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# Antibacterial Activity of Date Palm (*PhoenixDactylifera*L.) Fruit at Different Ripening Stages

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## Abstract

*In vitro* and *in situ* antibacterial activity of aqueous, ethanol, and ether extracts of three varieties of palm date (Khulase, Sheshi and Rezaz) in different maturation stages (Biser, Rutab and Tamer) were evaluated against some food borne pathogens. Gram positive bacteria showed higher sensitive against most extracts than Gram negative bacteria. Among the Gram positive bacteria, *Listeria monocytogenes*ATCC 7644 and *Staphylococcus saprophyticus* ATCC 15305 recorded highest sensitive against most extracts. The ethanol followed by water extracts of Biser stage had a stronger antibacterial activity than other maturation stage for all varieties against *Staphylococcus saprophyticus* ATCC 15305. The results of Minimum Inhibitory Concentration (MIC) shows that *Staphylococcus saprophyticus* was very sensitive to the lowest concentration of ethanol extract of Rezaz variety at Biser stage (RBOH), (MIC, and 3.75 mg/ml). RBOH was able to reduce the count of *Staphylococcus saprophyticus* in minced meat by above of 1 log cycle during storage at 5°C. This extracts (RBOH) contained highest amount of phenolics (2035.3 mg/100g) compared to other extracts.

**Keywords:** Date palm; Date varieties; Date ripening stages; Antibacterial activity; Food borne pathogens

## Introduction

Elevation of pathogenic bacterial contamination is one of the challenge factors which still cause food deterioration and serious diseases to humans around the world. Food borne illnesses are still a major concern for consumers, the food industry and food safety authorities. Meanwhile, consumers have been questioning the safety of synthetic preservatives of food. In recent decades an increasing tendency towards the use of natural substances instead of the synthetic ones has been observed. As the synthetic materials and products are more complex in comparison to natural substances, it will take a long time for them to complete their natural cycles and return to natural; thus causing a lot of environmental pollution. Also with the increase in the price of raw materials, the problem of cost benefits for chemical production is becoming more considerable. Food antimicrobials are the added or presented compounds in foods that hinder microbial growth or kill microorganisms. The functions of food antimicrobials are to inhibit or inactivate spoilage and pathogenic microorganisms. These functions have increased in importance in the past 10–15 years as food processors search for more and better tools to improve food safety especially those depending on the use of natural derivatives as antimicrobial agents [1].

Dates (*Phoenixdactylifera* L.) are an important nutritional source for many countries of the world, because the dates containing different nutrients such as carbohydrates, vitamins and minerals. From pollination to final date-fruit ripening takes 200 days. Date palm flowering and fruiting were recognized to have distinct stages over the ripening period. Hababauk, female flowers and immediate post-pollination period when the very young fruits are creamy white in color; Biser, sometimes named Kimri, green fruit undergoing rapid growth; Khalal, fruit grows slowly to full size, sugar content increasing while moisture content decreases, hard, glossy, red or yellow in color; Rutab, fruits ripening to a soft stage, brown in color; Tamar, fruits fully ripened, wrinkled, brown or black in color. All of the dates in different ripening stages containing various qualitative and quantitative amount of phytochemicals.

In recent decades an increasing tendency towards the use of natural substances as food additive instead of the synthetic ones has been observed. Natural phytochemicals, such as phenolic compounds, which extracted from many types of plants, are gaining importance that adds to foods, due to their benefits for human health, showed best antioxidant activity[2-9]. In addition to antioxidant activity, several studies demonstrated the antibacterial activity of phenols and/or phenolic extracts [2-9,10-13]. Besides nutritional values, dates are rich in phenolic and phytochemical components which changes during ripening stage [14-19] and may possessing antimicrobial activity. Several studies have been done on the antioxidant activity of dates [20-23,15,17,18] but the literature review are poor about studies on the antibacterial activity of dates. Thus, the purpose of the present investigation was to investigate the antibacterial activity of dates ether, ethanol, and water extracts in different ripening stages against some Gram-positive and Gram-negative bacteria *in vitro* and *in situ* (in food) in order to establish their biological activity for value.

## Materials and Methods

### Palm date

Three varieties of palm date (Khulase, Sheshi and Rezaz) were collected during maturation stages (Biser, Rutab and Tamer) during June to November, 2012, from Private El Ghanem farm. Date samples were dried directly after collection in under vacuum oven at 50°C, milled and freezing storage until analysis.

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## Preparation of extracts

One hundred gram portions of dried date were separately homogenized (IKA, T18 basic Ultra-Turrax, for 5 min) with 100 ml solvents having an increasing polarity; diethyl ether, ethanol and distilled water. The mixtures were then left, in the dark, at room temperatures overnight. Theprior to filtration (Whatman No. 1) and centrifugation (Sorvall RC-5, Dupont, USA) at 5000 rpm for 10 min at 5°C. The clear extracts were membrane filtered (0.45 µm) followed by dried under reduced pressure at 40°CThe dried extracts were refrigerated storage until use. The preparation of date extracts were done three times. The extraction yield is shown in Table 1.

## Microorganisms and growth conditions

Table 2 lists microorganisms which were used to evaluate the antimicrobial activity of ether, ethanol and Water. Microbial strains were maintained at 5°Cin slants of Brain Heart Infusion (BHI, HIMEDIA, and Mumbai, India) plus 1.5% Bacto-agar (Difco, USA). The other media used were Tryptic soy agar (TSA, Fluka, Switzerland) and Mueller- Hinton broth (Himedia, India) with 1.7% agar (MHA). Working cultures were activated in the respective broth at 30°Cfor 18 h.

## *In vitro* evaluation of antibacterial activity of palm date extracts by agar well diffusion assay

The agar well diffusion method was used for determination of *in vitro* antibacterial activity of palm date extracts. An actively growing indicator bacteria in a soft nutrient agar (Oxoid, Hampshire, UK) of 24 h culture at 35°Cwere spread on the surface of a nutrient agar plate medium (thickness of 5 mm) with a sterile glass spreaders (hockey sticks) which was rotated several times. The plates were left for about 30 min to ensure an even distribution of inoculum. 0.1 g of each dried extractwas dissolved in 5 mL of methanol and water mixture (60: 40, v/v), (20 mg/mL), then filter-sterilized by 0.45 µm syringe filter. Three holes were punched out of the agar, by using a sterile cork borer of 6 mm diameter. The well was filled by 100 µL of the sterilized supernatants of each extract (2 mg/well). The plates were incubated until zones of inhibition have clearly developed. The diameter of the complete inhibition zones was measured, including the well diameter. Zones are measured to the nearest whole number in millimeter, using transparent ruler. The antibacterial activity of date extractswas compared to the activity of both antibiotics, chloramphenicol (Riyadh Pharma, Saudi Arabia) and sulfadimidin (interchemie, Holland). The results are presented as the means of duplicate.

| Cultivars | Ripening stages | Extraction yield (g/100g) on dry weight basis* | | |
|---|---|---|---|---|
| | | Ether | Ethanol | Water |
| Khulase | Biser | 10.15 ± 1.21 | 29.56 ± 0.99 | 13.84 ± 1.65 |
| | Rutab | 10.01 ± 0.92 | 32.28 ± 2.65 | 31.29 ± 3.56 |
| | Tamer | 8.25 ± 0.73 | 78.45 ± 3.98 | 66.48 ± 4.58 |
| Sheshi | Biser | 12.17 ± 1.01 | 29.12 ± 2.11 | 25.75 ± 2.35 |
| | Rutab | 10.48 ± 1.32 | 32.70 ± 2.99 | 26.93 ± 1.56 |
| | Tamer | 10.55 ± 0.52 | 81.61 ± 4.65 | 80.52 ± 5.23 |
| Rezaz | Biser | 9.14 ± 0.75 | 29.49 ± 1.62 | 12.28 ± 2.31 |
| | Rutab | 12.23 ± 0.81 | 28.51 ± 2.51 | 25.51 ± 2.56 |
| | Tamer | 8.47 ± 0.28 | 67.83 ± 3.93 | 73.25 ± 5.18 |

*(Mean values ± SD), n = 3

**Table 1:** The extraction yield of date cultivars ether, ethanol, and water extracts at various ripening stages.

## Minimum inhibitory concentration (MIC)

The agar dilution method of Clinical and Laboratory Standard Institute, CLSL, (2006) was adopted to perform the MIC using Mueller-Hinton Agar, (MHA, CM0337, Oxoid) against *Staphylococcus saprophyticus* ATCC 15305. These bacterial strain and chloramphenicol were, respectively, used as the control strains and reference antibiotic. The stock solution of the antibiotic was prepared in potassium phosphate buffer (pH 8.0, 0.1 M) and diluted in buffer (pH 6.0, 0.1 M) to make working solutions (1000 and 100 mg/L). Those solutions were sterilized with 0.45 µmAcrodiscfilter (Gelman, USA).The media Muller-Hinton Agar (MHA) were aseptically added to sterile glass tubes containing extracts or the antibiotic. Negative controls water/methanol (40/60, v/v) were also used under the same conditions. The content (final volume 20 ml) of each tube was gently mixed and poured in Petri plates. The final concentrations of extracts were 0–20 mg/mL whereas that of chloramphenicol was 0.001 and 0.005 mg/mL. After hardening, the agar media were spotted with 5 µL (104cfu) of the tested bacterium. The spots were left to dry and then plates were inverted followed by incubation at 30°Cfor 12–48 h. The MIC was defined as the lowest concentration (mg/mL) of the extract resulting in no growth of bacteria. The test was conducted three times.

## *In situ* (In food) evaluation of antibacterial activity of palm date extracts

The *in situ* assay was performed against *Staphylococcus saprophyticus* ATCC 15305 in minced camel meat as food module. Pure colonies of *Staphylococcus saprophyticus* ATCC 15305 taken from plate count agar (Oxoid CM 463, UK) were suspended in 0.85% NaCl, and the concentration adjusted with the Spectrophotometer to give $3\times10^8$cfu/mL (as 0.5 ± 0.01 OD at 625 nm equal $3\times10^8$)[24]. This suspension was then serially diluted in maximum recovery diluent to give a suspension of $1\times10^6$cfu/mL. Under aseptic condition, 200 g of the meat (obtained from local super market) was placed into stomacher plastic page, inoculated with 20 ml of cell suspension (give an initial contamination rate of around $1\times10^5$cfu/g of meat) and mixed well with the Stomacher Lab Blender (Lab Blender, 400\UK). The meat divided into two portions, each portion was 100g in stomacher plastic pages, the first

| Microorganism | Strain | Origin | Gram Stain |
|---|---|---|---|
| *Escherichia coli* | ATCC 25922 | American Type Culture Collection | G- |
| *Pseudomonas aeruginosa* | ATCC 27853 | American Type Culture Collection | G- |
| *Salmonella enterica* | ATCC 13076 | American Type Culture Collection | G- |
| *Listeria monocytogenes* | ATCC 7644 | American Type Culture Collection | G+ |
| *Staphylococcus saprophyticus* | ATCC 15305 | American Type Culture Collection | G+ |
| *Staphylococcus aureus* | ATCC 25923 | American Type Culture Collection | G+ |
| *Staphylococcus aureus* | ATCC 29213 | American Type Culture Collection | G+ |

**Table 2:** Microorganisms which were used to evaluate the antimicrobial activity.

one was resaved as control and the other portion was mixed with the 5 g of dried ethanol extract of Rezaz variety at Biser stage dissolved in 10 ml saline solution. The samples were refrigerated storage and the counts of *Staphylococcus saprophyticus* were measured every 3 days.

## Determination of total content of phenolic compounds

The Total Content of Phenolic Compounds (TPC) in samples was determined according to the method reported by Boyer and Hai Liu [25] with some modifications. One ml of extract was mixed with 1 mL of 10% Folin-Ciocalteu reagent in distilled water and 4 ml of 7.5% sodium carbonate solution. The samples were maintained at room temperature for 30 min with periodical mixing, the absorbance at 765 nm was measured. The calibration curve was constructed within the concentration range 0.075–0.6 mg/mL of gallic acid. Mean values were calculated from three parallel analyses. Results were calculated as gallic acid equivalents in mg/100 g of dry plant material using the following equation:

$$C = a \times \gamma \times (V/m) \times 100$$

Where: C: total amount of phenolic compounds, mg/100g as gallic acid; a: dilution number; γ: concentration obtained from calibration curve (mg/mL); V: volume of aqueous ethanol used for extraction; m: weight of sample (g).

| Cultivars | Ripening stages | Extracts | Diameter of inhibition zone (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | *E. coli* ATCC 25922 | *Pseudomonas aeruginosa* ATCC 27853 | *Salmonellaenterica* ATCC 13076 | *Listeria monocytogenes* ATCC 7644 | *Staph. saprophyticus* ATCC 15305 | *Staph. aureus* ATCC 25923 | *Staph. aureus* ATCC 29213 |
| Khulase | Biser | Ether | ND | 10.5 ± 1.0$^{cd}$ | ND | 12 ± 0.5$^{gh}$ | 13.5 ± 0.0$^{g}$ | 17.5 ± 0.5$^{c}$ | 16.5 ± 0.0$^{c}$ |
| | | Ethanol | ND | 9 ± 0.5$^{d}$ | ND | 20 ± 1.0$^{c}$ | 35 ± 0.1$^{b}$ | 15 ± 0.5$^{d}$ | 15 ± 1.0$^{d}$ |
| | | Water | ND | ND | ND | 16.5 ± 0.0$^{de}$ | 14 ± 0.5$^{f}$ | ND | ND |
| | *Rutab* | Ether | ND | ND | ND | 13 ± 0.5$^{f}$ | 9.5 ± 1.0 | 8.5 ± 1.0$^{g}$ | 11 ± 0.5$^{fg}$ |
| | | Ethanol | ND | ND | ND | 13 ± 0.0$^{f}$ | 31 ± 0.0$^{c}$ | ND | ND |
| | | Water | ND | 8.5 ± 0.5$^{d}$ | ND | 15 ± 0.5$^{e}$ | 10 ± 0.5$^{h}$ | 9 ± 0.5$^{fg}$ | 12 ± 0.0$^{ef}$ |
| | *Tamer* | Ether | ND | 11 ± 0.5$^{c}$ | ND | 12.5 ± 0.5$^{fg}$ | 10 ± 0.5$^{h}$ | ND | 12 ± 0.5$^{ef}$ |
| | | Ethanol | ND | ND | ND | 14.75 ± 1.0$^{ef}$ | 29 ± 0.5$^{d}$ | ND | ND |
| | | Water | ND | 10.25$^{cd}$ | ND | 12.5 ± 0.0$^{fg}$ | 29.5 ± 0.5$^{cd}$ | ND | 10 ± 0.5$^{gh}$ |
| Sheshi | Biser | Ether | ND | 9 ± 1.0$^{d}$ | ND | 17.5 ± 0.0$^{d}$ | 17 ± 0.0$^{e}$ | 12.5 ± 1.0$^{e}$ | 11.5 ± 0.5$^{fg}$ |
| | | Ethanol | ND | 10 ± 0.5$^{cd}$ | ND | 16 ± 1.0$^{de}$ | 30 ± 0.5$^{cd}$ | 12.5 ± 0.5$^{e}$ | 16.5 ± 0.5$^{c}$ |
| | | Water | ND | 9 ± 0.0$^{d}$ | ND | 15.5 ± 1.5$^{e}$ | 29 ± 1.0$^{d}$ | 9.5 ± 0.5$^{fg}$ | 10.5 ± 0.0$^{gh}$ |
| | Rutab | Ether | ND | ND | ND | 12 ± 0.0$^{gh}$ | ND | 9 ± 0.0f$^{g}$ | 10 ± 0.0$^{gh}$ |
| | | Ethanol | ND | ND | ND | 12 ± 0.0$^{gh}$ | 31 ± 0.5$^{c}$ | ND | 9 ± 0.0$^{h}$ |
| | | Water | ND | ND | ND | 15 ± 0.5$^{e}$ | 9 ± 0.0$^{h}$ | ND | 11 ± 0.0$^{fg}$ |
| | Tamer | Ether | ND | 13.5 ± 1.5$^{b}$ | ND | 9.5 ± 0.0$^{h}$ | ND | ND | 9 ± 0.0$^{h}$ |
| | | Ethanol | ND | ND | ND | 12.5 ± 1.0$^{fg}$ | 35.5 ± 1.0$^{b}$ | ND | 8 ± 0.0$^{h}$ |
| | | Water | ND | ND | 9 ± 0.0$^{d}$ | 12.5 ± 0.5$^{fg}$ | 29.5 ± 0.5$^{cd}$ | ND | 8.5 ± 0.0$^{h}$ |
| Rezaz | Biser | Ether | ND | ND | ND | 15.5 ± 0.5$^{e}$ | ND | 10 ± 0.0$^{f}$ | 10.5 ± 1.0$^{gh}$ |
| | | Ethanol | ND | ND | ND | 15.5 ± 1.5$^{e}$ | 37.5 ± 0.5$^{a}$ | ND | 10.5 ± 1.0$^{gh}$ |
| | | Water | ND | ND | ND | 14.5 ± 1.0$^{ef}$ | 30 ± 0.5$^{cd}$ | ND | 8.5 ± 0.5$^{h}$ |
| | Rutab | Ether | 11 ± 1.0$^{c}$ | ND | ND | 12 ± 0.0$^{gh}$ | ND | ND | 8.5 ± 0.5$^{h}$ |
| | | Ethanol | ND | ND | 12 ± 0.0$^{c}$ | 13 ± 0.5$^{f}$ | ND | ND | ND |
| | | Water | ND | ND | 11 ± 1.0$^{c}$ | 15.5 ± 0.5$^{e}$ | ND | ND | ND |
| | Tamer | Ether | ND | ND | ND | 12 ± 0.5$^{gh}$ | 12.5 ± 0.0$^{g}$ | ND | 13 ± 0.5$^{e}$ |
| | | Ethanol | ND | ND | ND | 12.5 ± 0.0$^{fg}$ | ND | ND | 8.5 ± 0.5$^{h}$ |
| | | Water | ND | ND | ND | 11 ± 0.0$^{h}$ | ND | 13 ± 0.0$^{e}$ | ND |
| | | Chl | 44 ± 1$^{a}$ | 33 ± 1.0$^{a}$ | 48 ± 0.0$^{a}$ | 60 ± 0.0$^{a}$ | ND | 46 ± 0.0$^{a}$ | 46 ± 1.0$^{a}$ |
| | | Sul | 30 ± 1$^{b}$ | ND | 26 ± 0.0$^{b}$ | 30 ± 0.0$^{b}$ | 38 ± 0.0$^{a}$ | 32 ± 0.0$^{b}$ | 33 ± 1.0$^{b}$ |

$^{a\text{-}h}$Mean values ( ± SD; n = 6) within the same column bearing different superscripts are significantly different (*P>0.05*)
Chl: chloramphenicol, 25 mg/ml
Sul:sulfadimidin, 100 mg/ml
ND: Not Detected

**Table 3:** : Antibacterial activity (Diameter of inhibition zone mm) of date cultivars extracts at various ripening stages against pathogenic bacteria.

### Determination of pigments content

To prepare the extracts, a modified version of a previously published method [26] was used. Samples were soaked in methanol (1 g plant material per 2 mL methanol) for 24 h in refrigerator. The liquid was removed, and solid parts were soaked again in methanol. Extracts were combined and stored in refrigerator prior to analysis. The carotenoids fraction was measured in a UV spectrophotometer at 470 nm [27]. To determine the concentration of anthocyanin's the absorbance at 550 nm was determined and anthocyanin concentration was calculated using an extinction coefficient of 33000 mol-1 cm-1[28].

## Statistical Analysis

Three independent experiments were performed. All analysis and enumeration were done in duplicate. All data were analyzed by ANOVA using the general models procedure of SAS (1989) [29]. Differences among means were tested for significance (P>0.05) by Duncan's multiple range test.

## Results and Discussion

### *In vitro* Antibacterial activity of extracts

The antibacterial activity of date cultivars extracts at various ripening stages against some pathogenic bacteria are tabulated in Table 3. The results of the disk diffusion method indicated that ether, ethanol and water extracts of the three cultivars dates in different maturation stages showed different degrees of growth inhibition, depending on the bacterial strains. All tested extracts recorded stronger antibacterial activity against Gram positive bacteria than Gram negative bacteria. It has been stated that Gram negative bacteria are more resistant to various antibacterial against than Gram positive bacteria due to their outer lipopolysaccharide membrane cell [30,12,31].However, *Listeria monocytogenes* and *Staphylococcus saprophyticus* was very sensitive towered most date extracts than other Gram positive bacteria. Generally, *Staphylococcusaureus* ATCC 25923 was more resistance than other Gram positive bacteria. Whereas, *Staphylococcus saprophyticus* was more sensitive towered most date extracts than other Gram positive bacteria. The ethanolic extracts of the three tested cultivars (Khulase, Sheshi and Rezaz) in Biser ripening stage showed the stronger antibacterial activity by inhibiting growth of*Staphylococcus saprophyticus* (the diameter of inhibition zone, 35, 30 and 37.5 mm, respectively), also, the aqueous extracts of cultivar Khulase in Tamer ripening stage, Shashi in Biser and in Tamer stage and Rezaz in Biser stage showed the stronger antibacterial activity by inhibiting growth of *Staphylococcus saprophyticus* (the diameter of inhibition zone, 29.5, 29, 29.5 and 30 mm, respectively). Thus the ethanolic extracts of date illustrated the stronger antibacterial activity than aqueous extracts against *Staphylococcus saprophyticus*. The total phenols and pigments (carotenoids and anthocyanins) of date cultivars ethanol and water extracts at various ripening stages are tabulated in Table 5. The ethanolic extracts of all date's cultivars in Biser ripening stage recorded higher amount of phenols compared with the other stage (Rutab and Tamer). The methanolic extracts of Rezaz cultivar in Biser stage had highest contents of phenols (2035.3 mg/100g). Al- Qurashi [16] and Awad [17] reported that the total phenols concentration in date palm fruit sharply decreased during ripening to a low level means Biser stage containing the highest amount of phenolic compounds. Polyphenols play an important role as antibacterial activity through the precipitation of proteins and inhibition of enzymes of microorganisms [31,32]. The pigments (carotenoids and anthocyanins) in all date's cultivars increased with the increasing of ripening stages for both ethanolic and water extracts. Lutein is the major carotenoid pigment present in dates followed by β-carotene [14].Parshanth[33] and Al-Zoreky[34] proved that methanolic extract of pomegranate peels were more active than water extracts against some pathogenic bacteria. Shen[13] found antimicrobial effect of blueberry (are rich in phenolics) extracts against *Listeriamonocytogenes* and *SalmonellaEnteritidis*. The antibacterial activity of date extracts may be due to the phenolic compound which more extracted with ethanol and water than ether [2-9].

| Cultivars | Ripening stages | Extracts | MIC (mg/ml) *Staph. saprophyticus* ATCC 15305 |
|---|---|---|---|
| Khulase | Biser | Ethanol | 10 |
| | Rutab | Ethanol | 5 |
| | Tamer | Ethanol | 10 |
| | | Water | 10 |
| Sheshi | Biser | Ethanol | 5 |
| | | Water | 10 |
| | Rutab | Ethanol | 5 |
| | Tamer | Ethanol | 5 |
| | | Water | 5 |
| Rezaz | Biser | Ethanol | 3.75 |
| | | Water | 10 |

**Table 4:** Minimal inhibitory concentration (MIC) of most effective date cultivars extracts at various ripening stages against Staph. saprophyticus ATCC 15305.

### Minimal Inhibitory Concentration (MIC) of extracts

The minimum inhibitory concentration of the most active date cultivars extractsagainst the most sensitive bacteria (*Staphylococcus saprophyticus*) are presented in Table 4. The results shows that *Staphylococcus saprophyticus* was very sensitive to the lowest concentration of ethanol extract of Rezaz variety in Biser stage (MIC 3.75 mg/ml). While these bacteria appeared medium sensitive to the ethanolic extracts of khulase cultivar at Rutab stage and Sheshi cultivar at Biser, Rutab and Tamer stage (MIC, 5 mg/ml). However, the tested bacteria showed resistant to the other dates extracts (MIC, 10 mg/ml). The strong effect of ethanol extract of Rezaz variety in Biserstage may be due to the higher phenolic compounds content than other extracts (Table 5).Allaith (2008) found the total phenolics at Biserwere 196 ± 72.1 mg/100g but at Rutab stage were 116.7 ± 44.1 mg/100g based on fresh weight. *Staphylococcus saprophyticus* is often implicated in urinary tract infection [35].Haman [36] found that *Staphylococcus saprophyticus* was found to contaminate 16.4% of the various food samples with a high prevalence of 34% in raw beef and pork.

### In situ antibacterial activity of extract

*Staphylococcus saprophyticus* were common found in meat and carcasses as well as worker's protective gloves in slaughterhouse [36]. The survivalof *Staphylococcus saprophyticus* during refrigerated storage in minced camel meat medium effected by presence of ethanol extract of Rezaz variety at Biser stage (RBOH), (as a more effective extract) are showed in Figure 1.During storage *Staphylococcus saprophyticus* was not detected in non-inoculated meat used in trials. The obtained results revealed that the RBOH possessed an immediate inhibition (0.1 $\log_{10}$cycles) at zero time against tested bacteria in meat. However, *Staphylococcus saprophyticus* was gradually increasing in control sample (meat infected by *Staphylococcus saprophyticus*), reaching more than 5.4 $\log_{10}$cfu/g after 12 days of storage. Addition of RBOH significantly reduced the count of bacteria in meat gradually during storage. The

| Cultivars | Ripening stages | Total phenols (mg/100g) | | Carotenoids (mg/100g) | | Anthocyanins (mg/100g) | |
|---|---|---|---|---|---|---|---|
| | | Ethanol | water | Ethanol | water | Ethanol | water |
| **Khulase** | **Biser** | 1608.6 | 776.3 | 2.83 | 1.73 | 29.40 | 29.40 |
| | **Rutab** | 1384.8 | 944.2 | 5.41 | 3.25 | 39.41 | 33.40 |
| | **Tamer** | 1042.1 | 754.6 | 6.85 | 7.63 | 77.15 | 45.09 |
| **Sheshi** | **Biser** | 1657.6 | 629.46 | 4.58 | 2.85 | 31.73 | 29.06 |
| | **Rutab** | 902.2 | 511.3 | 4.16 | 3.81 | 32.40 | 47.76 |
| | **Tamer** | 937.2 | 552.4 | 6.85 | 4.39 | 47.43 | 46.76 |
| **Rezaz** | **Biser** | 2035.3 | 552.5 | 4.46 | 4.82 | 31.75 | 31.08 |
| | **Rutab** | 879.5 | 532.1 | 5.28 | 6.17 | 92.85 | 41.41 |
| | **Tamer** | 888.2 | 425.6 | 20.26 | 8.71 | 153.30 | 46.09 |

**Table 5:** The total phenols and pigments of date cultivars ethanol and water extracts at various ripening stages.

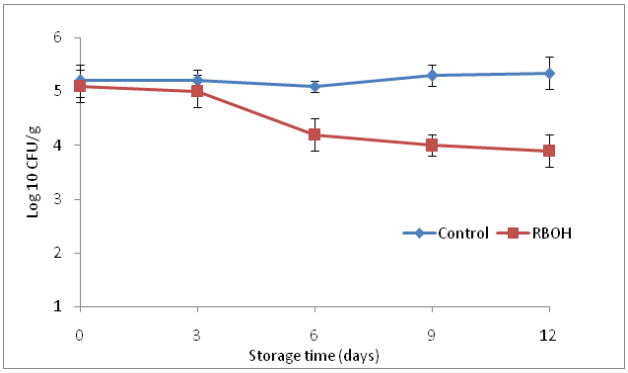


**Figure 1:** The survival of *Staphylococcus saprophyticus* during refrigerated storage in minced camel meat medium effected by presence of ethanol extract of Rezaz variety at Biser stage (RBOH).

viable cell numbers of *Staphylococcus saprophyticus* remained below the inoculation level (5 $\log_{10}$ /g) until the end of storage time. The presence of RBOH reduced 1, 1.2 and 1.3 $\log_{10}$cfu/g of *Staphylococcus saprophyticus* after 6, 9 12 days of storage period, respectively, in treated meat compared to the control sample. The inhibition ability of RBOH in treated meat could be attributed to the impact of phenolic compounds on the pathogen. Polyphenols played an important role in protein precipitation and enzyme inhibition of microorganisms [31,32].

## Conclusion

The results obtained indicate that the ethanol extract of Rezaz variety at Biser stage RBOH may be used for natural food preservation and may become important in the obtainment of a noticeable source of compounds with health protective potential and antimicrobial activity.

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