

## INFLUENCE OF SOLVENT SYSTEMS ON THE DPPH RADICAL SCAVENGING POTENTIAL AND PHYSICAL PARAMETERS OF INDIGENOUS *PHOENIX DACTYLIFERA* L. CULTIVARS

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### Abstract

The date palm (*Phoenix dactylifera* L.) is a vital agricultural asset in Pakistan, particularly in the Khairpur District, which is recognized as a global hub for date cultivation. The purpose of this research was to assess the various physical attributes, as well as the antioxidant potentials of five varieties of native dates; Kashkari, Hussani, Allie, Mazawati and Otakin at their peak ripening stage (Tamar) found in the Taluka of Kingri/which include; (length, width, mass, moisture content) for each sample which were taken and then the morphometric measurements determined considerable amount of morphometric variation. The Allie type had the largest length (17cm), while the Kashkari was found to be the heaviest (70.1g) and have the highest moisture content (40.3%). Antioxidant capabilities were determined using the DPPH-radical scavenging assay with five different solvents (pure methanol, pure ethanol, distilled water, hydro-methanol & hydro-ethanol). The antioxidant activity showed a dose related scavenging action for all of the varieties. Pure methanol and pure ethanol were generally found to be the best solvents, however in the case of the Hussani date palm, the hydro-methanol solution provided the maximum antioxidant activity (i.e. the lowest IC<sub>50</sub>: 21.206 µg/ml). These results demonstrate the genetic diversity of the date palms in the Kingri area, as well as the importance of selecting the ideal solvent to obtain the maximum recovery of bioactive antioxidant compounds for use in food products and/or pharmaceuticals.

### INTRODUCTION

Dates (*Phoenix dactylifera* L.) have long been referred to as the “Tree of Life” and are historically significant in fulfilling the nutritional needs of those who reside in arid areas (Sivapragasam, et al., 2025). Their most notable characteristic is that they contain a high-quality source of energy. The sugar pair found in dates (glucose and fructose) is surrounded by a huge amount of dietary fiber,

thus allowing for a gradual release of energy into the bloodstream (Al-Shaaib, 2022). This helps to avoid energy surges that can disrupt metabolism and digestion (M Abo-El-Saad, et al., 2024). In addition to calories, dates contain a substantial amount of minerals, including potassium, magnesium, and copper; these are essential for body health (i.e., heart and bone health). From a biochemical standpoint, the second most important component of dates

and the focus of most DPPH studies regarding their radical scavenging ability, are their high concentrations of secondary metabolites, e.g., phenolic acids, flavonoids, and carotenoids, which all possess high levels of antioxidant activity (Fernández-López, et al., 2022). These compounds help neutralize body free radicals, reducing oxidative stress and chronic disease risk (e.g., neurodegenerative disorders and certain cancers) (Djaoudene, et al., 2024).

The indigenous cultivars of plants provide great medicinal benefits, as they have adapted to extreme environmental stressors, such as high salinity and excessive heat, over thousands of years (Sharma, 2024). As a result of these adaptations, indigenous plants typically have significantly higher concentrations of various bioactive compounds than commercial (non-indigenous) versions of these same plants. In addition, dates are known to provide some specific physiological benefits, such as supporting the health of expectant mothers during the later portion of their pregnancy (e.g., facilitating natural labor) by alleviating some of the symptoms associated with both inflammation and swelling in joints and tissues (Salajegheh, et al., 2024).

The use of the entire date is one of the most notable aspects of food science and industry; therefore, both the date pulp (used as a whole food (nutrient-rich) and also as a naturally sweetening ingredient) and the date seeds (which are usually thrown away) have special properties, such as having high levels of oil and polyphenols (many of which can be used in cosmetic and nutraceutical applications) (Alu'datt, et al., 2025). The biological mechanism of action for these various beneficial properties largely depends upon how effectively the beneficial compounds are extracted from the dates. The type of solvent used in the extraction process of the beneficial compounds from the fruit dictates the level of the fruit's antioxidant potency, thus demonstrating the importance of the study of indigenous cultivars as an avenue for further research, which links traditional agricultural practices to contemporary pharmaceutical practices (El-Saadony, et al., 2025).

In Pakistan, date palm (*Phoenix dactylifera* L.) is a major contributor to agricultural diversity and food security in dry lands (Khan, et al.,

2025). Nutritionally speaking, date palm is an ideal source of diet for humans, and it is very important to support this claim with strong scientific background. Dried dates (the fruit of the date palm) are usually referred to as a "complete food." They contain many important nutrients (including macro and micro) and bioactive compounds that lead to tremendous health benefits for humans (Al-Karmadi, et al., 2024). To a degree, the health benefits of date palm have also been confirmed by research showing that date palms may provide anti-inflammatory and antioxidant activity. For example, animal studies have reported that date palms can help protect against drug-induced damage (Al-Habsi, 2025). As one of the largest producers of dates in the world, Khairpur District is called the "date palm capital" or "date palm region" of Pakistan and serves as a worldwide centre for date palm cultivation because of its unique agroclimatic characteristics (Solangi, et al., 2024). The abiotic conditions in Khairpur are very favourable for many varieties of dates. The region has clay loam soils, which are very well suited for growing dates; the temperature during the summer months can exceed 50°C (122°F), which leads to favourable growing conditions for more than 300 varieties of dates. The Aseel variety is the most common and commercially important type of date grown in Khairpur. However, Khairpur is a very important genetic centre for date palm; it has many still other varieties of date palm, both local to the area (e.g., Karbalain, Kupro, and Fasli) and from other regions of the world. Thus, the date palm is an extremely important significant (and vital to agriculture and providing food) crop of the Khairpur region because of its historic value and potential future role in promoting food security worldwide. Date occurrence physical characteristics – length, width, and weight – are key parameters to be used to evaluate fruit quality and marketability (Jagirani, et al., 2024). This study was conducted at the end of the Tamar phase of date palm fruit development to assess the Tamar's final phase of fruit maturation and to document the physical characteristics at this stage (Ghonimy, et al., 2025). This study focuses on the Tamar stage, which represents the final ripening phase where

the fruit reaches peak maturity (Shi, et al., 2025).

At this stage of the fruit's life, there is extremely high moisture loss and peak levels of glucose, fructose, and sucrose (Hassan, et al., 2025). Thus, measuring moisture levels and physical characteristics at this point in time is key, as these two variables play a large role in determining the quality and shelf life of the fruit (Alqahtani, et al., 2025). The quantification of phenolic compounds and their bioactivity is currently a key area of research at the intersection of modern pharmaceutical and medicinal science (Zhang, et al., 2022). The rate at which dates exhibit antioxidant activity is most frequently measured with the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. However, there is a strong correlation between the efficiency of extracting the bioactive compounds and the type of solvent system used during extraction (Ouamnina, et al., 2024).

#### Research Suggests That:

- Solvent polarity (whether pure methanol or some binary mixture thereof, such as methanol and ethanol) can determine the total concentration of phenolics and flavonoids that are extracted from a plant
- i. The solvent system used to extract antioxidant compounds will also affect the activity of those antioxidant compounds, and therefore the scavenging power (IC<sub>50</sub> values) of the extracts will differ based on the solvent system used
  - ii. Most previous work has focused on aqueous or ethanolic extracts, but the level of antioxidant recovery from some indigenous cultivars needs to be compared with the use of different extraction solvent systems

#### Research Objective

While Khairpur has many different kinds of dates, there have not been very many comparisons made on the characteristics of the

different kinds of dates or the antioxidant activity of the dates using different solvents—to help establish those parameters for cultivar diversity. Therefore, by researching some cultivars of dates (including Otakin, Mazawati, Allie, Hussani and Kashkari), this study aims to determine the best extraction techniques for each type of date and characterise their unique physicochemical properties at peak ripeness.

## MATERIAL AND METHODS

### Study Area of Taluka Kingri District Khairpur

Khairpur District is globally known as "the date palm capital of Pakistan". Taluka Kingri is an important agricultural centre located in the Khairpur District, which has a very specific set of agro-climatic conditions, including clay loam soils and extreme summer heat that can frequently exceed 50 °C. This type of high heat is essential for the natural ripening of fruit until it reaches the Tamar stage. Kingri's agriculture is characterized by rich genetic diversity of more than 300 cultivars, with Aseel being the most widely used and consumed cultivar, along with regions' other local varieties such as Karbalian, Fasli & Kupro. Date production is a primary contributor to the local economy supported by the substantial irrigation system fed by the Sukkur Barrage irrigation system. Kingri date growers are consistently faced with obstacles to the production of their crops especially from the July/August monsoon rains that can inflict trauma on mature crops. Growers must frequently harvest early to mitigate this threat to their crops to produce dried dates referred to as Chuhara. Additionally, local orchards are highly susceptible to infestations from Lesser Date Moth and Red Palm Weevil pests, prompting the continued research into pest-resistant varieties of Kingri date palms to maintain the high levels of yield and nutritional quality for which Kingri date palms are known (Magsi, et al., 2023).



### Physical Characterization of Date Fruits

A random sample of 10-20 date fruits were taken from a batch of Date Fruits. Each sample fruit was thoroughly cleaned and examined to light debris from their exterior surface. All fruits were handled in a manner that prevented bruising or otherwise damaging the surface of the fruit. Each sample fruit was measured for length and width using a digital Vernier calliper. The sample fruit was oriented lengthwise between the calliper jaws and the maximum linear straight line distance from the stem end (calyx) to the tip end (apex) of the fruit was determined. The calliper jaws were then gently closed until the jaws made contact with the surface of the fruit, but did not damage or crush the fruit. The length of the fruit was recorded in millimetres. Next, the sample fruit was weighed on an analytical balance by turning the balance on, allowing it to warm-up, and waiting for it to stabilise before placing the fruit on the balance (i.e. waiting for the balance to settle on zero). Following this, the maximum circumference (diameter) of each sample fruit was determined by orienting the fruit perpendicular to the jaws of the Vernier calliper at its maximum circumference (equatorial) location. This was also measured in millimetres.

The weighing pan had a clean weighing boat on it, and the tare button was pressed to subtract the weight of the weighing boat. Next, one date fruit was placed on the weighing boat carefully.

Once the reading was stable, the weight was written down in grams (g) to two decimal places (0.01 g). We measured the length, width, and weight of each fruit in the sample set again. The data were systematically entered into a prepared data sheet next to the fruit or sample number so that they could be used for statistical analysis later (Ghonimy, et al., 2025).

### Determination of Moisture Content

The oven-drying method was used to find out how much moisture was in the date fruits. We turned on the analytical balance and let it settle. Weigh each clean, dry Petri dish or aluminum dish separately and write down the weight as the initial weight of the empty dish (W1). About 5 to 10 grams of fresh date fruit sample, cut into small pieces, was carefully weighed and put into each dish that had already been weighed, while weighing the dish and the fresh sample together is represented by W2. Then, the samples were put in a hot air oven that stayed at  $105\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  for 24 hours or until they reached a constant weight. After they had dried, the dishes were taken out of the oven and put in a desiccator to cool to room temperature. This was done to keep moisture from getting back into the dishes. The last weight of the dish and the dried sample was written down (W3) (Mohammed, et al., 2022).

To figure out the moisture content, we used the following formula to find the percentage of weight loss based on the fresh weight:

$$\text{Moisture Content (\%)} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where:

W1 = Weight of empty dish (g)

W2 = Weight of dish + fresh sample (g)

W3 = Weight of dish + dried sample (g)

### Preparation of Extracts

To produce sample extracts, 0.1 g of date pulp was solubilised in 50 ml of separate solvent systems consisting of pure methanol (CH<sub>3</sub>OH), pure ethanol (C<sub>2</sub>H<sub>5</sub>OH), distilled water (H<sub>2</sub>O), and binary mixtures of methanol with water and ethanol with water (50:50 v/v for both mixtures). Reflux extraction was the method used to extract the products of date pulp from solution. The date pulp and the solvent solution were placed in a round-bottom flask

and heated at reflux for extraction. After refluxing, the dates and solvents were allowed to cool to ambient temperature. Following this, the cooled extracts were filtered using Whatman No 1 filter paper to remove any solid matter and produce clear filtrate as an initial extract or "filtrate". The final extracts were placed into airtight containers for future analysis ((Halabi, et al., 2022).

### Determination of Antioxidant Activity (DPPH Radical Scavenging Assay)

The antioxidant activity of extracts from fruit of various degrees of ripeness were evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The stock solution of DPPH was prepared by dissolving 2.4 mg in 100 mL of methanol to give a 0.1 mM concentration. The stock solution was stored in the dark to prevent degradation. A reaction was created by mixing 1.0 mL of each extracted date fruit with 2.0 mL of the DPPH solution; the

two solutions were mixed completely by vortexing each sample then allowed to react for 30 minutes in the dark at room temperature. The samples were subsequently analyzed for absorbance at 517 nm with a UV-Visible spectrophotometer.

A blank solution was prepared to be used as a control for each extraction. The blank solution was created by mixing 2.0 mL of DPPH solution with 1.0 mL of the corresponding extraction solvent and measuring its absorbance.

The following formula was used to determine the percentage of DPPH radical inhibition:

$$\text{Inhibition (\%)} = \frac{(Ab - As)}{Ab} \times 100$$

Where:

Ab = Absorbance of the blank (control) at 517 nm

As = Absorbance of the sample at 517 nm

All assays were performed in triplicate, and the results were expressed as mean percentage inhibition  $\pm$  standard deviation. A higher percentage of inhibition indicated greater antioxidant activity of the date extract (Gulcin, and Alwasel, 2023).

### Preparation of DPPH Stock Solution and Working Standard

To prepare the DPPH (2,2-diphenyl-1-picrylhydrazyl), it is important to protect the reagent from light. Therefore, all steps were completed in low light, with the DPPH being covered by aluminum foil. The first step in this procedure was to prepare a stock solution of 0.01M DPPH. To do this, it required the accurate measurement and dissolution of 0.0394g of DPPH (as calculated using its molecular weight of 394.32g/mol) into an appropriate volume of methanol. To stop photodegradation, the solution was moved to a 100 mL volumetric flask covered with aluminum foil. To guarantee that the DPPH was completely dissolved, the flask was shaken vigorously and the volume was adjusted with methanol. The working standard solution of 164  $\mu$ M was made by repeated dilution from this concentrated stock solution (0.01 M). In order to achieve a working concentration of 164  $\mu$ M, an aliquot from the stock solution was transferred to a different volumetric flask sealed in foil and diluted with methyl alcohol each day to form fresh working solutions and then stored out of direct sunlight until needed for testing antioxidant activity (Hassan, et al., 2022).

### Assessment of Antioxidant Capacity (DPPH Assay)

To measure the antioxidant activity of date palm fruit, an experiment was performed using DPPH as a free radical scavenger with a method based on the procedure developed by Gulcin and Alwasel, (2023) (Reference 21). Date fruit extracts (1.0 mL) were prepared using 5 different solvent systems - methanol (CH<sub>3</sub>OH), ethanol (C<sub>2</sub>H<sub>5</sub>OH), water (H<sub>2</sub>O), methanol-water (50:50 v/v) and ethanol-water (50:50 v/v). Two milliliters of a freshly prepared DPPH solution (164  $\mu$ M) were added to each of the date fruit extracts. The resulting solutions were then thoroughly mixed and allowed to react in the dark at room temperature for thirty minutes. Following the incubation period, the absorbance of each reaction mixture was determined at 517 nm with a double beam UV-Vis spectrophotometer. The standard antioxidant used for comparison was ascorbic acid; a blank of the appropriate solvent was used for baseline calibration.

Inhibitory concentration (IC<sub>50</sub>) which determines the concentration of the sample (in  $\mu$ g/mL) that will produce a 50% reduction in free radical DPPH scavenging was calculated from the percentage of inhibition plotted

against extract concentration, where linear regression analysis was also performed in order to yield the IC<sub>50</sub> values. The means of IC<sub>50</sub> values ± standard deviation were reported and

every experiment was completed in triplicate. A lower value of IC<sub>50</sub> indicates greater antioxidant activity (Belfar, et al., 2022).



Figure: 2 Concentration of antioxidant in date fruit extracts

## RESULTS AND DISCUSSIONS

### Physical Characteristics of Selected Date Varieties

The fruit morphology of the palm cultivar varieties from the Taluka Kingri area shows substantial morphological diversity among the five native palm varieties at the stage of Tamar fruiting. Morphological characteristics such as width, length, mass and percentage moisture content are essential to determine market value, fruit quality and consumer acceptance. The results demonstrate large variances from fruit measurement and weight. Length measurements of all cultivars were highest for Allie (17 cm) and Hussani (15.9 cm) indicating that both belong to the group of long-fruited cultivars. However, Mazawati and Otakin all had shorter fruit measurements at 2.8 cm and 2.7 cm respectively, indicating they both are characteristic of the small/medium group of palm fruit types from the local area. The width of the fruit was similar in that Kashkari and Allie both had the widest fruit measurements at 11.6 cm and 11.11 cm, while Mazawati and Otakin both had narrow fruit measurements.

The weight and water content of different varieties of table dates have so much variation in general. The variety that weighs the most is Kashkari (70.1 g), which can be attributed to its very high water content (40.3%). Because of its high level of water content at the Tamar stage, it is likely to have a soft texture; however, it may require special care after harvest to avoid fermentation. In sharp contrast, the Hussani variety has the lowest level of water (12.9%) and one of the lighter weights (20.11 g), suggesting it has a dry, fibrous fruit; this type is generally preferred for extended storage and for producing dried dates. The Allie variety represents a balanced profile because of its relatively high weight (36.87 g) and moderate water content (30.2%). The various physical parameters between cultivars represent the great genetic diversity of the Kingri region. These differences in physical characteristics directly affect the overall physiologic behavior of the fruit and their potential for diverse uses within the table date industry and the date palm industries in the Khairpur District (Table: 2, Figure: 2(a-d)).

Table: 2 Physical Parameters of Various Date fruit varieties collected from district Khairpur

| S. No | Date fruit Variety | Width (cm) | Length (cm) | Mass (g) | H <sub>2</sub> O (%) |
|-------|--------------------|------------|-------------|----------|----------------------|
| 1     | Kashkari           | 11.6       | 9.1         | 70.1     | 40.3                 |
| 2     | Hussani            | 8.9        | 15.9        | 20.11    | 12.9                 |
| 3     | Allie              | 11.11      | 17          | 36.87    | 30.2                 |
| 4     | Mazawati           | 1.3        | 2.8         | 66.15    | 28.31                |
| 5     | Otakin             | 1.4        | 2.7         | 23.1     | 26.3                 |

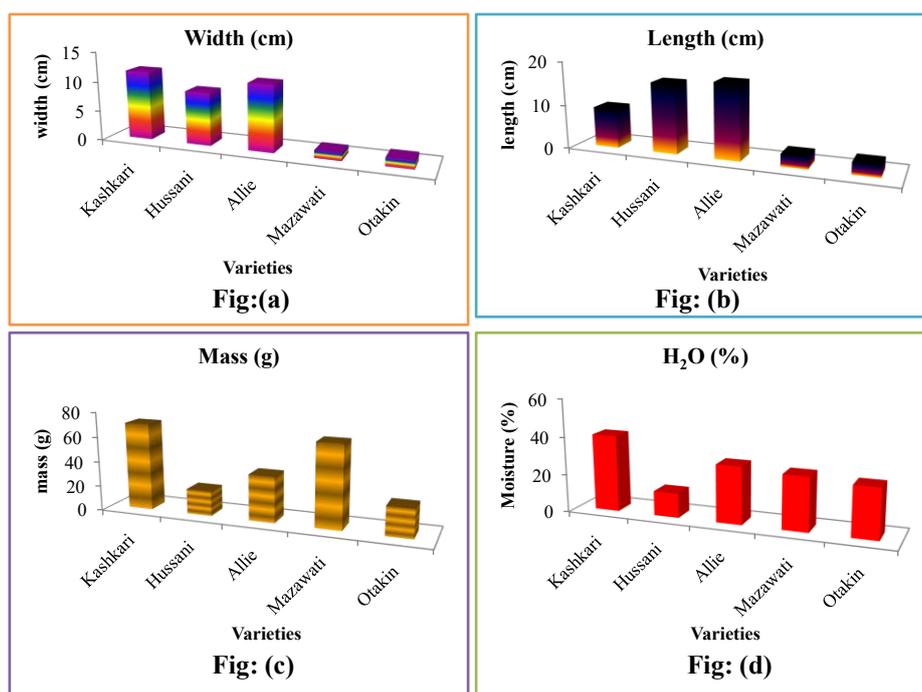


Figure: 3 Physical Parameters of Various Date fruit varieties collected from Taluka Kingri, district Khairpur, Sindh

**Antioxidant Activity**

**Kashkari Date Variety**

The DDPH radical scavenging assay was used to test the antioxidant activity of Kashkari dates grown in Taluka Kingri over five solvents in a manner that yielded a dose-dependant curve, with increasing inhibition percentages corresponding to increasing concentrations of extracts. The two best solvents for extracting bioactive compounds were pure ethanol and methanol, as shown by their IC-50 (µg/ml) values of 26.635 for methanol and 27.33 for ethanol, respectively. While D/W showed an initial high inhibition percentage at the maximum concentration of 222 µg/ml, it was

not as efficient overall compared with ethanol and methanol. It is interesting to note that mixing methanol with D/W and ethanol with D/W increased the IC-50 values (especially the methanol/D/W mixture, 37.907 µg/ml), possibly indicating that the amount of specific antioxidant phenolic compounds extracted from this indigenous variety may be reduced when diluted in water. The low IC-50 values obtained using any of the solvents indicated that Kashkari dates at the Tamar are very good natural antioxidant sources and that using pure organic solvents was the best extraction method for maximizing the radical scavenging capacity (Table: 3; Figure: 4).

Table: 3 Antioxidant activity of Kashgri date fruit variety at Tamar stage collected from Taluka Kingri, district Khairpur, Sindh

| Conc:µg/ml | CH <sub>3</sub> OH %Inhibition | H <sub>2</sub> O %Inhibition | CH <sub>3</sub> OH+H <sub>2</sub> O %Inhibition | C <sub>2</sub> H <sub>5</sub> OH %Inhibition | C <sub>2</sub> H <sub>5</sub> OH+H <sub>2</sub> O %Inhibition |
|------------|--------------------------------|------------------------------|---|--|---|
| 222        | 88.87                          | 89.38                        | 72.79   | 85.4   | 86.9  |
| 111        | 75.74                          | 82.45                        | 63.24   | 72.78  | 68.37   |
| 55.5       | 64.71                          | 63.6                         | 51.61   | 65.53  | 58.93   |
| 27.27      | 49.79                          | 44.31                        | 47.98   | 48.08  | 48.83   |
| IC-50value | 26.635                         | 31.678                       | 37.907  | 27.33  | 32.062  |

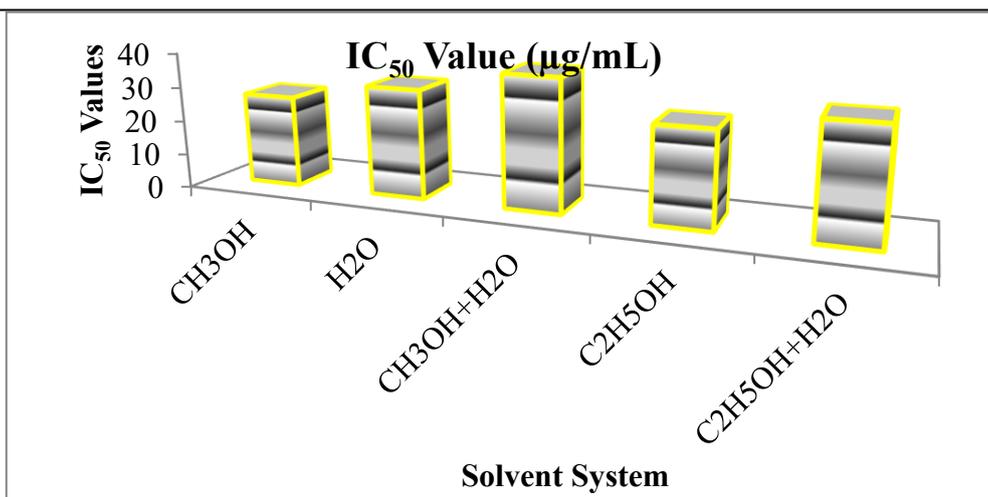


Figure: 4 Antioxidant activity of Kashgri date fruit variety at Tamar stage collected from Taluka Kingri, District Khairpur, Sindh

#### Hussani Date Variety

Antioxidant analysis of the Hussani date variety from taluka kingri shows significant variation based upon radical scavenging capacity as a function of the solvent matrix utilised. Notably, the Hussani date variety demonstrated superior antioxidant efficacy under a binary methanol-water solvent and an extremely low IC-50 value of 21.206 µg/ml. These results would indicate that specific bioactive phenolics found in hussani dates are liberated more effectively within a hydro-alcoholic medium relative to their liberation within pure organic solvents. Pure ethanol also exhibited a high level of

antioxidant activity at the maximum concentration achieving an inhibition rate of 90.58% ±0.2315 and a competitive IC-50 value of 27.968 µg/ml. While distilled water and pure methanol showed lower relative antioxidant doses with higher IC-50 values. All overall summary doses showed a consistent dose dependent relationship. Therefore, the Hussani date variety represents an excellent source of natural antioxidants as its radical scavenging potential is more accessible when extracted via a mixture of methanol and water (Table: 4; Figure: 5).

Table: 4 Antioxidant activity of Hussani date fruit variety at Tamar stage collected from Taluka Kingri, district Khairpur, Sindh

| Conc:µg/ml | CH <sub>3</sub> OH<br>%Inhibition | H <sub>2</sub> O<br>%Inhibition | CH <sub>3</sub> OH+H <sub>2</sub> O<br>%Inhibition | C <sub>2</sub> H <sub>5</sub> OH<br>%Inhibition | C <sub>2</sub> H <sub>5</sub> OH+H <sub>2</sub> O<br>%Inhibition |
|------------|-----------------------------------|---------------------------------|--|---|--|
| 222        | 75.24                             | 87.94                           | 83.52  | 88.7  | 90.58  |
| 111        | 65.85                             | 70.33                           | 70.03  | 78.41   | 78.16  |
| 55.5       | 53.15                             | 55.07                           | 60.45  | 66.29   | 67.29  |
| 27.27      | 48.67                             | 41.54                           | 55.61  | 47.15   | 43.89  |
| IC-50value | 34.723                            | 42.54                           | 21.206   | 27.968  | 31.038   |

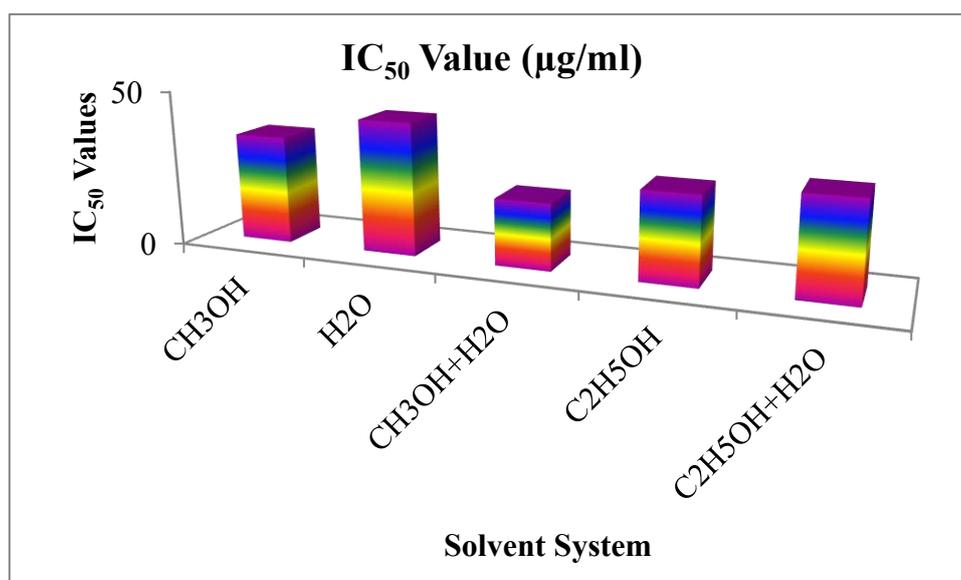


Figure: 5 Antioxidant activity of Hussani date fruit variety at Tamar stage collected from Taluka Kingri, district Khairpur, Sindh

#### Allie Date Variety

The Allie date-specific antioxidative potential measured through the Taluka Kingri area showed a considerable capacity to scavenge radicals. The extraction solvent used has a significant influence on the efficacy of extraction; of those tested, pure methanol was the most efficacious in extracting the phenolic compounds responsible for this antioxidant activity from Allie dates. The IC-50 value for pure methanol was 24.829 µg/mL, indicating that it was the most effective extraction solvent. Although a binary solution of ethanol and distilled water demonstrated the highest individual inhibition percentage (89.81%) at

the maximal concentrations tested, it showed a higher (-35.45 µg/mL) IC-50 than that of pure methanol for the overall quantification of inhibition throughout all of the concentrations tested. As such, distilled water performed poorly as an extraction solvent, yielding the lowest peak inhibition and an IC-50 value of 44.78 µg/mL. All of the extracts tested possessed dose-dependent activities; thus, it was concluded that the antioxidant activity of the Allie date variety would be optimally liberated by using polar organic solvents, specifically methanol, at the Tamar maturity stage (Table: 5; Figure: 6).

Table: 5 Antioxidant activity of Allie date fruit variety at Tamar stage collected from Taluka Kingri, district Khairpur, Sindh

| Conc:µg/mL | CH <sub>3</sub> OH<br>%Inhibition | H <sub>2</sub> O<br>%Inhibition | CH <sub>3</sub> OH+H <sub>2</sub> O<br>%Inhibition | C <sub>2</sub> H <sub>5</sub> OH<br>%Inhibition | C <sub>2</sub> H <sub>5</sub> OH+H <sub>2</sub> O<br>%Inhibition |
|------------|-----------------------------------|---------------------------------|--|---|--|
| 222        | 89.61                             | 71.25                           | 87.47  | 78.39   | 89.81  |
| 111        | 80.9                              | 63                              | 71.74  | 65.04   | 72.45  |
| 55.5       | 61.59                             | 53.41                           | 58.49  | 55.76   | 63.37  |
| 27.27      | 52.89                             | 42.71                           | 51.55  | 50.15   | 40.87  |
| IC-50value | 24.829                            | 44.78                           | 28.743   | 31.225  | 37.129   |

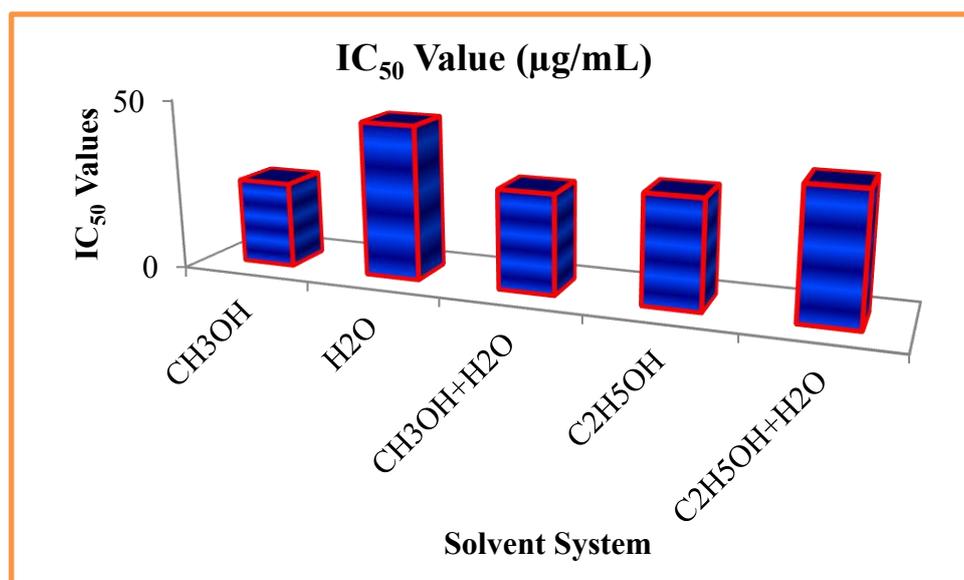


Figure: 6Antioxidant activity of Allie date fruit variety at Tamar stage collected from Taluka Kingri, district Khairpur, Sindh

#### Mazawati Variety

The Mazawati variety from Taluka Kingri has demonstrated strong antioxidant activity using a wide range of different extraction media at the Tamar stage. As such, pure methanol was found to be the most effective solvent for this cultivar, with the lowest IC-50 value being 24.611 µg/mL, indicating its high ability to extract radical scavengers from the Mazawati variety. Pure ethanol also had the greatest percentage of specific inhibition at the lowest concentration (53.38% at 27.27 µg/mL); however, its higher overall IC-50 value (35.708

µg/mL) demonstrates a different extraction mechanism for this solvent in comparison to the methanolic and aqueous extracts. In addition, chloroform and hydro-methanol mixtures produced very similar efficiencies, with IC-50 values near 30 µg/mL, reflecting that the bioactive constituents from Mazawati dates should be relatively easily accessible using more traditional solvent systems. These findings support that Mazawati dates represent a diverse source of natural antioxidants with optimal potential obtained by methanolic extraction (Table: 6; Figure: 7).

Table: 6Antioxidant activity of Muzawatidate fruit variety at Tamar stage Taluka Kingri, district Khairpur, Sindh

| Conc:µg/mL | CH <sub>3</sub> OH<br>%Inhibition | H <sub>2</sub> O<br>%Inhibition | CH <sub>3</sub> OH+H <sub>2</sub> O<br>%Inhibition | C <sub>2</sub> H <sub>5</sub> OH<br>%Inhibition | C <sub>2</sub> H <sub>5</sub> OH+H <sub>2</sub> O<br>%Inhibition |
|------------|-----------------------------------|---------------------------------|--|---|--|
| 222        | 84.47                             | 82.87                           | 85.76  | 85.35   | 75.10  |
| 111        | 73.24                             | 74.45                           | 70.97  | 75.31   | 69.36  |
| 55.5       | 63.06                             | 64.63                           | 62.43  | 65.68   | 58.91  |
| 27.27      | 48.79                             | 45.39                           | 46.88  | 53.38   | 47.44  |
| IC-50value | 24.611                            | 29.906                          | 31.115   | 35.708  | 30.257   |

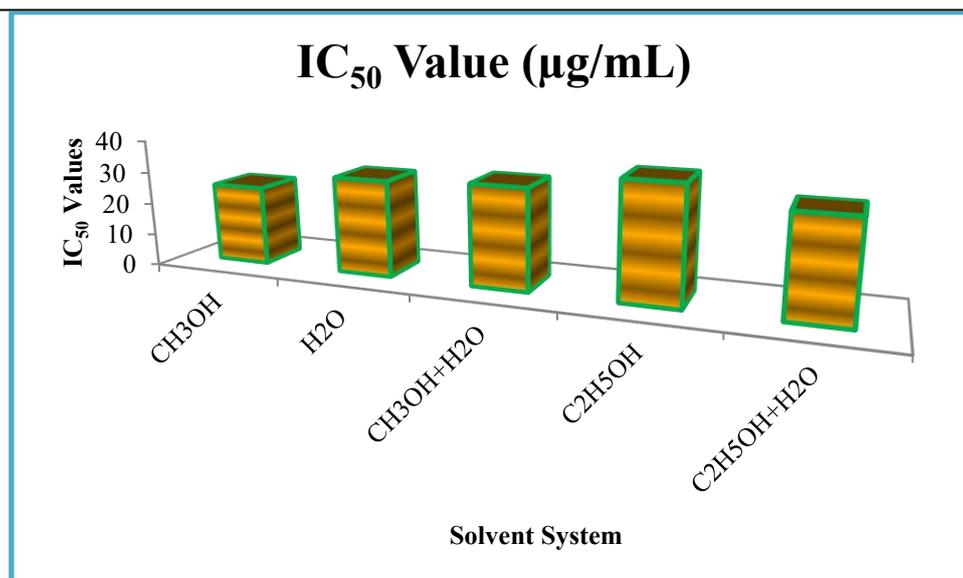


Figure: 7 Antioxidant activity of Muzawati date fruit variety at Tamar stage collected from Taluka Kingri, district Khairpur, Sindh

#### Otakin Date Variety

The radical scavenging capacity of the Otakin date variety from Taluka Kingri was assessed using an antioxidant profile and the solvent system used for the extraction had a large effect on the results. The pure methanol solvent system produced the lowest IC-50 value (25.481 µg/ml) for the sample which indicates it is an excellent medium to extract antioxidants. The IC-50 value found for distilled water was 42.779 µg/ml and although it gave the highest inhibition (89.06%) at the highest concentration tested (222 µg/ml), it

considerably reduced its activity as the concentration decreased. The ethanolic and hydroalcoholic extracts gave moderate inhibition but the ethanol-water mixture gave consistent inhibition at high concentrations. Overall, the results indicate that the indigenous Kingri variety of Otakin dates is a significant source of natural antioxidants and that it would be best extracted with pure methanol because of the predominance of polar bioactive compounds located in the date fruits (Table: 7; Figure: 8).

Table 7 Antioxidant activity of Otakaindate fruit variety at Tamar stage collected from Taluka Kingri, district Khairpur, Sindh

| Conc:µg/ml | CH <sub>3</sub> OH<br>%Inhibition | H <sub>2</sub> O<br>%Inhibition | CH <sub>3</sub> OH+H <sub>2</sub> O<br>%Inhibition | C <sub>2</sub> H <sub>5</sub> OH<br>%Inhibition | C <sub>2</sub> H <sub>5</sub> OH+H <sub>2</sub> O<br>%Inhibition |
|------------|-----------------------------------|---------------------------------|--|---|--|
| 222        | 83.91                             | 89.06                           | 85.78  | 77.86   | 88.88  |
| 111        | 76.91                             | 74.86                           | 76.87  | 68.54   | 76.85  |
| 55.5       | 66.37                             | 59.7                            | 61.78  | 59.82   | 57.62  |
| 27.27      | 48.28                             | 36.29                           | 42.56  | 46.79   | 45.09  |
| IC-50value | 25.481                            | 42.779                          | 34.859   | 31.534  | 35.343   |

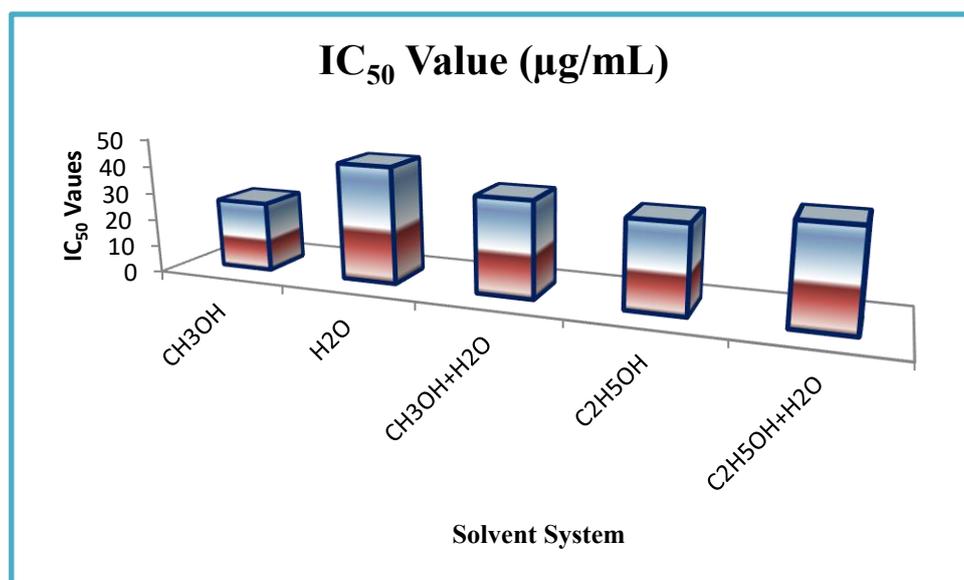


Figure: 8 Antioxidant activity of Otakain date fruit variety at Tamar stage collected from Taluka Kingri, district Khairpur, Sindh

#### Comparison of IC<sub>50</sub> Values

The analysis of IC<sub>50</sub> results among the five date fruit varieties located in the Kingri Taluka demonstrated considerable differences in antioxidant activity depending on solvent system used for extraction. Lower IC<sub>50</sub> values indicate greater radical scavenging abilities, as shown here, where both Methanol and Methanol+Water systems exhibited the strongest overall results. Allie and Mazawati represented the strongest antioxidant activity for pure Methanol, with IC<sub>50</sub>=24.829 and 24.611 respectively; these two varieties also contained the greatest amount of methanol soluble bioactive compounds.

As such, the majority of date fruit varieties demonstrated superior results in organic or

semi-organic solution; however, the Hussani variety demonstrated preference for extraction method involving Methanol + Water, producing an IC<sub>50</sub> of 21.206, while the other varieties produced much higher values when extracted in aqueous solvent alone (for example, with Allie's and Hussani's IC<sub>50</sub>s being 44.78 and 42.54 respectively). This indicates that aqueous extraction is less efficient for recovering the main source of antioxidant components in these date varieties. Kashkari exhibited a fairly consistent physical format across both Ethanol and Methanol; however, this variety demonstrated poorer performance with his other date fruit varieties when using Methanol + Water during extraction (Table: 8; Figure: 9).

Table: 8 Comparison of IC<sub>50</sub> Values among different varieties of Date Fruit collected from Taluka Kingri, District Khairpur, Sindh

| Solvent System | Kashkari<br>IC <sub>50</sub> Values | Hussani<br>IC <sub>50</sub> Values | Allie<br>IC <sub>50</sub> Values | Mazawati<br>IC <sub>50</sub> Values | Otakin<br>IC <sub>50</sub> Values |
|----------------|-------------------------------------|------------------------------------|----------------------------------|-------------------------------------|-----------------------------------|
| Methanol       | 26.635                              | 34.723                             | 24.829                           | 24.611                              | 25.481                            |
| Water          | 31.678                              | 42.54                              | 44.78                            | 29.906                              | 42.779                            |
| Methanol+Water | 37.907                              | 21.206                             | 28.743                           | 31.115                              | 34.859                            |
| Ethanol        | 27.33                               | 27.968                             | 31.225                           | 35.708                              | 31.534                            |
| Ethanol+Water  | 32.062                              | 31.038                             | 37.129                           | 30.257                              | 35.343                            |

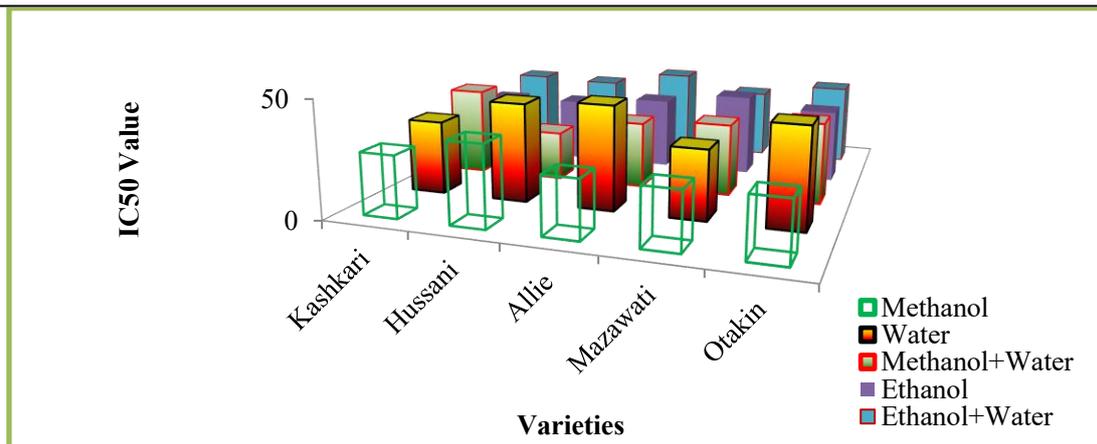


Figure: 9 Comparison of IC50 Values among different varieties of Date Fruit collected from Taluka Kingri, District Khairpur, Sindh

## CONCLUSION

This study describes the extensive testing and analysis of the antioxidant activity and chemical profile of traditional date palm varieties from Kingri, Pakistan at their highest levels of ripeness. The results indicate that the different forms of dates are highly variable in relation to their physical characteristics, and highlight the differences between the elongated female dates (Allie & Hussani) with small, compact dates (Mazawati & Otakin). The Kashkari female date was found to exhibit relatively high mass and moisture content and therefore softer texture than the Hussani female date which had considerably lower moisture content and therefore would be dryer and better for storage long term. The results from this study also showed that the indigenous date fruits demonstrate substantial capacity to provide a source of natural antioxidants. The quantity of antioxidant activity extracted from the indigenous date varieties was found to be strongly dependent on the solvent used to extract the antioxidants; thus, organic solvents (specifically methanol and ethanol) produced consistently higher values for the radical scavenging activity for the majority of the date cultivars. The distinctive extraction data for the Hussani cultivar in a hydro-methanolic solvent indicates that the different cultivars may contain various classes of phenolic compounds (i.e., varying polarity) and therefore would yield different antioxidant activity when using different extraction methodologies. The results of the study confirm the potential nutritional and therapeutic value of the large and varied

genetic resources of date palms from Kingri, Pakistan, and provide the basis for developing the best possible extraction techniques in order to produce date-derived functional foods and natural health products.

## Author Contribution

Sobia Shaikh and Ali Ibrahim Shar conceptualized, collected samples, designed experiments, Ammat - ur - Rehman Soomro, Dr. Abdul Raheem Shar and Farzana Mangrio prepared the draft of the article. Seema Sarwar Ghumro and Tahmina Fakhr-U-Nisa Abbasi interpreted the data, Prof. Dr. Ghulam Qadir Shar supervised the whole work, all authors read, revised, and approved the final version of the manuscript.

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## Conflict of Interest

The authors declare no conflict of interest

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