

## Analysis of Bioactive Compounds in Nabeez Water

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Dates (*Phoenix dactylifera* L.) are a unique fruit because they have many health benefits. Ajwa dates are one of the dates that many people are interested in for ingestion. Making date-infused water or nabeez water is one technique to ingest dates as exemplified by the Prophet SAW and is believed to have many benefits for digestion. As a result, there has never been any research on the analysis of active compounds from nabeez water, particularly Ajwa dates themselves, so the goal of this study was to determine the content of active compounds in nabeez water as well as the total content based on qualitative and quantitative analysis. The nabeez water from dates was tested qualitatively and quantitatively using phytochemical tests and UV-Visible Spectrophotometry. The results show that the nabeez water contains active ingredients of flavonoids, saponins, alkaloids, and tannins, according to qualitative analytical results. The quantitative analysis showed that nabeez water has a total concentration of flavonoid compounds of 5.749 g/mL and a concentration of tannin compounds of 52.934 g/mL.

**Keywords:** Active compounds; Nabeez water; Phytochemical test; Qualitative; Quantitative; Spectrophotometry.

Dates (*Phoenix dactylifera* L.) are a popular food in Islamic countries and are a staple in the Middle East. Date palms are one of the many types of palm trees that belong to the Arecaceae family, which includes over 2.500 species and 200 genera<sup>1</sup>. The date palm is the most significant cultivated plant in North Africa and the Middle East, and it is one of the world's oldest cultivated plants<sup>2</sup>. This is based on the nutritional value and benefits of dates, which are well-known around the world for their high-quality nutrients and healthpromoting characteristics<sup>1,3</sup>.

According to prior research, dates contain flavonoids, carotenoids, glucose, fructose, sucrose, protein, ascorbic acid, phenolic acid, potassium

(K), magnesium (Mg), phosphorus (P), sodium (Na), and iron (Fe)<sup>4-6</sup>. Dates also known to have antioxidants, antihyperlipidemic, antimutagenic, anti-inflammatory, hepatoprotective, and nephroprotective properties, making them beneficial as an immunomodulator<sup>7-10</sup>.

Dates have a unique feature in that they are one of 20 fruits mentioned in the Qur'an. One of the Islamic teachings for its believers is to consume all products that are permissible (halal) and good (thayyib) according to the relevant specifically the Quran and the Hadith<sup>11</sup>. Dates are in great demand because they have many health benefits and are halal. The Ajwa type, sometimes known as Prophet dates, is widely grown in Madinah and

is also commonly found in Indonesia. Prophet Muhammad strongly advises that this sort of date be consumed every day because it has a compound that is beneficial for the health of the body<sup>12,13</sup>.

The Prophet Muhammad demonstrated one method of digesting dates by soaking the dates flesh in water in a closed container overnight, then drinking the soaked water the next morning. The outcome of this immersion is known as Nabeez water. It is well known that Nabeez water is beneficial for removing acid from the stomach, avoiding flatulence, neutralizing uric acid, launching the digestive system, and serving as a detox element because it works to remove toxins from the body<sup>14-17</sup>.

Based on the various benefits of Nabeez water, and the absence of studies discussing the analysis of compounds in Nabeez water, particularly from Ajwa dates themselves, it can be used as an opportunity for research related to Nabeez water. The purpose of this research was to determine the content of the compounds in Nabeez water based on qualitative and quantitative analysis using the UV-Vis spectrophotometry.

## MATERIAL AND METHODS

### Material Preparation

The dates were purchased from a store that sells ajwa type dates, and then the dates were separated from seeds. 5 g of date flesh was soaked in 100 mL of water in a closed container and allowed to stand for 18 hours at room temperature ( $\pm 25$  C), and then filtered to get Nabeez water.

### Qualitative Analysis of Nabeez Water

#### Flavonoid Test

5 mL of Nabeez water was added to a sterilized test tube, then 0.05 mg of magnesium (Mg) powder and 1 mL of concentrated hydrogen chloride (HCl) 37% were added, and shaken vigorously. The positive result of flavonoids are indicated by the change of the original color of nabeez water (muddy brown) to red, yellow, or orange in nabeez water<sup>18,19</sup>.

#### Saponin Test

1 mL of Nabeez water was placed in a test tube, followed by 10 mL of hot water, cooled, and vigorously shaken for 10 seconds. Positive results if the foam is formed which persists for not less than 1 minute as high as 10 cm, or if 1 drop of 2N HCl

is added, the foam does not disappear, indicating the presence of saponins<sup>20,21</sup>.

#### Alkaloid Test

Add 1 mL of Nabeez water into a test tube, then add 5 mL of 2 N HCl and heat it in a water bath for 2 minutes. Then added 3 drops of Dragendrof LP reagent (Sigma-Aldrich, Germany). A positive result if a yellow-orange to brick-red precipitate is formed indicates the presence of alkaloids<sup>21,22</sup>.

#### Tannin Test

Take 2 mL of Nabeez water and then heated for  $\pm 5$  minutes. Followed by adding a few drops of 1% *ferric chloride (III)* (FeCl<sub>3</sub>). Positive results are indicated by the formation of a greenish-brown or blue-black color in the solution<sup>22,23</sup>.

### Quantitative Analysis of Nabeez Water

#### Total Flavonoid Content Test

In the process of making the standard curve, 10 mg of standard quercetin was weighed and put in a 10 mL volumetric flask and dissolved with methanol: aquadest (8:2). Pipette the solution as much as 0.5 mL and then dissolve it with 10 mL of distilled water to obtain a concentration of 50 ppm. Furthermore, 0 mL; 0.3 mL; 0.5 mL; 0.7 mL; and 0.9 mL of the 50 ppm solution was pipetted to obtain concentration 0 ppm; 3 ppm; 5 ppm; 7 ppm; and 9 ppm. Next, the absorbance was measured at a wavelength of 430 nm. In addition, the supporting reagent was made by weighing 0.1 g of plumbum (Pb) acetate and 0.06 g of Plumbum(II) oxide (PbO). Then put into a beaker glass and dissolved in 10 mL of distilled water, stirring until dissolved and a cloudy solution is formed.

The total flavonoid content was determined by putting 0.05 g/mL with an immersion time of 18 hours into as much as 0.2 mL, put in a beaker glass, and dissolved with 5 mL of methanol, sonicated for 30 minutes. Then 0.1 mL of support reagent was added and centrifuged for 10 minutes at 3500 rpm. 1 mL of this solution was pipetted and 1 mL of 2% aluminium chloride (AlCl<sub>3</sub>) was added. Samples were incubated at room temperature for 30 minutes. After incubation, the absorbance of the samples was measured using a UV-Vis spectrophotometer at a wavelength of 430 nm. The sample absorbance calculation was repeated three times for each analysis so that the average absorbance value was obtained.

#### Total Tannin Content Test

In the process of making a standard curve,

10 mg of standard gallic acid was weighed, then put in a 10 mL volumetric flask and dissolved with methanol: aquadest (8:2). Pipette the solution as much as 0.5 mL and then dissolve it with 10 mL of distilled water to obtain a concentration of 50 ppm. Furthermore, 0 mL; 0.3 mL; 0.5 mL; 0.7 mL; and 0.9 mL of the 50 ppm solution were pipetted to obtain concentration of 0 ppm; 3 ppm; 5 ppm; 7 ppm; and 9 ppm. Next, the absorbance was measured at a wavelength of 765 nm. In addition, the supporting reagent was made by weighing 0.1 grams of Pb acetate and 0.06 grams of PbO. Then put into a beaker glass and dissolved in 10 mL of distilled water, stirring until dissolved and a cloudy solution is formed.

The total tannin content was determined by putting 0.05 g/mL with an immersion time of 18 hours as much as 0.1 mL, put in a beaker glass, and dissolving it with 5 mL of methanol. Then sonicated for 30 minutes. Then 0.1 mL of support reagent was added and centrifuged for 10 minutes at a speed of 3500 rpm. From the sample solution, 1 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, Germany) was added, shaken, and allowed to stand for 5 minutes. Then 2 mL of 15% Na<sub>2</sub>CO<sub>3</sub> solution was added, shaken until homogeneous, and allowed to stand for 5 minutes. Next, demineralized water was added to the exact volume of 10 mL, homogenized, and allowed to stand for 90 minutes. Then the absorbance was observed at a maximum wavelength of 765 nm. The tannin content was obtained from the average absorbance value for three repetitions of the analysis.

#### Data Analysis

Analysis of the data on the results of the qualitative test will be compared with the standard of qualitative testing based on the literature. Then the results of the quantitative test will be obtained in the form of primary data obtained from the absorbance of the standard solution.

Then a calibration curve is made to obtain a linear regression equation. The calculation of the total content of the compound was obtained by substituting it into the linear regression equation  $y = ax + b$  which was obtained by comparing the calibration curve, and the calculation results were expressed in units of g/mL.

## RESULTS AND DISCUSSION

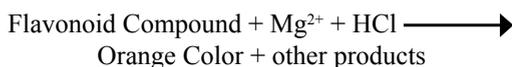
Nabeez water with a concentration of 0.05 g/mL was utilized in this investigation as the test sample. To eliminate sample selection problems and to verify that the correct dates were utilized, the dates were recognized and identified as raw materials before performing Nabeez water preparations. Nabeez water is made using the maceration extraction method, in which the dates' flesh is immersed in a water-based solvent<sup>24</sup>.

Before testing for quantitative analysis, the qualitative analysis was used to determine the presence of secondary metabolites in nabeez water. After being provided reagents for the flavonoid, saponin, alkaloid, and tannin components, this qualitative examination took the form of a phytochemical test, which looked for any organoleptic alterations (color, precipitate formation, foam development)<sup>25-26</sup>. Table 1 summarizes the findings of the qualitative analysis.

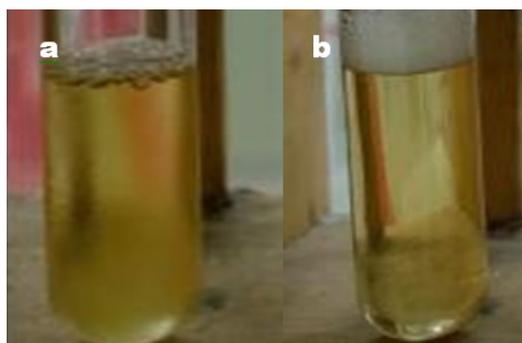
According to the results of the qualitative test above, the Nabeez water contains active substances such as flavonoids, saponins, alkaloids, and tannins. A change in the hue of the sample to yellow showed the presence of flavonoid molecules (Figure 1). The combination of Mg and HCl is intended to decrease the benzopyron core in the flavonoid structure, allowing flavilium salts to form<sup>19,21,27</sup>. The equation for the reduction of active flavonoid compounds by Mg and HCl is:

**Table 1.** Nabeez Water Qualitative Analysis Results

Identification of Compounds	Reagent	Positive Indication	Conclusion
Flavonoids	Mg + HCl	Formation of red, yellow, or orange color	Positive
Saponins	Aquadest + 2N HCl	Foam is formed	Positive
Alkaloids	Dragendrof LP	A yellow-orange to brick-red precipitate is formed	Positive
Tannins	FeCl <sub>3</sub>	Formation of greenish-brown or blue-black color	Positive

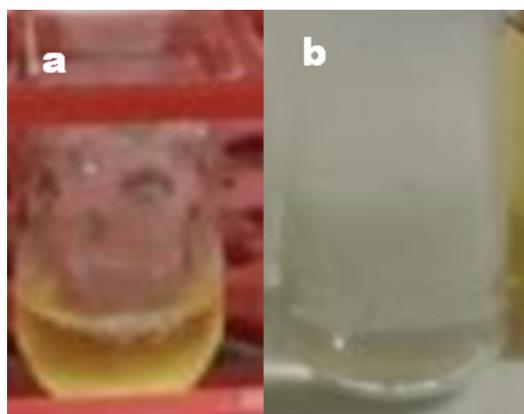


The qualitative examination of Nabeez water revealed that saponins were also present, as evidenced by the development of foam when the sample was mixed with HCl and shaken (Figure 2). Saponins froth when shaken because hydrophilic groups connect to water while hydrophobic groups bind to air. The addition of HCl solution increases the polarity of the combination, making the interaction of the hydrophilic groups of saponins with water more stable, as well as the foam generated. The foam is formed when the polar groups face outward and the nonpolar groups face inward in the micelle structure<sup>21,28</sup>.



**Fig. 1.** Flavonoid Compounds Qualitative Test Results. (a) Before administration of reagents; (b) After administration of reagents

A qualitative test on nabeez dates water showed positive results containing alkaloid compounds with Dragendrof LP reagent indicated by the presence of orange-yellow precipitate (Figure 3). Because the alkaloids are alkaline, before adding Dragendrof's reagent, HCl was added first, so the extraction was carried out with a solvent containing acid. When the Dragendrof's reagent is added, bismuth nitrate dissolves in HCl so that the bismuth salt is not hydrolyzed to bismuth ions (BiO<sup>+</sup>) as the reaction equation:



**Fig. 2.** Saponin Compounds Qualitative Test Results. (a) Before administration of reagents; (b) After administration of reagents

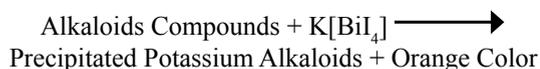
**Table 2.** Results of Determination of Total Flavonoid Levels in Nabeez Dates Water

Replication	Absorbance (y)	Initial Total Flavonoid Level (µg/mL)	Total Flavonoid Level (µg/mL)	Percent Content of Total Flavonoid (%)
1	0.012±0.001	0.225	5,858	0.0006
2	0.010 ± 0.000	0.206	5,365	0.0005
3	0.012±0.001	0.232	6,023	0.0006

**Table 3.** Results of Determination of Total Tannin Levels in Nabeez Dates Water

Replication	Absorbance (y)	Initial Total Tannin Content (µg/mL)	Total Tannin Content (µg/mL)	Percent Content of Total Tannins (%)
1	0.186 ± 0.000	1.048	53.443	0.0053
2	0.187±0.001	1.051	53.596	0.0054
3	0.181 ± 0.001	1.015	51.763	0.0052

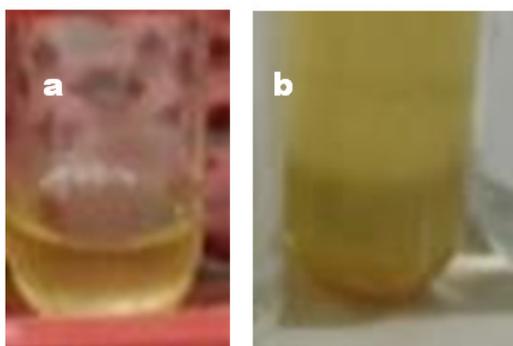
The Bi<sup>3+</sup> ion from reacting bismuth nitrate with KI forms a black precipitate of bismuth(III) iodide, which dissolves in excess KI to form potassium tetraiodobismuthate. The use of nitrogen in this test aims to form a coordinate covalent bond with K<sup>+</sup> as the metal ion<sup>21,28</sup>. The following is an approximate the equation for the reaction of alkaloids with Dragendroff's reagent:



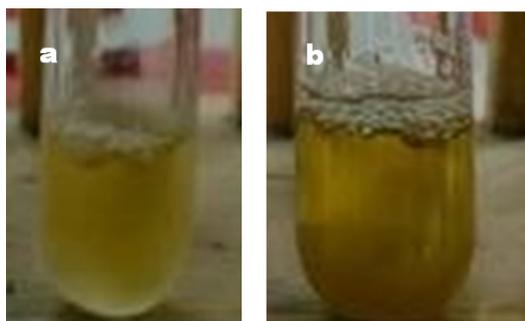
The color solution changed hue to greenish-brown after adding FeCl<sub>3</sub> solution to Nabeez water indicating that the water contained

tannins (Figure 4). This implies that the added FeCl<sub>3</sub> solution will react with one of the hydroxyl groups in the tannin molecule to generate a complex compound with Fe<sup>3+</sup> ions due to the presence of phenolic compounds in the form of tannins in the water sample of Nabeez dates. To identify phenolic substances such as tannins, the FeCl<sub>3</sub> reagent is commonly used<sup>21,29</sup>.

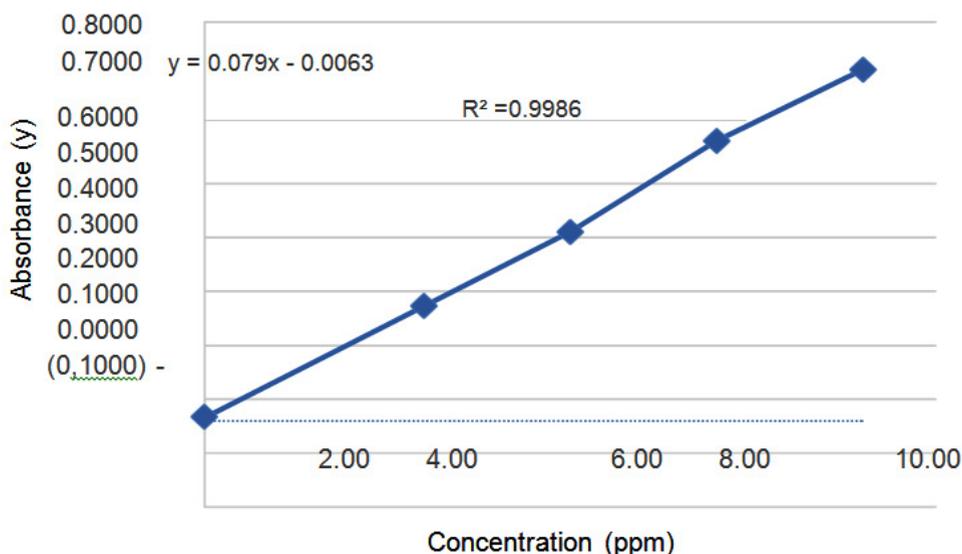
Meanwhile, quantitative analysis was carried out to determine the total levels of active compounds contained in the Nabeez water dates using the UV-Vis spectrophotometry method. The total levels of compounds tested were the total levels of flavonoid compounds and tannin compounds based on the standard curve for each



**Fig. 3.** Alkaloid Compounds Qualitative Test Results. (a) Before administration of reagents; (b) After administration of reagent



**Fig. 4.** Results of Qualitative Test of Tannin Compounds. (a) Before administration of reagents; (b) After administration of reagents



**Fig. 5.** Quercetin Standard Calibration Curve

compound. In determining the total flavonoid content using the results of the standard curve values. The standard curve obtained with the linear regression equation is  $y = 0.079x - 0.0063$  and the value of  $r$  squared is 0.9986 (Figure 5).

The total levels of flavonoid compounds can be determined using the test parameters of total quercetin equivalent levels and the result of the regression equation obtained. The flavonoid concentration of the Nabeez water was 5.749 g/mL on average (Table 2). Flavonoid molecules contain antioxidant and diuretic properties, as well as anticholesterol properties<sup>30,31</sup>.

In the quantitative test for the determination of tannin levels, the regression equation is obtained, namely  $y = 0.1673x + 0.0116$  and the value of  $r$  squared is 0.9971 (Figure 6). The average content of tannins contained in the Nabeez water with the total test parameter of gallic acid equivalent level was 52,934 g/mL (Table 3). Tannin compounds are astringents that are useful as antidiarrheal, hemostasis, and anti-inflammatory, especially in the oral mucosa, as well as antiseptics due to the presence of phenolic groups<sup>30,32</sup>.

The Nabeez water of the Ajwa type were discovered to include flavonoid chemicals, saponins, alkaloids, and tannins, according to the results of qualitative and quantitative analyses. According to Hamad *et al* (2015)<sup>4</sup> study, the level of flavonoid components in 100 g of dry weight extract of Ajwa dates was 27.8 g/g. The levels of active compounds in the water samples of Nabeez water are lower than the levels of active compounds in the extracts of dates, according to these findings. This is possible because the Nabeez water dates is only made from the results of soaking the flesh of the dates (water extract of dates fruit) so that the total levels of active compounds are lower.

## CONCLUSION

Based on the results of the research that has been done, it can be concluded that the Nabeez water contains active compounds of flavonoids, saponins, alkaloids, and tannins. Nabeez water have an average level of total flavonoids with quercetin equivalent test parameters of 5.749 g/mL and average levels of total tannins with gallic acid equivalent test parameters of 52,934 g/mL. As for supporting this research, it is necessary to analyze

the content of other compounds in water nabeez dates, because there is still little research related to water nabeez dates, it is important to know the overall content of any compound in Nabeez and can be used as a reference to see the potential of compounds for health..

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## Conflict of Interest

All authors declare there is no conflict of interest in this manuscript.

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