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The nutritional evaluation of *Ruthana* date fruits (*Phoenix dactylifera* L.) with its role in controlling free radicals and anti-inflammatory activities

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ABSTRACT

In this study, the polyphenol content was measured and the potential therapeutic value of the methanolic extract of *Ruthana* dates grown in Saudi Arabia was analyzed. The results showed that the *Ruthana* dates contain a high percentage of phenols and flavonoids, reaching 119.2 %, and 55.6 %, respectively. The antioxidant activity was also measured by several methods. The methanol extract of *Ruthana* showed significant effectiveness in inhibiting free radicals in the DPPH test. The highest inhibition rate was (79%) with an IC50 value of (3.97±0.21 mg Trolox/g plant), and (3.9035±0.380 µg/ml) on nitric oxide radical. The FRAP and ABTS tests showed higher sensitivity to antioxidants with IC50 values (4.08±0.26 mg of Trolox/ g of plant extract, and 4.18±0.26 mg of TEAC/ g of plant extract), respectively. The antioxidant activity was significantly correlated with the anti-inflammatory activity, the methanolic extract showed activity in preventing heat-catalyzed protein denaturation with a ratio of (74%), and IC50 (3.835±0.380 µg/ml), and at RBC'S membrane stabilization the effectiveness of the extract reached (68%) with IC5 value (6.215±0.238 µg/ml). The results showed a concordance between the phenolic content and the antioxidant and anti-inflammatory activity. Accordingly, the fruits of *Ruthana* dates constitute a promising possibility for isolating the active compounds and introducing them into the pharmaceutical industries in the future.

1. Introduction

Natural phytochemical compounds in plants have inspired drug development researchers to find largely safe treatments. It was noted that approximately 50% of the treatments used clinically are of natural origin (vegetable, animal, microorganisms) (Newman and Cragg, 2016). Moreover, plant products, whether fresh, dried, or their extracts, are considered functional food in the diet of individuals that supports general health and enhances the immune system, thus protecting or treating some health degenerations. Many natural products have been used since ancient times in traditional medicine to treat various ailments. The World Health Organization has defined traditional medicine as a series of skills, knowledge, or practices that draw on the theories, experiences, and beliefs of different cultures in protecting human health, whether physical or mental disease prevention or treatment (WHO, 2013). Traditional treatment methods are either taking plant extracts or using their oil for massage or spiritual therapies, and healing practices are adopted according to the culture of the country (WHO, 2013). This type of medical practice is spread in areas with limited capabilities, where 80% of health care is adopted in some countries in Asia and Africa on traditional medicine (WHO, 2013). According to WHO statistics, 60% of cases of fever in children believed to be caused by malaria are treated with herbs in Nigeria, Mali, and Ghana (WHO, 2004). Data on patients infected with human immunodeficiency virus (HIV/AIDS) revealed that two-thirds of those infected in developing countries use traditional

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medicines to relieve symptoms and strengthen immunity (UNAIDS, 2000). Other health problems in which herbs and medicinal plants are commonly used include digestive disorders, diabetes, and pressure, sickle cell anemia, high cholesterol and obesity, headaches, microbial infections, respiratory and bronchial diseases, fever, and skin infections as well as menopause (Calixto, 2000; WHO, 2004). A large number of plant compounds have been developed and used in pharmaceutical drugs, such as Camptothecin, which is extracted from the bark of a tree that grows in China called the Chinese happy tree (Dai et al., 2016), and vincristine alkaloids, which are originally from the periwinkle plant (Qu et al., 2019). Some of the herbal plants used in traditional medicine since ancient times are ginger, cinnamon, cumin, and cloves (Pérez-Jiménez et al., 2010).

Often the whole plant is not used, but some of its parts (fruits, seeds, bark, leaves, stems, etc.) may give each part a different value and effectiveness (Leonti, 2011), and this actually corresponds to medical products and nutritional supplements that contain extracts of plant parts, or even their use In spices and healthy drinks (Mamedov, 2012). On the other hand, some plants contain microbial content that has been exploited in the synthesis of biologically active compounds for use in resisting new pathogens that resist drugs (Adeleke and Babalola, 2021; Kraupner et al., 2018), such as the use of (*Penicillium notatum*) discovered by Alexander Fleming in the synthesis of antibiotics (Kim and Cha, 2021). This awakened researchers to expand the fields of scientific research on biologically active metabolites present in the plant's inner envelope or in the roots (Gupta et al., 2020).

Also, the exploration of active compounds increases the selection of food options in the daily diet, thus creating a healthy diet pattern that protects the human body from health risks and reducing or dispensing with the costs of treatment and health care, as it was indicated that chronic diseases worldwide will constitute an economic burden estimated at 17.3 trillion US dollars between 2011 and 2030 (Mozaffarian, 2016). Therefore, the prevention of health risks is very necessary to reduce these economic crises at the individual and international levels (Micha et al., 2017). Therefore, food menus now call for a varied diet that includes all nutrients, especially vegetables and fruits, with more than five servings per day, in addition to nuts, whole grains, seafood, and reduced processed red meat (McGuire, 2016; Mozaffarian, 2016).

In our current studies, we have highlighted the date fruit, which is one of the most important fruits of trees (the date palm tree) that grows in the Middle East and is used as a basic fruit, as well as in traditional medicine.

The study aimed to analyze the fruits of *Ruthana* date in vitro, to achieve the following objectives:

1. Measurement of the content of phenols and flavonoids.
2. Antioxidant activity.
3. Anti-inflammatory efficacy.

2. Literature review

The date palm tree (*Phoenix dactylifera*) produces 12 types of date fruit. It is one of the oldest trees whose cultivation spreads throughout the world such as the Middle East, South America, and North Africa, as well as areas of Europe, Pakistan, and India (Al-Shahib and Marshall, 2003; Tang et al., 2013), with more than 2000 variety worldwide (Al-Hooti et al., 1997).

Date fruit grows in five stages until it reaches maturity (*Hanabauk, Kimri, Khalal, Rutab, and Tamr*). The fruits differ from one variety to another according to their color, size, taste, and texture, as well as the proportions of the internal ingredients (Ahmed et al., 1995). Date fruit carries many nutrients that give it nutritional and health value, such as carbohydrates, minerals, vitamins, alkaloids, and tannins, as well as the most biologically active compounds of phenols, and flavonoids (cinnamic acids, flavonoid glycosides, and flavonols) (Biglari et al., 2008). Previous studies indicated the fact that date fruit has the potential to control various health setbacks, as it treats digestive disorders such as diarrhea and stomach ulcers, as well as plays a role in cardiovascular diseases, bacterial and viral infections, and cancer diseases. Therefore, it is considered a safe extract when used with fewer side effects (Baliga et al., 2011).

As for date seeds, they are a by-product that has several uses, including as animal feed. It is rich in magnesium, potassium, and dietary fiber (Basuny and Al-Marzooq, 2011). When ground and boiled, it is considered a caffeine-free coffee with a good taste (Habib and Ibrahim, 2009). It was recently indicated that it is a rich source of polyphenols and antioxidants (Ardekani and Naeini, 2010).

Previous studies indicated the importance of *Ruthana* dates among the different types of dates. It showed a high nutritional value due to contains vitamins, minerals, and many biologically active compounds. Also provides a high energy content between (288 kcal/100 g, to 358 kcal/100 g) due to the proportions of carbohydrates, which are the main source of energy in the body (Al-Farsi et al., 2005). As well as a significant antioxidant activity (El-Mergawi et al., 2019); this was attributed to its richness in phenolic compounds. Containing antioxidants and fighting free radicals supports its effectiveness in the anti-inflammatory effect (Zhang et al., 2017). As well as the resistance to cancers or cytotoxicity of cancer cell lines that were recently studied, as inflammation and cancers are closely related to the formation of free radicals and reactive oxygen species (Jakubowska et al., 2016), but studies in this scope are still limited.

Several research results agreed on extracts of different types of date fruits, such as Ajwa and Khalas on prostate cancer cell lines and pancreatic

stellate cells. Both results showed the ability of the extracts to inhibit the mitochondrial membrane potential of cancer cells and the occurrence of programmed death (Al Alawi et al., 2020; Mirza et al., 2018). It was also indicated that the extracts of the fruits of *Ruthana* dates by Zhang et al. (2017) showed a significant effect in cytotoxicity on breast cancer cells, colon cancer cell lines, and stomach cancer. But the exact mechanism of cytotoxicity remains unclear.

According to Kahkashan et al. (2012), *Ruthana* fruit and leaf extracts showed significant activity on different gram-positive and gram-negative bacterial strains, such as (*S.aureus, S.epidermidi, Enterococcus faecalis, Klebsiella pneumoniae and E.coli*) and their effect was like that of the extracts of Barhi and Sukari cultivars. This gives it value in fighting microbial and bacterial diseases.

Date fruit contains carbohydrates with a low glycemic index, making it a food recommended for improving and controlling blood sugar levels. Studies have shown the ability of different types of dates (*Ruthana*, *Medjool*, *Khudari*, *Ajwa*, and *Sukkari*) to control the levels of sugar in the blood of diabetic rats (AlGeffari et al., 2016).

Due to the varied content of micro and macronutrients in date fruit, it provides an integrated functional food that can be adopted into the diet with different nutritional recipes that support the immune system and general health. Such as date syrup or yogurt fortified with dates or baked products and desserts made with date paste (Nwanekezi et al., 2015), date fruit jam (Besbes et al., 2009), and energy bars made from dates (Alla and Jithendran, 2018). They all provide nutritional and possibly therapeutic value.

3. Methodology

The method framework of the conducted study is illustrated in Fig. 1.

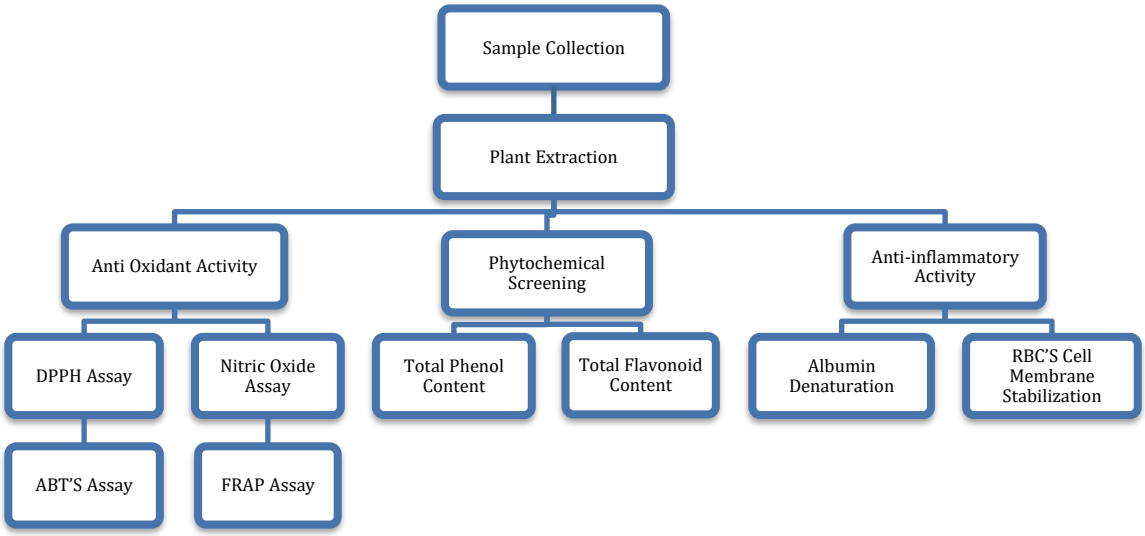

Fig. 1: Study method framework

3.1. Sample collection

The *Ruthana* fruit was collected during the harvesting season (beginning of May) (Table 1) from local farms located in the Medina region in the Kingdom of Saudi Arabia. The fruits were picked manually by a responsible farmer on the palm farm. Each pick of dates is wrapped with a very light cover and perforated to protect the fruits from some insects. Uniform-sized, matured, and non-damaged date fruits from the Tamr stage were selected for analysis and experimentation. The fruits it was kept at room temperature, without sunlight, and were extracted using a methanolic solution and stored until tested.

Table 1: Sample collection

Family	Scientific name	Common name	Time of collection	Part used
Aceraceae	*Phoenix dactylifera* L.	*Ruthana Rutab*	May-2021	Fruit (In full maturity)

3.2. Preparation extract

Date fruit methanolic extract (DFME) was prepared by mixing 25 g date without seeds in 250 mL methanol and left for three days with intermittent shaking using samples platform shaker at 25 °C. After that mixture was subjected to filtration under vacuum using Buchner Funnel. The crude methanolic extract was concentrated using a rotary evaporator by evaporation of the solvent at 65 °C with continuous rotation. Then the extract is stored at 4°C for the time of use (Ahmed et al., 2020).

3.3. Phytochemicals content

3.3.1. Total phenolic content

Polyphenols (e.g., phenols and flavonoids) are phytochemical compounds of great importance because they play a major role in antioxidant activity by inhibiting free radicals, and this activity is

attributed to their containing hydroxyl groups (Soobrattee et al., 2005). Measurement of plant polyphenols content is very important for estimating its association with antioxidant activities and anti-inflammatory efficacy.

The phenol content test in DFME was carried out according to the Folin-Ciocalteu reagent method (colorimetric method). To prepare standard Gallic acid, the different concentrations (25, 50, 75, and 100 µg/ml) were prepared. Then 1ml of Gallic acid was mixed with 5ml of Folin-Ciocalteu reagent (10%), and 4 ml of 7% Na_2 CO_3 to produce a blue color mixture. The mixture was incubated at room temperature (25 °C) for 30 min without light. After that, the absorbance was measured at 760 nm against a blank by a spectrophotometer. The average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve. The (DFME) samples were prepared in the same way as the standard at different concentrations. So that the final mixture consists of 5 ml Folin-Ciocalteu reagent (10%), 4 ml 7% Na_2Co_3, and different concentrations of DFME. The percentage of phenol was calculated based on a Gallic acid calibration curve.

3.3.2. Total flavonoids content

The total flavonoid content of date fruit extract was determined according to Karra et al. (2020). To prepare the Quercetin standard solution, different concentrations of standard quercetin (2.0, 1.0, 0.5, and 0.25 mg/ml) were prepared. 1 ml quercetin of each concentration was added to 4 ml distilled water. Then 0.3 ml of 5% $NaNO_3$ was added, after 5 min, 0.3 ml of 10% $AlCl_3$ was added and at 6 min, 2 ml of 1 M NaOH was added to the mixture. Immediately, the total volume of the mixture was made up to 10 ml by the addition of 2.4 ml of distilled water and mixed thoroughly. The absorbance of the pink color mixture was determined at 510 nm versus a blank containing all reagents except quercetin. The average absorbance values obtained at different concentrations of quercetin were used to plot the calibration curve (Pandey and Rajbhandari, 2014).

Samples of DFME were prepared in the same standard method, so that the test mixture consists of different concentrations of DFME, 4ml distilled water, 0.3 ml (5%) $NaNO_2$, 0.3 (10%) $AlCl_3$, and 2ml (1M) sodium hydroxide. The total flavonoid content was calculated based on the quercetin standard calibration curve.

3.4. Assessment of the antioxidant activity

The antioxidant activity of date fruit extract was studied using the most common methods (DPPH, FRAP, Nitric oxide, and ABTS radical scavenging assay), which work on the principle of the ability to inhibit free radicals.

3.4.1. Radical scavenging activity of *2,2-Diphenyl-1-Picryl Hydrazyl* (DPPH)

The DPPH test is one of the most common antioxidant tests performed in a short time. The principle of the test is based on the removal of DPPH by the antioxidant and the decolorization of a methanolic DPPH solution through reduction reactions. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical that reacts with hydrogen-giving compounds (antioxidants), and the maximum UV absorption of these compounds is 515 nm.

The free radical scavenging activity of the DFME was measured as described by Garcia et al. (2012) with minor modifications. 0.1 ml of different concentrations of test extract were mixed with 0.9 ml of 0.1 mM methanolic solution of DPPH. The mixture was shaken vigorously by hand and allowed to stand, in dark, at room temperature for 30 minutes. The change in absorbance was recorded at 517 nm. The assay was performed in triplicates and the percentage inhibition of free radical DPPH was calculated according to Sakat et al. (2010) as follows:

$$No.radicalscavenging(\%) = \frac{control_{OD} - sample_{OD}}{control_{OD}} \times 100 \quad (1)$$

3.4.2. Ferric reducing antioxidant power (FRAP)

Ferric-reducing antioxidant power was determined in the extract according to Benzie and Strain (1996) The principle of this method is based on the ability of the extract to reduce a ferric 2,4,6-tripyridyl-s-triazine complex (Fe3+-TPTZ) to its ferrous colored form (Fe2+-TPTZ) in the presence of antioxidants.

The FRAP assay was based on the reducing power of antioxidants in which a potential antioxidant will reduce the oxidized ferric ions to the ferrous ions, which form a blue-colored ferroustripyridyl triazine complex. The FRAP reagent was prepared by mixing 10 mM TPTZ (1ml) and 20 mM $FeCl_3$ (1ml) in 0.25 M acetate buffer (10 ml, pH 3.6). The DFME sample (50 µl) was added to 3 ml of the FRAP reagent (the final concentration of the date fruit extract in the solution was 100µg/ml). The tests were carried out in triplicate. The absorbance was measured at 593 nm after 8 min incubation at room temperature. Trolox (final concentration 0 to 1 µg/ml) was used as a standard for the construction of the calibration curve. The antioxidant capacity based on the ability to reduce ferric ions of the extract was expressed as mg Trolox equivalents per gram of plant extract.

3.4.3. $ABTS^+$ radical scavenging assay

The ABTS test proportionally measures the ability of antioxidant compounds to inhibit the free radical ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) that forms in the aqueous phase in reaction with a strong oxidizing agent such as potassium persulfate. The radical scavenging ratio of the studied sample is measured based on the

decrease in the absorbance by Trolox absorbance at a wavelength of 734 nm. ABTS was dissolved in deionized water to a 7 mM concentration and mixed with 2.45 mM potassium persulfate. Then the mixture was placed in the dark for 12 to 16 h at room temperature. The ABTS+• solution was diluted in deionized water or ethanol to an absorbance of (0.706) at 734 nm using a spectrophotometer.

Samples with different concentrations were prepared and mixed with ABTS in a 96-well plate. The mixture was kept in the dark at 37 °C for 6 min, whereas methanol was used as a blank, and Trolox was used as a positive control (Al-Qudah et al., 2014). The percentages of scavenging effects were calculated by the equation below:

$$Inhibition\ (\%) = (A1 - A2)/A1 \times 100\% \quad (2)$$

where A_1 is the absorbance of the control, and A_2 is the absorbance of the sample.

3.4.4. Determination of nitric oxide radical scavenging activity

Nitric oxide radical assay measures the concentration of nitric oxide resulting from the conversion of nitrate to nitrite via nitrate reductase by colorimetric detection.

3 ml of 10 mM sodium nitroprusside in 0.2 M phosphate-buffered saline (pH 7.4) was mixed with different concentrations of DFME extract and incubated at room temperature for three hours. After incubation time, 0.5 ml of Griess reagent (1% sulfanilamide, 0.1 % naphthyl ethylene diamine dihydrochloride in 2% H_3PO_4) was added. The absorbance was read at 546nm (Sakat et al., 2010).

The percentage radical scavenging activity of the samples (triplicate) was calculated as follows:

$$No.radicalscavenging\ (\%) = (control_{OD} - sample_{OD})/ control_{OD} \times 100 \quad (3)$$

3.5. Assessment of anti-inflammatory activity

Inflammation is scientifically defined as the body's own response because of a physical injury or microbial infection and is accompanied by symptoms of redness, fever, pain, and swelling because of a disturbance in the physiological functions of the affected organ. Non-steroidal drugs are the common treatment for infections, but they carry several side effects, such as stomach ulcers (Chandra et al., 2012). Modern medicine seeks to find natural anti-inflammatory alternatives, such as herbs and medicinal plants. In this section, we studied in vitro the effect of *Ruthana* date fruit extract on protein denaturation and erythrocyte (RBC's) membrane resistance from lysis.

3.5.1. Inhibition of albumin denaturation

The anti-inflammatory potential of DFME extract was evaluated by measuring the percentage of inhibition in Albumin denaturation according to the method of Mizushima and Kobayashi (1968) and then of Sakat et al. (2010) with minor modifications.

The reaction mixture was consisting of 1 ml of DFME at different concentrations added to 1 ml of 1% aqueous solution of bovine albumin (pH 5.3). The samples were incubated at 37°C for 20 minutes and then heated at 57°C for 20 minutes. The samples were left to cool after, and the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

$$Inhibition(\%) = (Control_{Abs} - Sample_{Abs}) \times \frac{100}{Control_{Abs}} \quad (4)$$

where, $Sample_{Abs}$ is the sample extract and the $Control_{Abs}$ is the absorbance control.

3.5.2. RBC's cell membrane stabilization test

Fresh whole blood was collected from laboratory animals (Rabbits) in the blood tubes with an anticoagulant. The tubes were centrifuged at 3000 rpm for 10 minutes; the pellets were washed three times with equal volumes of normal saline. The volume of the blood was measured and reconstituted as 10% suspension with normal saline.

Based on Shinde et al. (1999), the reaction mixture (2 ml) consisted of 1 ml of different concentrations of DFME sample, and 1 ml of blood suspension, instead of DFME only saline was added to the control test tube (negative control). Aspirin was taken as a standard drug-positive control). All preparations were incubated in a water bath at 56 °C for 30 minutes. After the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatants was taken at 560 nm.

4. Results and discussion

4.1. Percentage yield of plant extract

The proportion of crude methanolic extract of *Ruthana* date fruits was as shown in Table 2.

Table 2: Yield of MeOH crude plant extracts (%)

Scientificname	*Phoenix dactylifera* L.
Common name	*Ruthana* date
Dry weight [g]	25
Weight of MeOH extract [g]	4.62
Yield of MeOH extract (%)	18.48

4.2. Determination of phytochemicals content

4.2.1. Total phenolic content

The total phenol content of the studied date extract was measured using a colorimetric Folin-Ciocalteu assay which is a method based on the

transfer of electrons in an alkaline medium. Gallic acid was used as a standard compound. Absorbance ratios were read for different concentrations at 760 nm. Based on the calibration curve equation (Fig. 2) the final content was calculated and expressed as mg of gallic acid (GAE) per 100 grams of extract (Table 3).

Total phenolic content in the sample was calculated using the formula: C=cV/m where, C=total phenolic content mg GAE/100g dry extract, c=concentration of gallic acid obtained from calibration curve in µg/ml, V=volume of extract in ml, m=mass of extract in gram.

The phenolic content of the methanolic extract of *Ruthana* dates was 119.2±2.76. This value corresponds to the range of phenolic content of dates reported by Mohamed et al. (2016), ranging between (119±8.5 mg GAE/100 g DW of the Shalabe cultivar, which is approximately equal to the content of the *Ruthana* cultivar) and (247±14 mg GAE/100 g DW of Safawi cultivar). And the highest content of the Tunisian types of dates that were studied by Chaira et al. (2009) where the percentages of phenolic content ranged between (12.05±91.28 mg/100 g FW for Nefzaou dates) and 54.66±91.21 mg/100 g FW for Korkobbi dates).

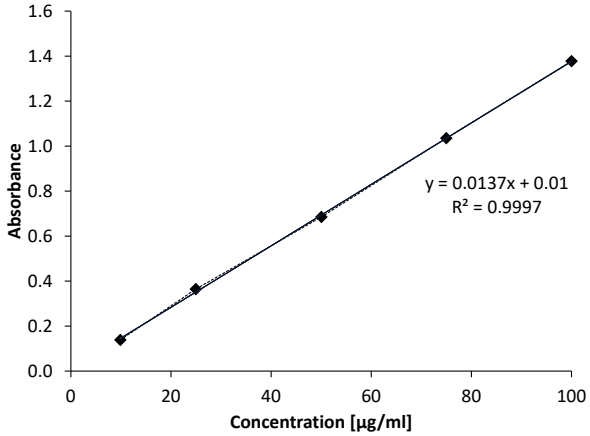

Fig. 2: Gallic acid standard curve

4.2.2. Total flavonoid content

The content of flavonoids in the plant extract was measured based on the aluminum chloride colorimetric method, in which the reaction with sodium nitrite is carried out, which later develops into the formation of a flavonoid and aluminum complex when aluminum chloride is added in an alkaline medium. Quercetin was adopted as a standard compound, and the absorbance was measured at 510 nm at different concentrations. Based on the standard calibration curve equation (Fig. 3), the flavonoid content of *Ruthana* dates was calculated as mg of quercetin equivalent (QE) per gram of extract (mg/100g dry weight) (Table 3).

Total flavonoid content is calculated by using the formula: C=cV/m where, C=total flavonoid content mg QE/g dry extract, c=concentration of quercetin obtained from calibration curve in mg/ml, V=volume of extract in ml, m=mass of extract in gram.

The flavonoid content of the methanolic extract of *Ruthana* dates was 55.584±1.31. This variety of dates is rich in flavonoids compared to Rotbi (8.64 ±90.08 QE/100 g FW), (Khlase 27±1.5 mg Q E/100 g DW), (Barni 52±2.2 mg Q E/100 g dry weight) (Mohamed et al., 2016).

Table 3: Total phenols and flavonoids content

TPC (mg GAE/100gm of plant)	119.2±2.76
TFC (mg QE/100gm of plant)	55.584±1.31
QE/GAE (%)	46

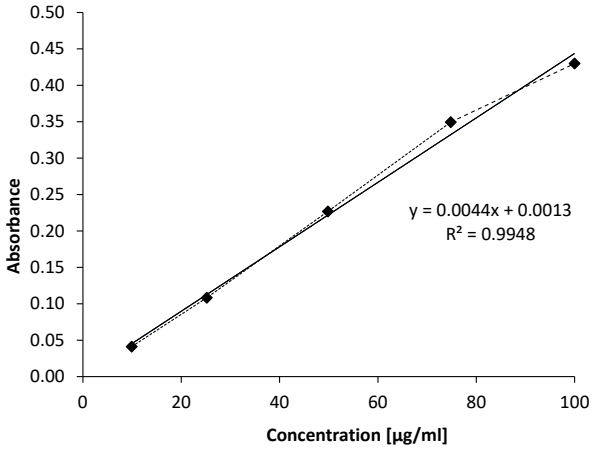

Fig. 3: Quercetin standard curve

4.3. Assessment of the antioxidant activity

Antioxidant activities of DFME were assessed by detecting their ability to scavenge the free radicals using the DPPH method, FRAP assay was used as a direct test of total antioxidant power, also ABTS method and nitric oxide radical scavenging assays.

4.3.1. DPPH radical scavenging activity

DPPH radical was used for the evaluation of free radical-scavenging properties of the plant extract. To measure the Trolox equivalent antioxidant capacity (TEAC), a Trolox standard curve was established using ten concentrations of Trolox standard solution (0 to 1.5 µg/ml) (Fig. 4). The activity of date fruit extract as a free radical scavenger was measured and expressed as TEAC [mg Trolox/g of plant extract] (Table 4). The results showed that date fruit extract has high effectiveness in inhibiting free radicals up to 79% at a concentration of 100 µg/ml (Fig. 5), with an IC50 value of (3.97±0.21 mg Trolox/g plant). This value is consistent with the antioxidant efficacy evaluated by El-Mergawi et al. (2019) in the same tested method and the IC50 value for *Ruthana* dates was (3.37±0.22). In line with these results, Mohamed et al. (2016) evaluated the antioxidant activity of a number of different date cultivars, and the effectiveness ranged between (1.65±0.04 for Ajwa date extracts and 8.25±0.21 for Khalas date extracts). The free radical inhibiting activity is attributed to the phenolic compounds and flavonoids.

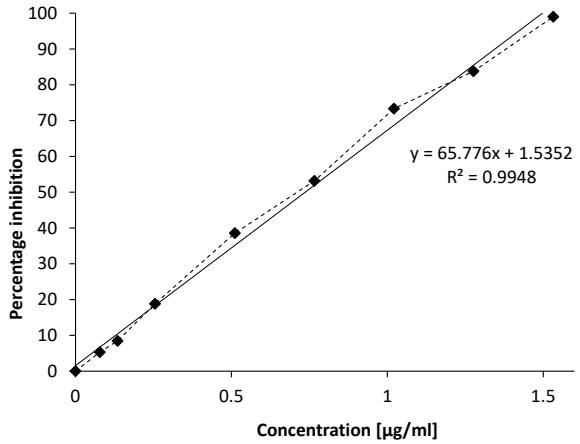

Fig. 4: Trolox standard curve for the TEAC measurement of plant extract in the DPPH method, data expressed by mean±SD, n=3

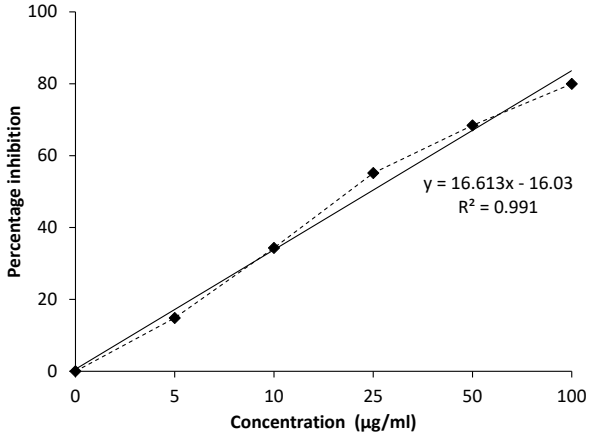

Fig. 5: The effect of DFME, on DPPH radical scavenging activity

4.3.2. Ferric reducing antioxidant power (FRAP)

The ability of methanolic date extract to reduce ferric ions as an indicator of antioxidant activity was measured using the FRAP assay. The antioxidant reacts with the ferrous triethyltriazine complex (Fe3+-TPTZ) and this reaction produces the colored ferrous trimethyltriazine (Fe2+-TPTZ). Thus, it breaks the free radical chain by donating a hydrogen atom and lowering Fe3+-TPTZ to blue. At a pH equal to 3.6. To calculate the antioxidant equivalent capacity (TEAC), the Trolox curve was generated as a reference curve (Fig. 6). The activity of the methanolic extract was calculated and expressed in mg Trolox/g of plant extract.

The results showed that the extract had a significant activity scavenging ability equal to 4.08 mg of Trolox/ g of plant extract (Table 4). It was noted that the effectiveness of the FRAP test is higher than that of the extract in the DPPH test.

Among the various plants studied, FRAP levels ranged between (0.88-18 mg/gdw). The value we obtained in this test was lower than the value reported (El-Mergawi et al., 2019). But in contrast, it is higher than a large number of plants such as Vitexnegundo (2.69±0.11mg/gdw) and Gymnema Sylvestre (1.00±0.07 mg/gdw) (Rajurkar and Hande, 2011). These values differ based on their relation to the content of bioactive compounds and it was observed that their concentration is higher in dried date fruit extracts than in fresh extracts (Al-Farsi et al., 2005).

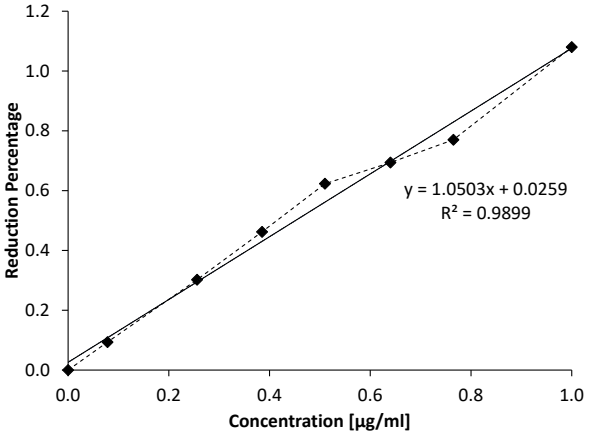

Fig. 6: Trolox standard curve for the TEAC measurement of methanolic date fruit extract in the FRAP method, data expressed by mean±SD, n=3

4.3.3. ABTS⁺ radical scavenging assay

The ABTS positive radical decolorization test showed the ability of the methanolic extract of date fruits to inhibit free radicals, by eliminating ABTS radical cation which was produced in the stable form using potassium persulphate, and the antioxidant capacity was calculated by comparing it with the reference Trolox (Fig. 7). The TEAC value was (4.18±0.262 mg TEAC/g dry weight) (Table 4). It was observed that the TEAC value obtained with the ABTS test is higher than that of the DPPH, and approach to the FRAP test. This indicates that the FRAP and ABTS tests are more sensitive to polyphenols than the DPPH assay.

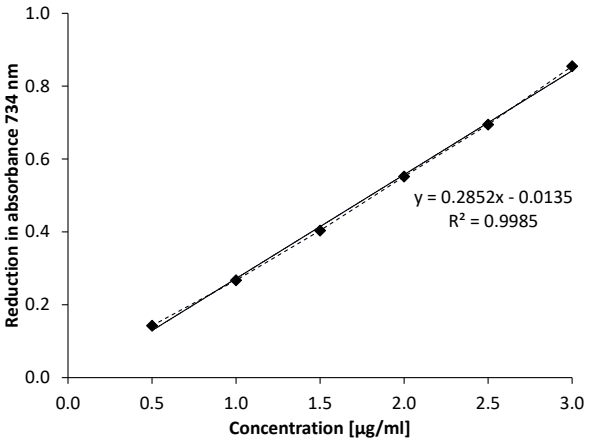

Fig. 7: Trolox standard curve for the TEAC measurement of methanolic date fruit extract in the ABTS method, data expressed by mean±SD, n=3

4.3.4. Determination of nitric oxide radical scavenging activity

The ability of the tested extracts to neutralize the produced nitric oxide radical in aqueous solutions and thus reduce the formation of nitrite ions is

considered a criterion to nominate plant extracts as sources of antioxidant compounds. methanolic date fruit extract exhibited antioxidant activity by reducing the number of resulting nitrites due to oxidation of the nitric oxide. The antioxidant compounds sources caused 50% inhibition of the oxidation reaction with IC50 equal to 3.90351± 0.380 in Table 4. The highest radical inhibition was reached (77.1% at 500 µg/mg concentration) (Fig. 8). This activity is higher than that of other types of dates (Al-Mamary et al., 2014), and the total antioxidant value of dates exceeds that of more than 20 fruits consumed in China (Guo et al., 2003).

Table 4: IC50 of antioxidant activity of DFME

Assay method	IC50 value
TEAC DPPH (mg Trolox/g plant)	3.97±0.21
TEAC FRAP [mg of Trolox/g of plant extract]	4.08±0.269353
TEAC ABTS [mg of TEAC/g of plant extract]	4.18±0.262
(NO) Nitric oxide radical scavenging (µg/ml)	3.90351±0.380

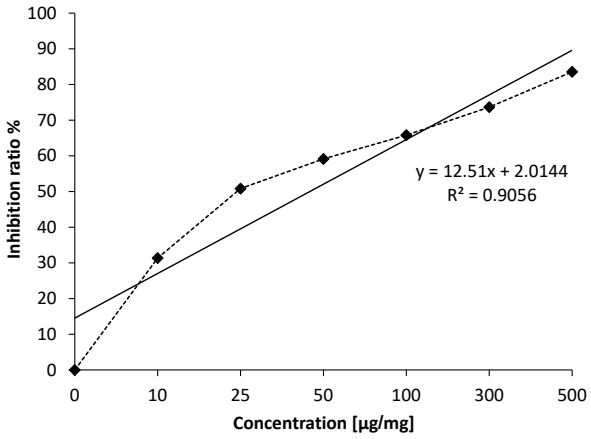

Fig. 8: the effect of DFME on nitric oxide

4.4. Anti-inflammatory activity

4.4.1. Inhibition of albumin denaturation

Various inflammatory diseases occur because of protein denaturation and the production of autoantigens may be caused by denaturation in the case of osteoarthritis. Therefore, it is imperative to know and develop anti-inflammatory agents. The ability of the methanolic extract to prevent heat-induced membrane denaturation was evaluated by forming a reaction mixture consisting of bovine serum albumin with phosphate-buffered saline at a pH of 5.3 and by adding variable concentrations of methanolic date fruit extract and using distilled water of the same volume as a control.

Methanolic date fruit extract showed anti-inflammatory potential, by preventing heat-induced denaturation of albumin. The efficacy was related to an increase in the extract concentration, where the maximum efficacy reached (74%) at a concentration of 100 µg/ml (Fig. 9), with an IC50 value equal to 6.215 ± 0.238 µg/ml (Table 5). These results are supported by Taleb et al. (2016) who indicated the role of date fruits in vivo and in vitro, the study showed that date fruits contain anti-inflammatory and activity related mainly to secondary metabolites as well as to the mechanism of antioxidants.

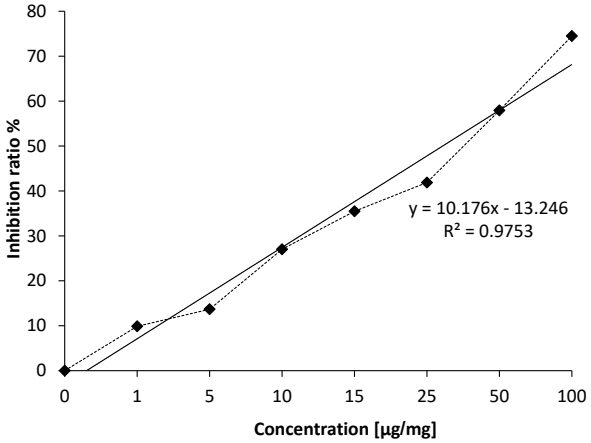

Fig. 9: The effect of DFME on albumin denaturation

4.4.2. RBC'S membrane stabilization test

An erythrocyte membrane stabilization technique was used to detect anti-inflammatory activity. A non-steroidal anti-inflammatory drug (aspirin) was used as a reference standard. Hemoglobin content was estimated using a spectrophotometer at 560 nm. Fresh whole blood samples drawn from laboratory animals (rabbits) were used, considering that they had not taken any antibiotics during the previous month. The results indicated that the methanolic extract of date fruit exhibited an activity that contributed to preventing membrane dissolution when exposed to heat with a ratio of IC50 (µg/ml 5.486±0.253) correlated with the concentration in Table 5, and the highest percentage of heat-induced membrane inhibition reached (68.8±0.014) (Fig. 10).

Applying aspirin to the RBCs hemolysis test showed the protecting ability of this drug to stabilize the membrane at IC50 of 2.335±0.010 µg/ml with a maximum inhibition in thermally induced blood cell lysis up to 86.226±0.22 percent at 200µg/ml as shown in Fig. 11. Worthy to mention, aspirin does induce hemolysis in RBCs at concentrations of more than 200 µg/ml at room temperature after 30 min.

Previous literature confirms the effectiveness of date fruit in reducing inflammation of all kinds, as it shows neuroprotection that resists oxidative stress and protects neurons (Maryam et al., 2015). Dates reduce the expression of pro-inflammatory cytokines (IL-6, IL-10, and TNF-α), due to their richness in antioxidants, phenols, carotenoids, and anti-oxidant enzymes (Al-Yahya et al., 2016).

5. Conclusion

This study concluded the health potential provided by date fruit because it contains a high percentage of biologically active compounds (phenols and flavonoids), which are the source of antioxidant activity and inhibit free radicals, thus

controlling inflammation and preventing its development. *Ruthana* date fruits are considered a suitable potential choice for introduction in food industries and pharmaceutical applications.

Table 5: The anti-inflammatory activity of DFME

Assay method	Highest scavenging activity (%)±SD	IC50 value
Inhibition of albumin denaturation	74.496±0.238	6.215±0.238
RBC'S membrane stabilizing (µg/ml)	68.87±0.0141	5.486±0.253

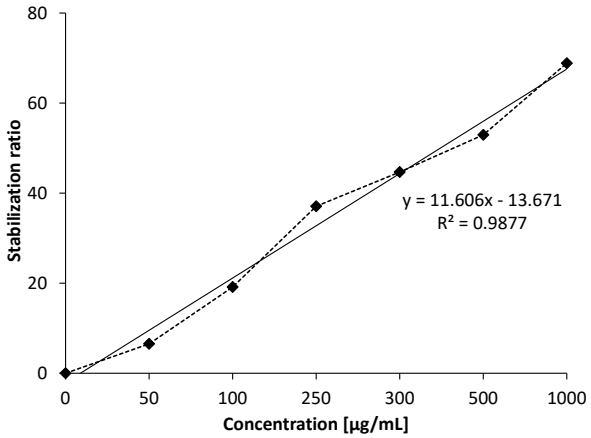

Fig. 10: Effect of DFME on RBC'S membrane stabilizing

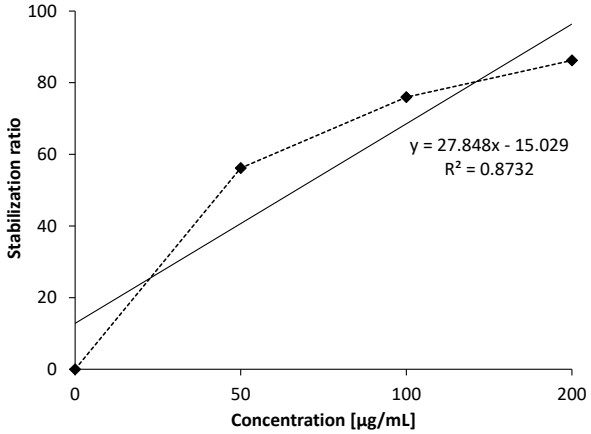

Fig. 11: Effect of aspirin as standard on RBC'S membrane stabilizing

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Compliance with ethical standards

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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