanullah (2008) and Aly, Maraei, & Ayadi (2018) referred that the impact of gamma irradiation on the total content of phenolic (increase or reduce) may be due to many factors, such as plant or cultivar species, environmental and geographical circumstances, sample type (solid or dry), phenolic structure, extraction solvent, method of extraction, temperature and gamma irradiation dose level. In addition, in line with these results, Mansouri, Embarek, Kokkalou, & Kefalas (2005) discovered ferulic acid, sinapic acid, and p-coumaric as some cinnamic acid derivatives in date fruits from Ghardaia of Algerian. For Tunisian (Regnault-Roger, Hadidine, Biard, & Boukef, 1987) and Omani cultivars (Al-Farsi, Alasalvar, Morris, Baron, & Shahidi, 2005b), a comparable phenolic acid profile was reported. Interestingly, Al-Farsi, Alasalvar, Morris, Baron, & Shahidi (2005b) explained that in date cultivars from Oman ferulic acid was the most important phenolic acid. In the same concern, Maraei, Khaled, & Elsawy (2017) reported that gamma irradiation at different dose levels stimulated the biosynthesis of some phenolic compounds such as pyrogallol, gallic, catechol, chlorogenic, and ellagic acid. Tomas-Barberan & Clifford (2000) found that gallic acid had a favorable impact on cancer cells under in vitro circumstances. This acid is ingested in the human body all around, contrasted with other phenolic compounds (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005). Several researches showed that date palm contained flavonoids, for example, quercetin, apigenin and luteolin and phenolics such as ferulic acids, P-coumaric, sinapic, and cinnamic acid derivatives (Sanchez-Moreno, 2002). The present findings are in agreement with those obtained with Al-Farsi & Lee (2008), they stated that in date seeds nine of phenolic acids have been differentiated, of which four consisted of hydroxylated derivatives of benzoic acid (gallic acid, vanillic acid, protocatechuic acid, and P-hydroxybenzoic acid) and five were derivatives of cinnamic acid (caffeic acid, M-coumaric, ferulic acid, P-coumaric acid, and O-coumaric acid). So, it seems that irradiation dose levels 2.5 and 5 kGy might stimulate some chemical reactions in segments of dates, which perhaps debase or disintegrate huge particles into small phenolic compounds, that are readily soluble in solvents and can be also helpful for plant antioxidant characteristics (Ghadi, Ghara, & Ghanbari, 2015). As suggested by Radfar et al. (2019) the date seeds are good sources of phenolic compounds, including phenolic acids, and their derivatives such as p-hydroxybenzoic, protocatechuic, and m-coumaric acids were three major compounds.

3.5. Antioxidant activity using DPPH radical

Data presented in (Table 2) showed that the scavenging capability of the studied two date cultivars against the DPPH radical percentages considerably varied (p < 0.05). Sakouti cultivar demonstrated the largest of scavenging activity (DPPH %) by expanding the irradiation dose level to 5 kGy (82.85%) while in Bondoky cultivar it was 60.77% at 5 kGy compared with control samples. Increasing the dose to 10 kGy in both cultivars decreased significantly the DPPH % to 61.20% and 55.61%, respectively. The present examination uncovered plainly that the two types of date fruits are able to scavenge the free radicals strongly. It was observed that cultivar Sakouti had a potential scavenging activity more than Bondoky cultivar at dose 5 kGy. Gamma irradiation is suitable for breaking the chemical bonds of polyphenols and consequently discharging soluble phenolics of small molecular weights prompte the development of phenolics rich in antioxidants. The present results were in concurrence with those obtained by Abbes et al. (2013), who disclosed that the antiradical activity reported for Tunisian date fruits showed a broad range from 27.97 (Allig) to 76.40% (Deglet Nour). Higher accessibility of different antioxidants could clarify the DPPH scavenging capacity of date palm extract. Several studies have shown that palm dates include flavonoids such as quercetin, luteolin, and apigenin, as well as phenolics such as ferulic, sinapic, p-coumaric acids, and derivatives of cinnamic acid (Sanchez-Moreno, 2002). The radical DPPH in oil produced by un-irradiated and irradiated Moringa oleifera seed oil showed that scavenging activity gradually increased by increasing the amount of irradiation dose to 10 kGy (Aly, Maraei, & Ali 2016).

3.6. Antimicrobial activity

The antimicrobial activity of two date flesh Sakouti and Bondoky extracts was carried out against six pathogenic microbial strains (Staphylococcus aureus, Escherichia coli, Enterobacter cloacae, Aspergillus niger, Penicillium sp., and Candida albicans) and both Gentamycin and Nystatin were used as antibiotic control. Data in (Table 4) summarize the strong antibacterial activity of the date palm fruit extract for date flesh irradiated by gamma irradiation dose level 5 kGy against Staphylococcus aureus (16.0 and 15.0 mm) for the two cultivars Sakouti and Bondoky, respectively. In addition to information on zone inhibition, the most antibacterial activity against Gram-negative organisms demonstrated Escherichia coli and Enterobacter cloacae were more pronounced with Sakouti than Bondoky cultivar. On the other hand, for fungi (Aspergillus niger, Penicillium sp. and Candida albicans) the antifungal activity was stronger than the antibacterial for the two cultivars and the effective result registered by Penicillium sp. and the inhibition zone increased by increasing irradiation dose level in the two cultivars. Findings are in concurrence with the literature, antibacterial activity is similar to prior results. (Saleh &
Date flesh extracts of the two cultivars exhibited antimicrobial activity that may be attributed to its bioactive components, for example, phenolic and flavonoid compounds due to the phenolic compound. Polyphenols assume an important role as an antibacterial activity through the protein precipitation and microorganism enzyme inhibition (El-Beltagi, Mohamed, Abdelazeem, Youssef, & Safwat, 2019c; Metou, Essid, Bouzoumita, & Ferchichi, 2019). Moreover, Maraei & Hamoud (2019) observed an increase in antioxidant and antimicrobial activity was parallel to an increase in phenolic compounds and flavonoid content due to irradiation treatment. Otherwise, results obtained by Radfar et al. (2019) declared that increased phenolic contents can result in increased antimicrobial activity of the date extracts.

4. Conclusion

The results of this investigation concluded that γ-irradiation at dose 5 kGy enhanced the natural polyphenolic compounds, total phenolic contents, total flavonoid content, antioxidant scavenging activity (DPPH%), total soluble sugars (%), reducing sugars (%), and antibacterial potentials of two Egyptian date palm fruits. Irradiation treatment was observed to be unrivaled for improving the quality of date fruit. These fruits can be used for various food and food products as natural antioxidants and antimicrobials. It could be concluded that the dates by-product may be an outstanding source of antioxidants in the preparing of food and medicine.

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Table 4. Susceptibility of tested strains toward extract of gamma-irradiated date palm fruits with different dose levels (control, 2.5, 5, and 10 kGy) (zone of inhibition in mm) comparing to standard antibiotics.

| Microbial strains | Control | 2.5 | 5.0 | 10.0 | Control | 2.5 | 5.0 | 10 | *GN | **NS |
|-------------------|---------|-----|-----|------|---------|-----|-----|----|-----|------|
| Gram positive     |         |     |     |      |         |     |     |    |     |      |
| S. aureus         | 10      | 12  | 16  | 12   | 9       | 13  | 15  | R  | 15  | NT   |
| Gram negative     |         |     |     |      |         |     |     |    |     |      |
| Escherichia coli  | 9       | 12  | 17  | R    | 8       | 11  | R   | R  | 10  | NT   |
| Enterobacter Cloacae | 18     | 21  | 23  | 20   | 10      | 15  | 23  | 20 | 11  | NT   |
| Fungi             |         |     |     |      |         |     |     |    |     |      |
| Aspergillus niger | 8       | 9   | 14  | 12   | 10      | 13  | 16  | R  | NT  | R    |
| Pencillium sp.    | 18      | 20  | 22  | 21   | 15      | 16  | 18  | 21 | NT  | 10   |
| Yeast             |         |     |     |      |         |     |     |    |     |      |
| Candida albicans  | 13      | 14  | 15  | 12   | 10      | 12  | R   | R  | NT  | 12   |

*Gentamycin (GN 10 µg), **Nystatin (NS 100 µg), NT: not tested, R: resistant

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