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# Exploring the bioactivities and phytochemistry of *Phoenix dactylifera* L. (Aseel and Zahidi dates)

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**Abstract**
Date palm (*Phoenix dactylifera L.*), a monocotyledonous plant belonging to the Arecaceae family, has been utilized as a medicinal plant. In this study, we investigated and compared the cytotoxicity, antioxidant, antidiabetic, and antimicrobial properties of the fruit pulp extracts from two varieties of *Phoenix dactylifera*, namely Aseel and Zahidi. Solvent extraction was performed using the maceration method to extract the bioactive components. In this study, the antioxidant activity of two *Phoenix dactylifera* varieties was assessed by determining total flavonoid content (TFC), total phenolic content (TPC) and DPPH (2,2-diphenyl-picrylhydrazyl) assay. Alpha-amylase inhibitory assay confirmed anti-diabetic potential. Hemolytic assays measured cytotoxicity. The results of all activities were expressed as mean±S.D and a t-test was applied to check the significance of the results. *Phoenix dactylifera* (Aseel date) showed 61.4±5.9mg CE/100 g TPC, 28.03±0.72 mgGAE/100 g TFC and 69.0±1.0 DPPH scavenging activity. Zahidi variety has 49.1±2.51 mgCE/100 g TPC, 115.8±0.65 mgGAE/I00 g TFC and 76.6±0.48 DPPH scavenging activity. *Phoenix dactylifera* (Aseel date) inhibits *E. coli* and *Staphylococcus aureus* at 13mm and 15mm, respectively. Zahidi variety had no inhibition zone. Chemical characterization of the extracts was performed using Fourier transform infrared spectroscopy (FTIR).

**Keywords:** Antioxidant, antidiabetic, antimicrobial, hemolytic activity

## Introduction
According to the World Health Organization (WHO), 80% of the world's population relies on herbal remedies for specific conditions. Dates are high in phytochemicals like polyphenols, carotenoids, sterols, and tanning, which are essential for maintaining good health [1]. *Phoenix dactylifera* commonly known as date palm belongs to the family Arecaceae and order Arecales which has over 3,000 species in about 200 genera. It is a monocotyledonous, perennial tree species [2]. *Phoenix dactylifera* L. has high carbohydrate content and pantothenic, folate, pyridoxine riboflavin, thiamine, and niacin. According to studies, *Phoenix dactylifera* L. contains significant amounts of the important amino acids, like proline, leucine, histidine, lysine, aspartic acid, glycine and glutathione. Saponins, vitamins, alkaloids, tannins, steroids, and flavonoids were found in *Phoenix dactylifera* L. in a phytochemical analysis [3].
It has hepatoprotective, antioxidant, anti-diabetic, antihypertensive, anti-inflammatory, anti-ulcerative, anti-diarrheal, antifungal, antiviral, antibacterial and anticancer action in its barks, leaves, fruits, pits, and pollen [4]. Date fruit extracts (*Phoenix dactylifera*) are studied for their possible positive and negative effects on cypermethrin-induced male infertility [5]. Study was done to determine whether date palm pits of various Emiratis kinds had any antitumorigenic effects. Triple-negative breast cancer tissue-derived MDA-MB-231 cells used as a model [25-26]. There are currently more than 5000 different date types planted throughout the world. Aqueous date extract improved the clinical signs of diabetic neuropathy and stopped diabetic aggravation in diabetic rats [5]. The first in-vitro investigation into the antioxidant potential of dates revealed that date pulp aqueous extract was an effective scavenger of reactive oxygen species, including superoxide and hydroxyl radicals. Methanol and acetone extract of *P. dactylifera's* leaves and pit, which shows strong antibacterial activity [6]. In another investigation, date-fed volunteers' erythrocytes showed resistance to streptolysin O's hemolytic activity, but there was no decrease in the anti-streptolysin O antibody titer [7]. Aseel and Zahidi dates aqueous fruit extract is frequently used in traditional medicine.

Although investigations on the bioactivities of the date palm have been conducted, there are no recently published studies comparing these two kinds. The aim of present study is to compare the cytotoxicity, antioxidant, antidiabetic, and antimicrobial activities of *Phoenix dactylifera* (Aseel and Zahidi dates) to demonstrate their value for human use and determination of their chemical compounds through FTIR.

## Experimental

### Sample Collection, Preparation and Extraction

There are two varieties of date palm (*Phoenix dactylifera)* were obtained from locally known as Aseel and Zahidi dates, from Imtiaz supermarket of Faisalabad and identification was approved from the Department of Horticulture, University of Agriculture, Faisalabad, Pakistan.

*Phoenix dactylifera* date fruit pulp washed by the water and dried at room temperature for one week. Date fruit pulp converted into fine powder and grinded pulp of dates was extracted by using the distilled water (1:5 (w/v) on water bath for the 72hrs at the room temperature. The extract was filtered by the Whatman filter paper and stored at 2-8 $^{o}$C in refrigerator for the subsequent experiments.

### Antioxidant Profile

*Phoenix dactylifera* antioxidant profile was assessed by following methods.

### Total Phenolic Content (TPC)

For the estimation of antioxidant activities of date (*Phoenix dactylifera*) pulp extract were determined by colorimetric method by using the Folin-Ciocalteu reagent was used for the determination of total phenolic content (TPC) by using the gallic acid as a standard, added 100ul $Na_2CO_3$, 125µL of test samples and 10% of 25µL of diluted reagent were mixed and measured the absorption at 765nm after 2hrs incubation as shown in graph 2(a) [8].

### Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined by the AlCl3 (Aluminum chloride) assay, which was used as a standard, added 9.5µL $NaNO_2$ and 156µL distilled water and 19µL of 10% $AlCl_3$ sample was incubated for the 5min, measured the absorption by the Elisa reader at 570nm shown in graph 2 (b) [8].

### DPPH Radical Scavenging Assay

The DPPH radical scavenging assay was used to determine the level of free radical inhibition for the antioxidant assay activity. 2.5mL of plant extract was combined with 250mL of DPPH solution (0.004mg DPPH in 100mL of methanol), which was then wrapped in aluminum foil for 35 minutes to measure the absorbance at 517nm. Triton X was utilized as a positive control to compare the outcomes [8]. The radical scavenging capabilities were determined using the formula.

% DPPH scavenging = [A (Control)-A (sample)/A (control)]×100

### Antimicrobial Activity

### Agar well diffusion method

In this method, ethyl acetate (5mg) extract was mixed with 1mL DMSO solution and agar solution was prepared by adding 2.66g agar in 70mL aqueous solution in two separate flasks. Added 100µL bacterial strains, *staphylococcus aureus* in one flask and *E. coli* in another flask. 100µL of both samples were poured onto the two wells in both plates and the third well was filled with ciprofloxacin as positive control. Plates were incubated for 18hrs under the sterilized condition by using the laminar flow unit. Antibacterial activity was measured for the inhibition zone in mm [9].

### Antidiabetic Evaluation

### Alpha-Amylase Inhibition Assay

For the colorimetric approach, 96-well plates containing 30µL of sample testing and 0.5 mg/mL of standard acarbose were maintained at room temperature for 10 minutes before 10µL of amylase solution was dissolved. After preincubation, 40µL of a 1% starch solution was added to the reaction mixture and the mixture was incubated for 30 min. 20µL of 1M HCl was added into each well. After that, each well-received 75µL of iodine solution and then measured absorbance at 570 nm [9].

% inhibition= 1−A(control) A sample × 100

### Cytotoxicity Activity

A sterilized falcon tube having 15-mL of capacity was used to collect human blood which was then centrifuged for five minutes while being cleaned with 5mL of cooled PBS thrice. Then, RBC (180µL) and plant extract (20µL) were combined in Eppendorf tubes of 2mL. The supernatant (100 µL) from centrifuged tubes was diluted with 900µL cold PBS after 5 minutes of centrifugation. For the entire hemolysis of RBC, PBS was used as the negative control and Triton X-100 as the positive control. At 576 nm, the absorbance was observed [10].

### Structural Analysis

### Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed for the confirmation of active functional groups in the plant extracts. *Phoenix dactylifera* (Aseel and Zahidi dates) extract was employed in powder form along with potassium bromide (KBr), compressed dye and high pressure until a pellet formed. The pellet was then evaluated between 400 and 4000$cm^{-1}$ [11].

### Statistical analysis

All experiments were carried out in triplicate. The results of all activities were expressed as mean±S.D and a t-test was applied to check the significance of the results [12].

## Results and Discussion

### Antioxidant Potential

**Table 1:** Antioxidant potential of aqueous extracts obtained from two varieties of date palm (*Phoenix dactylifera*)

| Sample | Extract conc | TPC mgGAE/100g | TFC mgCE/100g | DPPH% |
|---|---|---|---|---|
| Aseel date | 125ul | 28.03±0.72 | 61.4±5.9 | 69.0±1.0 |
| Zahidi date | 38ul | 49.1±2.51 | 115.8±0.65 | 76.6±0.48 |
| Control | | - | - | 94.77±0.00 |

Data represented as mean ± S.E means sharing similar letters either in a column or in a row are statistically significant (p>0.05)

Table 1 shows different antioxidant assays including the TPC, TFC and DPPH inhibition assay. Results of Varieties of date palm (*Phoenix dactylifera*) Aseel and Zahidi were showed in TPC (28.03±0.72) (49.1±2.51) in mgGAE/100g and TFC

(61.4±5.9) (115.8±0.65) in mgCE/100g. Hence it was proved by the analysis that high phenolic contents have high antioxidant activity. Table 1 results showed that the DPPH% of Aseel and Zahidi extract ranged from 69.0±1.0 and 76.6±0.48, respectively and control was shown 94.77±0.00.

Nadeem *et al.* 2019 [13] obtained that the total phenolic content of Aseel date was 279.43±1.08 while our current study showed that TPC of Aseel variety of *Phoenix dactylifera* was 28.036±0.72 and that of Zahidi variety of *Phoenix dactylifera* was observed to be 49.12±2.510. The total flavonoid content of Zahidi variety of date was 3.26±0.28 while our study showed that it was 115.8±0.605. Biglari *et al.* 2008 [14] reported that the flavonoid content of kharak date variety was 81.79±14.27. In this study Aseel variety showed less flavonoid content as compared to Zahidi variety. Arshad *et al.* 2015 [15] stated that the DPPH% inhibition of the aqueous extract of 50g of date fruit was 71.74±0.10 which is closely related to Aseel variety of *Phoenix dactylifera* (69.07±1.0) and similar to Zahidi variety of *Phoenix dactylifera* (76.62651±0.48) The DPPH scavenging% of aqueous extract of Zahidi variety was 42.07% which is lower as compared to our results.

## Antimicrobial Activity

### Antimicrobial activity

**Table 2:** Inhibition of bacterial growth by aqueous extract of both varieties (Aseel and Zahidi date) of *Phoenix dactylifera L*

| Strain name | Control (Ciprofloxacin) | Inhibition zone of Aseel date | Inhibition zone of Zahidi date |
|---|---|---|---|
| *Escherichia coli* | 26mm | 13mm | 0mm |
| *S. aureus* | 26mm | 15mm | 0mm |

Antibacterial activity was performed by agar well diffusion method using *E. coli* (gram-negative) and *Staphylococcus aureus* (gram-positive). Aseel date showed inhibition zone of 15mm in *Staphylococcus aureus*, 13mm in *E coli* and no inhibition was observed of Zahidi date against the date palm extract, respectively (Table 2). Data has shown that ciprofloxacin (positive control) is most effective against bacterial strains. Results is clearly showed that *E coli* and *S. Aureus* is most sensitive pathogen to Date palm (Aseel) extract while the Zahidi extract showed no activity.

El Sohaimy *et al.* 2015 [16] reported that palm fruits extract has a strong antibacterial activity (for water and ethanol extracts) against *E. coli* (20±0.57 and 16±0.57 mm) and moderate inhibition against *staphylococcus aureus* (8±0.48 and 5±0.52 mm). Rafdar *et al.* 2019 [17] stated that date palm fruits extract shows antimicrobial activity of 12.2±0.05 which is closely related to our study in case of the Aseel variety. Zahidi variety shows no activity in *Escherichia coli.*

## Antidiabetic Evaluation

### Alpha-Amylase Inhibition Assay

**Table 3:** Alpha-amylase inhibition assay of aqueous extract obtained from both varieties (Aseel and Zahidi date) of *Phoenix dactylifera*

| Aqueous Extract | Mean% Inhibition |
|---|---|
| Aseel date | 52.87±0.03 |
| Zahidi date | 55.42±0.03 |
| Control | 81.471±0.00 |

Data represented as mean ± S.E showed that significant difference (p>0.05) was observed in the alpha-amylase inhibition assay

*In-vitro* antidiabetic activity of date fruit **(***Phoenix dactylifera)* extract was evaluated by the inhibitory effect of α-amylase inhibition assay. Table 3 shows alpha-amylase inhibition activity of two different varieties of *Phoenix dactylifera*. The Aqueous extract of Zahidi date showed highest inhibition of alpha amylase 55.428±0.03 activity. Least activity was shown by aqueous extract of Aseel date about 52.876±0.03.

Khan *et al.* 2016 [18] showed that the water extract significantly outperformed organic extracts (5.91±0.65 to 42.40±6.43) in terms of% inhibition (34.46±2.33 to 51.71±8.20) but the current study shows that the maximum α-amylase inhibition activity shows by Zahidi date which is 55.428±0.03 and least by Aseel date which is 52.876±0.03 (Table 3). Habib *et al.* 2022 [19] stated that the α-amylase inhibition activity of date fruit extract is 44.12±0.20 to 99.92±0.12% in which acarbose is taken as positive control but the alpha-amylase inhibition activity of Zahidi date is 55.428±0.03.

## Cytotoxic Activity

**Table 4:** Hemolytic assay of aqueous extract obtained from both varieties (Aseel and Zahidi date) of *Phoenix dactylifera* varieties

| Aqueous Extract | Mean% Hemolysis |
|---|---|
| Aseel date | 0.519±0.340 |
| Zahidi date | 2.22±0.445 |
| Control | 94.871±0.00 |

Data represented as mean ± S.E showed that significant difference (p>0.05) was observed in hemolytic activity.

The cytotoxic activity of date fruit (*Phoenix dactylifera)* extract was evaluated by using RBCs of humans through hemolysis assay. The% age of hemolysis of date fruit extract concentration was given in Table 4. The highest percentage of hemolysis of red blood cell was detected in Zahidi date extract 2.22±0.445 and in Aseel date extract 0.519±0.340. Triton X was used as standard drug control showed 94.871± 0.00 lysis of RBCs. Mechanical stability of RBCs showed better effect *in vitro* studies. The resulting% age of hemolysis of RBCs was calculated to be less than 5.0%, Hence proved that these date fruit extract has less cytotoxic effect.

Qasim *et al.* 2020 showed that the% hemolytic potential of 50g of aqueous date fruit extract is 2.22% which is closely related to Zahidi variety of *Phoenix dactylifera* while the hemolytic potential of Aseel variety of *Phoenix dactylifera* is 0.519%. Shahid *et al.* 2021 [20] stated the percentage of hemolysis caused by the date crude extract is 1.8±0.04 while our Zahidi extract hemolytic activity is 2.22% which is higher than crude extract.

## Structural Characterization

### Fourier Transform Infrared Spectroscopy

FTIR (Fourier transform infrared spectroscopy) is an instrumental technique used to pinpoint the functional groups present in both organic and inorganic molecules by measuring the infrared radiation's absorption across a spectrum of wavelengths. It is used to determine the presence of organic substances in samples, such as polyphenols and various other chemicals.

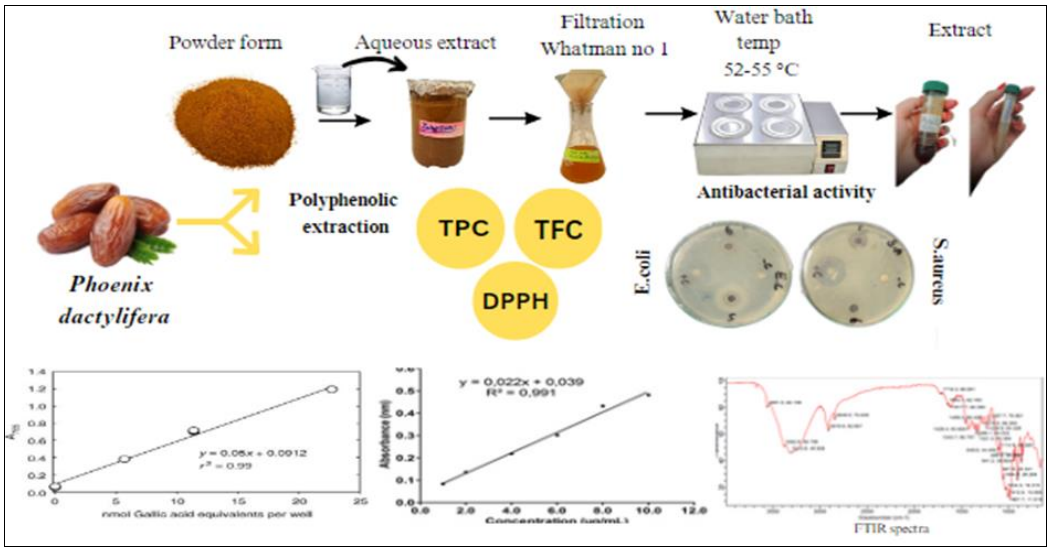


**Fig 1:** *Phoenix dactylifera* extraction and activities

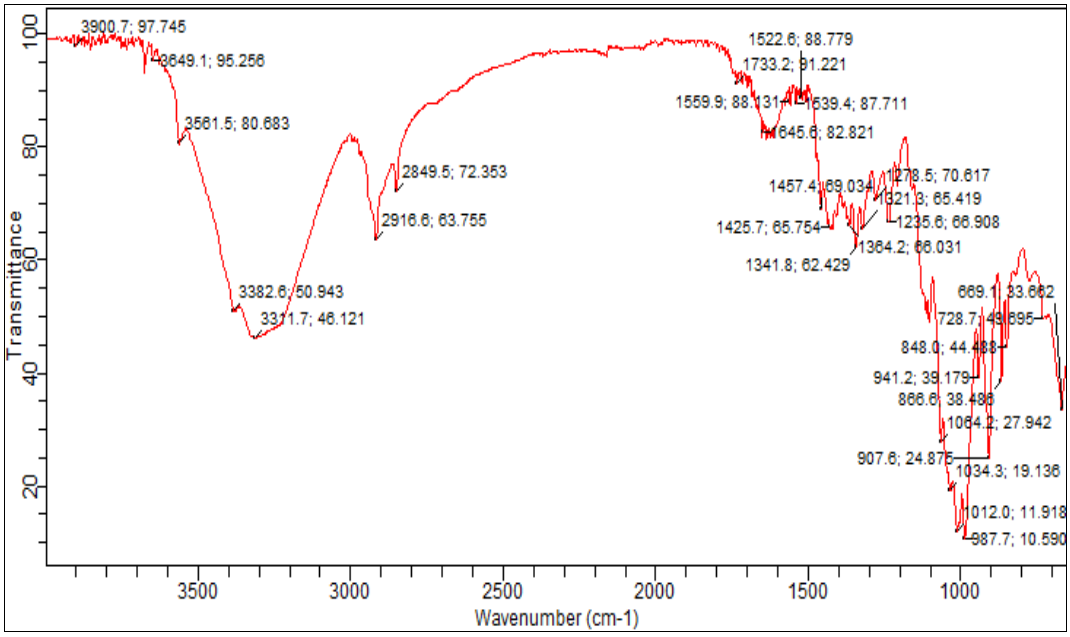


**Fig 2 (a):** FTIR Spectra of *Phoenix dactylifera* (Aseel date)

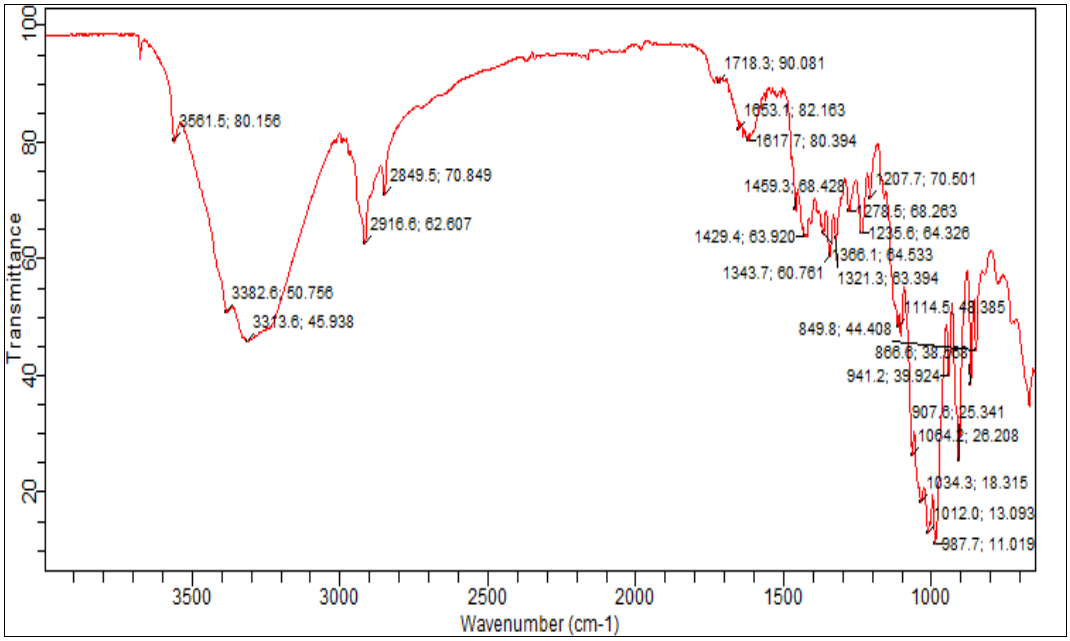


**Fig 2 (b):** FTIR Spectra of *Phoenix dactylifera* (Zahidi date)

**Table 5:** FTIR results of *Phoenix dactylifera* (Aseel and Zahidi date) for identification of functional groups

| *Phoenix dactylifera* (Aseel date) | | | *Phoenix dactylifera* (Zahidi date) | | |
|---|---|---|---|---|---|
| **Characteristics Absorption** | **Identified functional group** | **Compound Class** | **Characteristics Absorption** | **Identified Functional group** | **Compound Class** |
| 3900.7 | O-H | Alcohol | 3561.5 | O-H | Alcohol |
| 3649.1 | O-H | Alcohols, phenols, carboxylic acids | 3382.6 | O-H | H-bonded, carboxylic acids |
| 3561.5 | O-H | Alcohol (Stretch Free) | 3313.6 | O-H | Carboxylic acids |
| 3382.6 | O-H | H-bonded carboxylic acids | 2916.6 | C-H group | Alkanes, carboxylic acids |
| 3311.7 | O-H | Carboxylic acids | 2849.5 | C-H | Aldehyde, alkane, carboxylic acid |
| 2916.6 | C-H group | Alkanes, carboxylic acids | 1718.3 | C=O | Ketones, carboxylic acid |
| 2849.5 | C-H | Aldehyde, alkane, carboxylic acid | 1653.1 | C=N<br>C=O<br>C=C | Imines and oximes<br>Amide<br>Alkene |
| 1733.2 | C=O | Esters | 1617.7 | C=C | Alkene |
| 1645.6 | C=O | Alkenes, amide | 1459.3 | C-H | Alkane (bending) |
| 1559.9 | N-H | Primary and secondary amines and amides | 1429.4 | C-H | Alkane (bending) |
| 1539.4 | N-O | Nitro | 1366.1 | S=O | Sulfones, sulfonyl chlorides, sulfates, sulfonamides |
| 1522.6 | N-O | Nitro | 1343.7 | S=O | Sulfates, sulfonamides |
| 1457.4 | C-H | Alkane (bending) | 1321.3 | S=O | Sulfonamides |
| 1425.7 | C=C | Aromatics | 1278.5 | C-O, C-F | Ether, alkyl halide |
| 1364.2 | S=O | Sulfones, sulphates, sulfonamides | 1235.8 | C-O, S=O | Carboxylic acid, chlorides, sulphates, sulphonamides |
| 1341.8 | C-N | Amines | 1207.7 | C-O | Carboxylic acid, anhydrides |
| 1321.3 | S=O | Sulfates, sulfonamides | 1114.5 | C-O | Carboxylic acid |
| 1278.5 | C-F | Alkyl halide | 1064.2 | C=C, N-H | Alkene, amines and amides |
| 1235.6 | C-O, S=O | Anhydrides, sulphones, sulphonamides | 1034.3 | C-H, S=O | $-CH_3$(bend), sulfones, sulphonyl chlorides, sulphates, sulphonamides |
| 1064.2 | C=C, N-H | Alkene, Primary and secondary amines and amides (bend) | 1012.0 | C-O, C-N, C-X | Alcohol, ethers, esters, carboxylic acid, anhydrides, amines, fluoride |
| 1034.3 | C-H, S=O | $-CH_3$ (bend), sulfones, sulphonyl chlorides, | 987.7 | C-H | Alkenes |
| 1012.0 | C-O, C-N, C-X | Alcohol, ethers, esters | 941.2 | C-H | Alkenes |
| 987.7 | C-H | Alkenes | 907.6 | C-H | Alkenes |
| 941.2 | C-H | Alkenes | 866.6 | C-H | Aromatics |
| 907.6 | C-H | Alkenes | 849.8 | C-H | Aromatics |
| 866.6 | C-H | Aromatics | - | - | - |
| 1064.0 | C-H | Aromatics | - | - | - |
| 728.7 | C-X | Chloride | - | - | - |
| 669.1 | C-X | Chloride | - | - | - |

George *et al.* 2020 [21] described that O-H bond type and functional group hydrogen bonded alcohol phenols were observed at 3200cm$^{-1}$ is similar to our bond type and functional group observed at 3382cm$^{-1}$ (Table 5). In our studies first band observed at 3900cm$^{-1}$ indicated the presence of bond type O-H having functional group alcohol which does not exist in previous study while the last band observed at 669cm$^{-1}$ which is related to 750cm$^{-1}$ in previous research. Baker and Baseri *et al*, 2011 [22] stated that the intense bands occurring from 3419cm$^{-1}$ to 635cm$^{-1}$corresponds to O-H, C-H, C-O and C-CI stretching/bending vibrations respectively, which is similar to our peaks observed at 3561cm$^{-1}$, 2916cm$^{-1}$, 2849cm$^{-1}$, 1645cm$^{-1}$, 1421cm$^{-1}$, 1278cm$^{-1}$, 1064cm$^{-1}$, 1064 cm$^{-1}$ and 669cm$^{-1}$. Noorbaksh (2022) [23] reported the peaks at 1657cm$^{-1}$ and 2877cm$^{-1}$ are related to the stretching vibrations of the O-H bond of water molecules, respectively, whereas our peak at 1645cm$^{-1}$ is related to the symmetric and asymmetric frequency pattern of methyl groups. When compared to the Zahidi variety, the *Phoenix dactylifera* Aseel variety had more chemical compounds detected, according to FTIR data [22]. Nabili *et al.* 2014 [24] stated that the first band seen at 3367cm$^{-1}$ represents O-H stretching vibrations in hydroxyl groups, which is comparable to our research at 3561 cm$^{-1}$ while the last band seen at 870cm$^{-1}$ represents C-H rocking vibrations of cellulose, which is comparable to our bond type at 849cm$^{-1}$. George *et al.* 2008 [21] described that hydrogen bond vibrations and O-H stretching vibrations of the hydroxyl groups are in charge of a band ranging from 3600cm$^{-1}$ to 3200cm$^{-1}$, which is identical to our band detected at 3561cm$^{-1}$. Similar to our band which appears at 849cm$^{-1}$, the band at 910cm-1 displays C-H bending of syringyl units and aromatic ring antisymmetric out-of-phase ring stretching. The intense peak at 1650 cm$^{-1}$ is caused by primary amides C=O stretching and N-H bending vibrations, which is identical to our bond at 1653cm$^{-1}$. When compared to the Aseel variety, the *Phoenix dactylifera* Zahidi variety had fewer chemical compounds detected, according to FTIR data.

## Conclusion

This study showed that *Phoenix dactylifera* has a long history as a medicinal plant with a variety of therapeutic uses. The results of this investigation showed that *P. dactylifera* is a rich source of antioxidants, which are crucial function in regulating the conflict between the body's capacity to detoxify free radicals and balance reactive oxygen species and repair damage. Selected bacterial strains were subjected to extracts and concentrations that demonstrated antibacterial activity. Dates are suitable for usage as herbal medicine due to their low cytotoxicity. The extracts of the chosen *Phoenix dactylifera* (Aseel and Zahidi date) showed antidiabetic activity. An effective tool for visualizing the chemical makeup of several date palm varieties was FTIR. It would be beneficial to start a thorough scientific inquiry into this potentially valuable therapeutic herb and to encourage its widespread use.

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**Conflicts of Interest**
The author declares no conflict of interest.

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