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Original article

Comparative analysis of chemical, mineral and in-vitro antibacterial activity of different varieties of date fruits from Saudi Arabia

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ABSTRACT

Three important varieties of dates (Sukri, Barhi, and Rothana), grown in Saudi Arabia were analyzed for their chemical, mineral and anti-bacterial activity. Among all the varieties analyzed, the Sukri dates had the highest moisture (16%), carbohydrate (80.65 mg/g) and the phenolic content (1.24 mg GAE/100 g) whereas, the ash content was the lowest (2.22%). The mineral content analyzed by ICP OES reveals that all three varieties contain an appreciable amount of essential macro and micro nutrients. They contain a good amount of potassium; the maximum amount of K (8171.24 mg/kg) was recorded in Barhi dates. Besides that, all three varieties of dates contain a good amount of Se, Cu, P, Fe, Mn. The comparative analysis of the GC MS chromatogram reveals that there were only five compounds that were present in all three varieties. The bioactive compounds Mannoic and Lactone were detected in all three varieties. The fruits contain some important groups of sugar, alcoholic sugar, aromatic hydrocarbon, phenol(s) and esters. The extracts of dates were assed for their antibacterial activity against 6 important strains of human pathogenic bacteria. The strains of *E. coli*-ATCC25922, *B. subtilis*-ATCC6633, *S. aureus*-ATCC25923, were observed to be highly sensitive to most of the extracts whereas *S. pyogenes* and *S. flexeneri* (clinical isolates) showed moderate sensitivity to some extracts. The variety Sukri was found to be superior to the other two varieties in terms of sugar and phenolic contents, in addition, the methanol extract of Sukri dates showed excellent antibacterial activity against most of the bacteria evaluated. These varieties of dates prove to have immense potential to be utilized as a therapeutic agent for curing mineral deficiency also can be explored for developing antibacterial agents.

1. Introduction

Date palm (*Phoenix dactylifera L.*) has always been an important crop of the Middle East region. Although there is no clarity on the native region of the tree however it has been the part of Middle East flora more than 6000 years (Ortiz-Uribe et al., 2019). Date palm is considered an important profitable crop of the Gulf region, the date palm fruits also known as "Dates" are an essential part of the human diet of this region. The Arab peninsula is responsible for the 30% world production of dates, Egypt, Kingdom of Saudi Arabia and Iran are the main producer of this region (AbdulQadir et al., 2011). According to FAOSTAT; the production of dates in Saudi Arabia in the year of 2017 was 6.98 tons/ha (Jemni et al., 2019). A report claimed that more than 450 different varieties/cultivars of the date palm are grown in Saudi Arabia (Zhang et al., 2017). Dates are not only known for their sweet taste but also for health and beneficial values (Tahraoui et al., 2007; Tang et al., 2013). Dates consist of a hard seed referred to as "pit" and edible fleshy pericarp. Dates go through four stages of ripening; the green immature dates "Kimiri", yellow mature dates "Khalal", ripe soft form of dates "Rutab" and the fully matured "Tamar". The shelf life of Tamar is quite long as it contains comparatively less moisture, hence are consumed whole round of the year. This fruit possess many important nutrition, besides the rich source of simple and complex sugars they contain a decent amount of fibers, protein, and vitamins, dates also contain a good amount of important micro and macro minerals, (Al-Shahib and Marshall, 2003; Biglari et al., 2008). Tang et al. (Tang et al., 2013) have described dates as a good source of phenolic- and bioactive compounds. Several reports suggest the anti-oxidant, antimicrobial, anti-mutagenic and anticancer properties of dates. (Tang et al., 2013; Hong et al., 2006; Vayalil, 2012).

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The dates have always been an interest of research in the Middle-East due to the nutritional and religious importance (Ortiz-Uribe et al., 2019; AbdulQadir et al., 2011; Tang et al., 2013; Vayalil, 2012; Al-Alawi et al., 2017; Mohamed et al., 2014) but it has not gained enough global recognition. Recently, Zhang et al. (Zhang et al., 2017) reported the antioxidant, anti-inflammatory, and anti-tumor activities of 29 varieties of dates from Saudi Arabia. While Alghamdi et al (Alghamdi et al., 2018) assessed the nutritional values of different varieties of Dates from the Hail province of Saudi Arabia. Despite several articles on the great nutritive and pharmacological properties of dates, there are limited reports on the antibacterial activity of date fruits from the world (Shakiba et al., 2011; El Sohaimy et al., 2015; Samad et al., 2016; Mainasara et al., 2017; Farhana et al., 2017) and only a few from Saudi Arabia (Abdullah et al., 2019; Bhat and Al-Daihan, 2012; Al-Daihan and Bhat, 2012; Saleh and Otaibi, 2013). Saudi Arabian dates have been explored for their minerals, vitamins and bioactive compounds however the comparative analysis of nutritional and antibacterial properties of varieties: Rothana, Sukri, and Barhi is lacking. Therefore, the current study investigated the total carbohydrate, ash, total-phenol, moisture chemical composition, mineral content and antibacterial activity of the crude extracts of three varieties of Saudi Arabian dates. The obtained information will be of an advantage to the farmers, pharmaceutical industries, researchers as well as consumers.

2. Materials and methods

2.1. Sample collections

Three varieties of Dates (Rothana, Sukri, and Bharhi) were obtained from the farm of Al-Shamlan, located at Onaizah of Qassim region, Saudi Arabia. Labeled dates were stored at 18 °C and transported to the laboratory for further analysis.

2.2. Moisture content determination

After removing pits from the dates, 10 g sample were crushed and 3 g bits were transferred into the previously oven dried dish; the sample was kept in a vacuum oven till the constant weight was obtained. The sample was kept in the desiccator till it cooled down and again weight measured. The content of moisturizer percentage on the dry basis was calculated as follows

$$\text{Moisture content } (\%) = (\text{Change in weight/weight of dry matter}) \times 100$$

2.3. Ash content determination

Date fruit pulp (5 g) was heated on the Bunsen burner till the production of fumes was apparent after that the sample was transferred into the furnace at 550 °C and left in it till a grey colored sample is obtained (Vayalil, 2012; AOAC, 2000). The sample was cooled and the weight of the ash was measured. Following formula was used to calculate the ash content:

$$\text{ASH}(\%) = (\text{weight of ash/weight of sample}) \times 100$$

2.4. Sugar determination

Complete sugar was measured as per the Anthrone method. Fresh date (0.5 g) was boiled with 80% ethanol (10 ml) and filtered. The volume of the obtained filtrate was made up to 50 ml. A reaction mixture was prepared by adding 1 ml filtrates and 5 ml of anthrone reagent and placed into the water-bath for 15 mins at 100 °C. The reaction mixture left at room temperature for 20 mins after that optimal density was recorded at 620 nm. The blank sample was also performed in a similar way. From the standard curve, the soluble sugars were measured.

2.5. Total phenolic content determination

Folin-Ciocalteau reagent was method used to measure the total phenol (Rajvaidya and Markandy, 2006). The fruit extract was obtained by soaking 0.5 g fresh date pulp in 30 ml methanol for half an hour followed by filtering; the obtained extract was dried and dissolved again in 0.5 ml methanol. The final concentration was made for 25 ml by adding the distilled water. In a test-tube the fruit extract was diluted with distilled water (1:6) and into it 0.5 ml of Folin-Ciocalteau reagent of 1;1 was transferred. The sample was left on the bench for 3 min followed by the addition of 1 ml sodium bicarbonate (35%), the final volume of the obtained solution was made to 10 ml with distilled water. About 30 mins, tubes were incubated at room temperature in darkness and the reading was recorded at an absorbance of 600 nm. The measurement of the phenolic compounds was obtained through the standard curve of gallic acid. The experiment has been performed in triplicates for all three varieties of dates.

2.6. Determination of the chemical composition of dates by GC–MS analysis

To determine the chemical composition, the methanol extract of dates was used. The analysis was performed by using Gas Chromatography-Mass Spectroscopy (GC–MS). The equipment Perkin Elmer gas chromatography (GC) along with mass spectrometer (MS) equipped with RTx-5 column (30x0.32 nm) was used. The initial oven-temp was 110 °C for 2 min and then increased for 9 mins at 200–250 °C. Helium was the carrier gas (2 ml/min). None of the chemical standards were used and analysis was interpreted using the program NIST-database. Reverse match value (REV) of the compound was obtained by ignoring all peaks that were in the sample spectrum but not in the library spectrum (Perveen and Alwathnani, 2013).

2.7. Preparation of dry powder of dates for ICP-OES and antibacterial analysis

Fresh date samples were washed a couple of times with deionized purified water and with deionized double-distilled water to remove surface contaminants. After removing the pits from the dates again samples were washed with deionized water. The samples were dried at 60 °C for two of days in the oven. With the help of mill, dried samples were converted into the fine powder (IKA, Germany).

2.8. Determination of mineral content by ICP-OES

Inductively coupled plasma-optical emission spectroscopy (ICP-OES) was used to find out the element content of the dates. At first, powdered samples of the dates were subjected to acid digestion, 0.5 g powdered samples were digested with 3.5 ml each of conc. Sulphuric acid and 30% of H_20_2 of 30 mins of heating at 25 °C. Next after cooling 30% of 1 ml of H_20_2 aliquot was mixed and filtered. Using 20 ml of distilled water, filtrate was diluted and then the digested sample was measured through ICP-OES to record the content of calcium (Ca), Magnesium (Mg), Phosphorous (P), Potassium (K), Iron (Fe), Copper (Cu), Zinc (Zn), Manganese (Mn), Sodium (Na) and Selenium (Se) in three varieties of dates (Perveen et al., 2013).

2.9. Antibacterial activity of extracts of dates

2.9.1. Bacterial strains

Human pathogenic bacteria i.e. *Bacillus subtilis*-ATCC6633; *E. coli*-ATCC25922; *Pseudomonas aeruginosa*-ATCC27853; *Shigella flexeneri*, *Staphylococcus aureus*-ATCC25923 and *Streptococcus pyogenes* (Clinical isolates) were used to evaluate the antibacterial activity of extracts of dates.

2.9.2. Preparation of extracts of dates

The crude extract of dates was prepared by extracting in water, chloroform, methanol, ethanol, and acetone. The powdered sample (10 g) was soaked in 100 ml of each solvent. Each mixture was kept at 25 °C into incubator shaker for 3 days and extraction was filtered using Whatman filter paper. Filtrates were reduced for dryness by rotatory evaporator (BUCHI, Switzerland). Each dried crude extract (s) was scraped in the round bottom-flask; the powder was stored in universal bottles for −18 °C until the antimicrobial activity was performed. Di-methyl sulfoxide (DMSO) was used to prepare the stock solution and crude extracts.

2.9.3. Antibacterial activity of different extracts of dates

The agar well diffusion method was used for screening the crude extracts for numerous human pathogens (Khan et al., 2011). Randomly, between 3 and 5 colonies of test-organism were inoculated in the 10 ml of MHB and incubated for 1 day at 37 °C. The inoculum density of bacteria was made to 1.5×10^8 CFU/ml by comparing with 0.5 McFarland standard and adjusting with the sterile MHB. A 10 µl aliquot of bacteria inoculum was evenly spread on the surface of the MHA plate. Four wells were made on the agar surface of each plate and 50 µl of crude extract was transferred into the respective wells. Gentamycin and DMSO were opted as positive and negative controls, respectively. The plates were incubated at 37 °C for 24 h. The zone of inhibition around the well was measured to determine the extent of antibacterial activity exhibited by dates extracts.

2.9.4. Minimum inhibitory concentration

Extracts that showed a good zone of inhibition against tested bacteria were selected for assessing the minimum inhibitory concentration (MIC). For the analysis, the microplate dilution method was used. To determine the MIC, 100 µl stock solution (10 mg/ml) of crude extract was diluted with MHB by two-fold dilution into 96-well microtitration plate. The 50 µl inoculum of test pathogen (1.5×10^8 CFU/ml) was added to wells. A tube with MHB, DMSO and test pathogen was included as positive growth control. MHB, DMSO without test pathogen served as a negative control. The plates were incubated at 37 °C for 24-hours. After incubation 30 µl MTT (0.2 mg/ml) per well was added; plates were further incubated for two hours interpretation of MIC value was based on colour of the MTT, the well that shows no colour change was scored as above MIC value.

3. Results

Three important varieties of dates (Barhi, Sukri, and Rothana) grown in Saudi Arabia were analyzed for their chemical, mineral and antibacterial activity, results are summarised in Table 1. The data depicts that among all the tested varieties, the highest moisture content (16%) and the lowest ash (2.22%) content was present in Sukri dates whereas, in Rothana dates the content of ash (6.67%) was maximum and moisture content was the least (9.7%). The comparison of data on carbohydrate and phenolic content shows that variety Sukri contains the maximum amount of carbohydrate (80.65 mg/g) and phenolic content (1.24 mg GAE/100 g) followed by Barhi and Rothana (Table 1). The results of the mineral content of the dates analyzed by ICP-OES are presented in Table 2. Among all the minerals detected the content of Potassium (K) was highest in all three varieties, the next most abundant element was Ca in varieties Sukri and Rothana followed by Mg > P > Na > Fe > Zn > Cu > Mn > Se whereas in variety Barhi next to element K was Mg followed by P > Ca > Na > Fe > Zn > Cu > Mn > Se. Comparative analysis of mineral content of three varieties of dates showed that the maximum amount of K (8171.24 mg/kg) was present in Barhi dates, in addition, it contains the highest amount of iron, zinc, copper, and selenium. Dates of variety Rothana contains the maximum amount of Ca, Mg, P and Mn as compared to other tested varieties of dates; while among all the varieties analyzed the maximum amount of sodium (89.7 mg/kg) was detected in Sukri dates (Table 2).

The chemical compounds of the dates were identified by GCMS analysis, Tables 3 represents the list of compounds identified from the dates of Sukri, Barhi and Rothana. Total ten, nine and eight compounds were identified in Barhi, Sukri, and Rothana, respectively. The compound 2-furancarboxaldehyde, 5-(Hydroxymethyl)- occupied the highest peak area of the chromatogram of Sukri and Rothana while, chromatogram of Barhi shows that 4 h-Pyran-4-One,2,3-Dihydro-3,5-Dihydroxy-6-Methy l- has the highest peak area. The comparison of chemical compounds of three varieties reveals that there were five compounds that were present in all three varieties, besides that Sukri dates contain 1-Butanol, 4-(Hexyloxy)-; Oxaziridine, 2-Methyl-3-Propyl-; 2,5-Monomethylene-L-Rhamnitol and Sorbitol, whereas 2-Amino-Octadec-7-Ene-1,3-Diol Butaneboronate and 1,6;3,4-Dianhydro-2-O-Acetyl-Beta. -D-Allopyranose compounds were found in both Barhi and Rothana. The variety Barhi also contains 1,6;3,4-Dianhydro-2-O-Acetyl-Beta-D-Galactopyranose;
Propanal,2,3-Dihydroxy-, (S)- and D-Glucose,4-O-Alpha-D-Gluco pyranosyl while Rothana has D-Glycero-D-Ido-Heptose.

Crude extracts of dates were also subjected to antibacterial activity. The results on antibacterial activity of crude extracts of dates presented in Table 4 reveals that in general, the extracts were effective in controlling the growth of the evaluated bacteria however, they varies in the antibacterial potential. In the case of Sukri extracts, methanol extracts of dates were able to reduce the growth of all tested strains except *S. pyogenes*. While the ethanol extracts of Sukri exhibited good antimicrobial activity against all pathogenic bacteria except *P. aeruginosa* whereas, acetone extracts of Sukri were effective against all but *P. aeruginosa*-ATCC27853 and *S. flexeneri* (clinical isolate). The water extract of Sukri was quite effective in controlling the growth of *S. aureus*, *E. coli*-ATCC25922 and *B. subtilis*-ATCC6633. Similarly, the water, ethanol and acetone extracts of Barhi dates exhibited comparatively good antibacterial activity against *S. aureus*, *E. coli*, *S. pyogenes* and *B. subtilis*. While the methanol extract of Barhi was effective in reducing the growth of all tested pathogenic bacteria except *S. flexeneri*. Rothana, third and final variety of date extract reveals moderate levels of antibacterial activity against all the bacterial strains; good inhibitory effects against *S. aureus*-ATCC25923 were recorded by the methanol, ethanol and acetone extracts of Rothana.

The statistical analysis of antibacterial data of the zone of inhibition caused by the crude extracts of three varieties of dates reveals that in general, the bacterial strains *E. coli* ATCC 25922, *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923 showed high degree of sensitivity followed by *S. pyogenes* (clinical isolate) and *S. flexeneri* (clinical isolate) whereas *P. aeruginosa* was found resistant against most of the extracts tested. Among tested pathogens, the highest zone of inhibition (27.75 mm) by methanol extract of Sukri dates was observed against *S. aureus* and significantly ($P \leq 0.05$) no difference was noticed with the zone of inhibition (27.25 mm) exhibited by acetone extract of Sukri against *S. aureus*. The methanol

Table 1
Chemical properties of three varieties of dates (Sukri, Barhi, and Rothana).

Chemical properties of three varieties of dates				
Variety	Moisture (%)	Ash (%)	Carbohydrates (mg/g)	Phenol (mg GAE/100 g)
Sukri	16 ± 0.58	2.22 ± 0.0	80.65 ± 0.21	1.24 ± 0.03
Barhi	15.7 ± 0.00	3.33 ± 0.58	70.76 ± 0.32	1.07 ± 0.11
Rothana	9.7 ± 0.58	6.67 ± 0.77	24.34 ± 0.37	0.75 ± 0.02

Data are means ± S.D of three replicates.

Table 2
Mineral content of three varieties of dates (Sukri, Barhi, and Rothana) analyzed by ICP OES.

Variety	Mineral content (mg/kg)									
	Ca	Mg	Na	K	P	Cu	Fe	Mn	Zn	Se
Sukri	742.2 ± 0.1	730.96 ± 0.55	89.70 ± 0.06	6789.11 ± 0.61	650.66 ± 0.58	5.63 ± 0.15	13.73 ± 0.05	2.1 ± 0.1	9.6 ± 0.1	0.43 ± 0.06
Barhi	617.47 ± 0.42	731.33 ± 0.55	89.15 ± 1.00	8171.24 ± 0.75	661.50 ± 10.76	6.37 ± 0.40	17.47 ± 0.42	2.00 ± 0.10	10.13 ± 0.61	1.77 ± 0.23
Rothana	920.53 ± 1.55	851.17 ± 1.04	73.25 ± 0.66	7396.77 ± 1.08	726.93 ± 1.25	5.30 ± 0.10	14.80 ± 0.44	2.50 ± 0.10	10.03 ± 0.06	1.60 ± 0.26

Data are means ± S.D of three replicates.

Table 3
Comparative data of chemical composition of three varieties of dates; Sukri (S), Barhi (B) and Rothana (R) identified by GCMS analysis.

Compound name	Molecular weight	Molecular formula	Chemical identified in Variety
Formic Acid Hydrazide	60	CH_4ON_2	B, S, R
1-Butanol, 4-(Hexyloxy)-	174	$C_{10}H_{22}0_2$	S
4 h-Pyran-4-One,2,3-Dihydro-3,5-Dihydroxy-6-Methyl-	144	$C_6H_80_4$	B, S, R
2-Furancarboxaldehyde, 5-(Hydroxymethyl)-	126	$C_6H_6O_3$	B, S, R
Nitro-Tert-Butyl-Acetate	161	$C_6H_{11}O_4N$	B, S, R
Oxaziridine, 2-Methyl-3-Propyl-	101	$C_5H_{11}ON$	S
2,5-Monomethylene-L-Rhamnitol	178	$C_7H_{14}O_5$	S
3-Deoxy-D-Mannoic Lactone	162	$C_6H_{10}O_5$	B, S, R
Sorbitol	182	$C_6H_{14}O_6$	S
2-Amino-Octadec-7-Ene-1,3-Diol Butaneboronate	365	$C_{22}H_{44}O_2NB$	B, R
1,6;3,4-Dianhydro-2-O-Acetyl-Beta.-D-Allopyranose	186	$C_8H_{10}O_5$	B, R
1,6;3,4-Dianhydro-2-O-Acetyl-Beta-D-Galactopyranose	186	$C_8H_{10}O_5$	S
Propanal,2,3-Dihydroxy-, (S)-	90	$C_3H_6O_3$	S
D-Glucose,4-O-Alpha-D-Glucopyranosyl	342	$C_{12}H_{22}O_{11}$	S
D-Glycero-D-Ido-Heptose	210	$C_7H_{14}O_7$	R

extract of Rothana also showed significant inhibition of *S. aureus* growth (26.75 mm). The maximum growth of *B. subtilis* (25.75 mm) was rendered by the acetone extract of Barhi and statistically, no difference is found in the inhibition caused by the methanol extract of Sukri to *B. subtilis* (25.5 mm). The pathogen *E. coli* was observed to be most sensitive (24.75 mm) to the methanol extract of Barhi as compared to other extracts. *S. pyogenes* showed the maximum sensitivity to the ethanol extract of Sukri (12.5), methanol (12.0 mm) and acetone extract of Barhi (12.0 mm). The methanol extract of Sukri was noted to show maximum zone of inhibition against *P. aeruginosa* (18.75 mm) and *S. flexeneri* (24.5 mm) as compared to other extracts (Table 4).

Further crude extracts were analyzed to estimate the MIC value (Table 5). The methanol extract of Sukri showed the lowest MIC value (48.15 μg/ml) against *B. subtilis* and *S. aureus* ATCC 25,923 (48.75 μg/ml), while acetone extract of Sukri has MIC value of 97.5 μg/ml against *B. subtilis* and *S. aureus* ATCC 25923. Methanol and acetone extracts of Barhi have recorded the MIC value 97.5 μg/ml against *B. subtilis* ATCC 6633 and *S. pyogenes*. The rest of the tested extracts showed a MIC value above 100 μg/ml.

4. Discussion

The date palm tree is known as one of the oldest and important fruit trees in the Arabian world and grown throughout the world (Al-Alawi et al., 2017). The fruit of date palm has several nutritional and bioactive properties; this makes it an important fruit to be investigated. In the present study, three important varieties of dates grown in Saudi Arabia were analyzed for their chemical, mineral and antibacterial activity. Phytochemical analysis of dates of variety Barhi, Sukri and Rothana showed that all three varieties contain a good amount of carbohydrate. In the present study, the highest amount of total carbohydrates was recorded from variety Sukri followed by Barhi and Rothana dates. The variation in the total carbohydrate estimated for the three varieties of dates may due to different ripening stages or cultivation conditions (Al-Farsi et al., 2005). Zhang et al. (Zhang et al., 2015) reported that Sukkari variety of dates from the Qassim region contains fructose, glucose and disaccharide sucrose that makes 63.7% of sugar. Rothana dates contain fructose (34.35%) and glucose (31.62%). Another study claims that the carbohydrate in the Sukari dates was 69.9%, while Barhi dates contains 66.92% (Alghamdi et al., 2018). It has been reported that matured dates (Tamar) contain digestible sugars up to 70% (Al-Farsi et al., 2005). Analysis of moisture and ash content of three varieties shows that the ash content ranges between 9.7% and 16% and moisture between 2.22% and 6.67%. Previous studies have claimed that depending upon the ripening stage and variety of dates, moisture content in dates ranges between 10% and 25% whereas ash content 1.4–2.3%. (Al-Shahib and Marshall, 2003; Vayalil, 2012; Al-Farsi et al., 2005). The results showed that Rothana dates contain less moisture therefore they can be stored for a longer duration since low moisture content will give them protection against post-harvest damage caused by microbes. Contrary, to the previous report, the ash content in Rothana was much higher that may be due to variation in the cultivation conditions and ripening stage of the dates (Al-Farsi et al., 2005). The mineral content of the dates was analyzed by ICP-OES, it was noted that all three verities of dates contain varying amounts of the essential macro and micro-elements however; in all tested varieties the potassium (K) content was higher than the other elements. The results are in line with the previous reports that claimed a high

Table 4
Antibacterial activity of crude extracts of dates assayed by well diffusion.

Extracts	Variety	Bacteria					
		Ec	*Bs*	*Sp*	*Pa*	*Sf*	*Sa*
		Zone of inhibition (mm)					
Water	Sukri	19.5^{c}	20.5^{f}	0^{a}	0^{a}	0^{a}	23.25^{ef}
	Barhi	17.5^{b}	17.25^{b}	0^{a}	0^{a}	0^{a}	13.5^{a}
	Rothana	15.25^{a}	15.75^{a}	0^{a}	0^{a}	0^{a}	19^{b}
Methanol	Sukri	22.25 ^{fg}	25.5^{i}	0^{a}	18.75^{c}	24.5^{d}	27.75^{i}
	Barhi	24.75 ^{h}	23.5 ^{h}	12.0^{d}	18^{b}	0^{a}	20.75 ^{cd}
	Rothana	20.5^{d}	19.5^{d}	0^{a}	18^{b}	0^{a}	26.75 ^{h}
Acetone	Sukri	21.75^{efg}	26^{i}	10.0^{b}	0^{a}	0^{a}	27.25^{hi}
	Barhi	22.75 ^{g}	25.75^{i}	12.0^{d}	0^{a}	0^{a}	24.75 ^{g}
	Rothana	21d^{e}	20.25^{ef}	0^{a}	0^{a}	0^{a}	20.25^{ce}
Ethanol	Sukri	21.5^{ef}	20.5^{f}	12.5^{d}	0^{a}	20.75^{c}	22.75^{e}
	Barhi	21.75^{efg}	19.5^{c}	11.25^{c}	0^{a}	0^{a}	21.25^{d}
	Rothana	22.0^{efg}	21.5 ^{g}	11.5^{c}	0^{a}	19.25^{b}	24 f^{g}
Control		26.0 ^{i}	31^{j}	16.5 ^{e}	23^{d}	25.5^{e}	30 ^{j}

Each value is an average of four replicates.
Column with different letters are significantly different ($P \leq 0.05$) according to Duncan's multiple range test.
Ec: *E. coli* ATCC 25922; **Bs:** *Bacillus subtilis* ATCC 6633; **Sp:** *Streptococcus pyogenes* (clinical isolate); **Pa:** *Pseudomonas aeruginosa* ATCC 27853; **Sf:** *Shigella flexeneri* (clinical isolate); **Sa:** *Staphylococcus aureus* ATCC 2592.

Table 5
Minimum inhibitory concentration (MIC) of selected extracts of dates against human pathogenic bacteria.

Bacteria	MIC (μg/ml)				
	Extract of variety Sukri			Extract of variety Barhi	
	Methanol	Acetone	Ethanol	Methanol	Acetone
E. coli	>100	>100	>100	>100	>100
B. subtilis	48.15	97.5	>100	>100	97.5
S. pyogenes	> 100	>100	97.5	97.5	97.5
P. aeruginosa	> 100	>100	>100	>100	>100
S. flexeneri	> 100	>100	>100	>100	>100
S. aureus	48.75	97.5	>100	>100	>100

level of potassium content in dates (Mohamed et al., 2014; Alghamdi et al., 2018; Al-Farsi et al., 2005). Besides that, all three varieties of dates contain a good amount of Se, Cu, P, Fe, Mn that are required for the normal human body functioning thus these dates can fulfil the daily requirement of these minerals as reported earlier by Al Farsi and Lee (Al-Farsi et al., 2005). The GC–MS analysis of the methanolic extract of date fruit shows the presence of sugar, phenol, aromatic compounds and esters. The compound Mannoic Lactone, identified from all three extracts of dates has been documented to own the bioactive properties (Ghosh et al., 2015; Shobana et al., 2009).

Evaluation of the antibacterial activity of crude extracts of three varieties of dates reveals that they have the potential to reduce bacterial growth. However, the extent of inhibition varied among extracts and pathogens tested. The methanol extract of Sukri dates exhibited maximum inhibition of the growth of bacteria as compared to other extracts. Similar results are reported earlier also (Bhat and Al-Daihan, 2012; Al-Daihan and Bhat, 2012; Saleh and Otaibi, 2013). Moreover, it has been noticed that the extracts were more effective against gram-positive bacteria than gram-negative bacteria. It has been observed that methanol solvent is a considerably good solvent for the extraction of bioactive compounds from plants than other solvents (Eloff, 1998). A systemic review by Nurul et al. (Farhana et al., 2017) described that methanol extract of dates was much more effective in inhibiting the growth of bacteria than other solvents furthermore they confirm that gram-positive bacteria were more sensitive to the extract than gram-negative. The extract of Sukri dates exhibited appreciable bioactivity as well as recorded the highest amount of phenol content. Antibacterial activity of different extract results through the synergistic effect of their phenolic compounds (Cutter, 2000; Puupponen-Pimiä et al., 2001). The presence of phenolic compounds, flavonoids, aromatic, volatile compounds and esters in dates was confirmed by the GC–MS analysis. Dates are known for a good amount of phenolic compounds, alkaloids, flavonoids (Tang et al., 2013; Biglari et al., 2008; Alghamdi et al., 2018). These compounds render the bacterial growth by causing damage to bacterial cell membrane either structural or functional (Jharia, 2011).

5. Conclusion

The present findings confirm that three varieties of dates from Saudi Arabia contain appreciable amounts of carbohydrate, phenolic compounds, minerals as well as antibacterial potential. Furthermore, analysis of the acquired data reveals that dates of variety Sukri were superior to the other two varieties in terms of total carbohydrates, phenolic compound and antibacterial activity. However, there is a need to investigate the possible mechanism responsible for these effects and can be explored for developing antibacterial agents. Moreover, these varieties of dates prove to have immense potential to be utilized as a therapeutic agent for curing mineral deficiency.

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