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Anti-tumor and anti-cancer activity test from Nabeez Ajwa Dates (*Phoenix dactilifera L.*) water

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Abstracts

Nabeez dates water is one of the fruits that is used as infused water. Dates contain flavonoids, phenols, alkaloids, and beta D-glucans which have anticancer and anti-tumor activity. Ajwa Dates have a higher phenol content than Sukkari Dates and Khalas Dates. Cancer is the number 5 deadliest disease in Indonesia and number 2 in the world after cardiovascular disease. The use of natural ingredients is an alternative for the treatment of this disease. One of the natural ingredients can be used is ajwa dates. The method of testing for anti-tumor and anti-cancer activity uses the Brine Shrimp Lethality Test (BSLT) method with shrimp larvae of Artemia salina leach as animals for testing. The results showed that the water of ajwa nabeez dates immersion for 24 hours, 48 hours, and 72 hours has the potential as anti-tumor and anti-cancer because it has an LC50 value of 676.39 ppm; 720.84 ppm; and 903.20 ppm. Phytochemical test results have shown positive samples containing tannins, phenolics, flavonoids, saponins, and alkaloids. The test results of FTIR spectophotometry resulted in the appearance of wave numbers indicating the presence of functional groups -OH, C-H, C = O, C-C (sp^3), and C-O.

Keywords: Ajwa dates, anti tumor, anti cancer, toxicity test

1. Introduction

Cancer is the number 5 deadliest disease in Indonesia and number 2 in the world after cardiovascular disease [20,27]. Medical treatment is provided by cancer patients includes surgery, chemotherapy and radiotherapy. However, if the cancer cells have spread to other organs, surgery cannot be performed. Chemotherapy and radiotherapy require a lot of money. Therefore, the use of natural ingredients is an alternative for the treatment of this disease. One of the natural ingredients that can be used is ajwa dates.

Ajwa dates contain flavonoids, phenols, alkaloids, and beta D-glucans which have

anticancer and anti-tumor activity [26]. Research that has been conducted by Saleh, et al (2011) proves that Ajwa Dates have a higher phenol content than Sukkari and Khalas Dates, namely 455.88 mg / 100g. In the research of Fibonacci (2020) it has been proven that the water of nabeez dates of ajwa has antioxidant activity with an IC_{50} value of 223.6 ppm for 24 hours of immersion, 369 ppm for 48 hours of immersion, and 2530 ppm for 72 hours of immersion. The results of this study are one of the lessons learned from Rasulallah's habit of consuming Ajwa dates.

Rasulallah SAW. have a habit of consuming dates either eaten directly or processed first. As has been narrated in the Hadith History of Muslims Number 3739. The hadith has the following meaning: "Ubaidullah bin Mu'adz Al-Anbari had told us my father had told us the Syu'bah from Yahya bin Ubaid Abu Umar Al-Bahrani he said; I heard Ibn Abbas say, "Rasulullah SAW. made Nabeez juice at the beginning of the night, then he drank it in the morning, then in the evening, then the day after tomorrow and at night and the next day until just before Asr. If the feeling was still there, he ordered his servants to spilled it, or ordered it to be spilled."[1]

Anti-tumor and anticancer activity tests can be determined through toxicity testing, Toxicity test can be carried out by the Brine Shrimp Lethality Test (BSLT) method which uses Artemia salina leach shrimp larvae as animals for testing.. This method is the method most widely used to test anti-cancer compounds found in plants. The test results with the BSLT method have an influence on the potential for the cytotoxic power of anti-cancer compounds [19]. This method is also relatively easy to do, cheap, fast and effective [23]. The effectiveness of the toxic activity can be determined based on the LC_{50}. An extract can be said to be toxic if the LC_{50} value is <1000 ppm, while for a pure compound it has an LC_{50} value <30 ppm.[18]. The results of this study are expected to provide information about the potential of Nabeez Water as an anti-tumor and anti-cancer agent.

2. Research Methodology

Tools and Materials

The tools were glass beakers, balance sheets, a set of larvae hatching vessels, test tubes, dropper pipettes, porcelain dishes, FTIR spectrophotometry (Bruker), oven (Memmert), mortar and stemper.

This study used a sample of ajwa variety dates from the city of Medina through the agent Fatimah Azzahra. The ingredients are Artemia salina Leach shrimp eggs, pure salt, 5% $FeCl_3$, 3% formaldehyde: HCl (2: 1), Na-acetate, chloroform, anhydrous acetic acid, concentrated sulfuric acid, concentrated HCl, magnesium powder, methanol, H_2SO_4 2N, benzene, NaOH 2 N, $NaHCO_3$ 2 N, dilute ammonia, acetic acid, Dragendroff's Reagent, Mayer's Reagent, and Wagner's Reagent.

Sample Preparation

Ajwa dates was use still fresh, dark brown in color, and have an oval shape. Samples are whole dates and their seeds. Samples have been sorted and weighed then macerated using distilled water as much as 50 mL, then the concentration is calculated in ppm units. Maceration contact time varied 24 hours, 48 hours, and 72 hours [12].

Toxicity Test

Toxicity testing used the Brine Shrimp Lethality Test (BSLT) method which use Artemia salina Leach larvae as test animals. This method follows McLaughlin's procedure with some modifications [17].

Shrimp larvae hatching was done by preparing a vessel that has been given a perforated bulkhead. Artificial seawater is made by dissolving salt with distilled water at a concentration of 10%. One room in the vessel is equipped with a lamp to attract the attention of the larvae after hatching to separate them from the egg shells, while in the other room 50 mg of Artemia salina Leach shrimp eggs are inserted and then covered with aluminum foil. Hatching

time lasts for 48 hours in water that has a constant oxygen content [31].

A trial test or orientation test is carried out with this toxicity test. This was necessary to make it easier to determine the concentration of date palm extract that is most effective for killing shrimp larvae. The orientation test can be carried out with 5 mL of artificial seawater containing 10 shrimp larvae pipette and put into the test tube. 5 mL of sample solution with a concentration of 1000 ppm, 3000 ppm, 5000 ppm, 7000 ppm, and 9000 ppm which has been extracted with distilled water, each of which is entered into a test tube containing shrimp larvae. The control tube was also required without adding to the sample. The solution was shaken slowly until homogeneous, then allowed to stand for 24 hours at room temperature. After 24 hours, the number of dead and living larvae was observed. The experiment was carried out 3 times. The number of live and dead larvae was counted by adding up the 3 tubes at the same concentration.

After obtaining the orientation test data in the form of the number of live and dead shrimp larvae, then the sample concentration was determined for further testing. The concentration of the sample used for the next test for the smallest concentration that could kill shrimp larvae. The data obtained are used to calculate mortality with the following equation:

$$\text{Mortality} = \frac{Dead\ maggot\ accummulation}{Alive\ and\ dead\ maggot\ accummulation\ (total)} \text{ x } 100\% \quad (1)$$

The mortality value was used to determine the probit value which can be converted through a probit table [10]. Determination of the LC_{50} value is done by looking for the linear regression equation from the relationship curve where the x-axis is the log of concentration and the y-axis is the probit value. The linear regression equation is obtained as follows:

$$y = bx + a \quad (2)$$

Where y is the probit value, x is the log value of concentration, a is the intercept, and b is the slope. Where y is the probit value, x is the log value of concentration, a is the intercept, and b is the slope.

Phytochemical Test

Nabeez water has the optimum conditions phytochemical tests which carried out to determine the active compounds contained qualitatively. Phytochemical tests include tannins, phenolics, steroids and triterpenoids, flavonoids, anthraquinones, saponins, and alkaloids.

The total tannin test was carried out by reacting 3 ml of the sample with 3 drops of 5% $FeCl_3$, a positive test was indicated by a change in color to blackish green or dark blue. Analysis of catechol tannin content and error test was carried out by adding 3% formaldehyde: HCl (2: 1) into the test tube containing the sample. Then the solution is heated with a water bath at 90°C. The presence of catechol tannins is indicated by the formation of pink deposits. Then to find out the error tannins the solution is filtered. The filtrate is saturated with Na-acetate and then added with 5% $FeCl_3$. The presence of an error tannin is indicated by a change in color to ink blue or black [14].

The phenolic test is carried out by reacting 1-2 drops of extract with 2 drops of 5% $FeCl_3$ solution. A positive test results in a discoloration of green, bluish black, or strong black [24].

Steroid and terpenoid tests were carried out by two methods. The first method was done by evaporating 2 mL of sample. The residue was dissolved with 0.5 mL chloroform, 0.5 mL anhydrous acetic acid and 2 mL concentrated sulfuric acid was added through the tube wall. A positive test for triterpenoids was indicated by the formation of a red ring at the border of the solution, whereas if a reddish-brown ring appears, it indicates the presence of steroids. The second method is the Salkowski test. The salkowski test is performed by adding a sample with chloroform and a few drops of concentrated sulfuric acid. The positive test for triterpenoids is indicated by the formation of yellow color in the solution, whereas if a red color is formed on the lower layer, the extract contains steroids [5].

Flavonoid test used the Shinoda Test method. The shinoda test adds 1 mL of concentrated HCl to the magnesium powder. Then add 2-3 mL of the sample that has been dissolved with methanol. A positive test for flavonoids is indicated by the formation of a pink or red color in the solution [24].

The anthraquinone test was carried out by heating 2 ml of the sample with 5 ml of H_2SO_4 2 N for 1 minute. After the solution has cooled, 10 ml of benzene is added and shaken. A positive test is indicated by the appearance of a yellow color on the benzene layer. The positive test is added with NaOH 2 N with a positive test which indicated by the appearance of a red color in the water phase [11].

Saponin test was carried out with a sample added with one drop of $NaHCO_3$ 2 N. The mixture was shaken vigorously for 3 minutes and heated until bubbly. A positive result is indicated by the formation of foam in the solution which does not disappear for 2-4 minutes [24].

Alkaloid test is carried out by dissolving the sample in 10 mL of alcohol, then heating and filtering. 5 mL of the filtrate are added to 2 mL of dilute ammonia. 5 ml of chloroform is added and shake slowly to extract alkaloid. The chloroform layer was extracted with 10 ml of acetic acid, divided into 3 parts, and tested as follows : the dragendroff test is carried out with a few drops of dragendroff solution which added to the chloroform solution, the reddish brown precipitate indicates the presence of alkaloids. The mayer test is carried out with a few drops of mayer reagent addition to the chloroform solution, white deposits indicate the presence of alkaloids. Wagner's test is done with a few drops of Wagner's solution which added to the chloroform solution, if a brown precipitate is formed it indicates an alkaloid [5].

Ajwa Dates Characterization Test

The Ajwa Dates characteristic test used the FTIR instrument to determine the functional groups contained in Ajwa Dates. Sample preparation for this characterization test was carried out by refining Ajwa dates then dry at a temperature of 50°C for 24 hours.

3. Results and Discussion

Procedure for Making Ajwa Nabeez Water

Dates maceration is carried out without separating the seeds and flesh of the dates because it is in accordance with the Hadith of Muslim History Number 3739 that the Prophet made nabeez water from the immersion of whole dates. In addition, date seeds also contain flavonoid and alkaloids compounds which have anti-cancer and anti-tumor properties [3,26].

After the dates are sorted then the dates are weighed to find out the mass of the dates, because each one date palm has a different mass. The weighing of dates before the maceration process serves to calculate the concentration of macerate or nabeez water in units of ppm. The concentration of macerated Nabeez water can be seen in the following table:

Table 1. Nabeez Water Concentration

Soaking Time (hour)	Mass off Dates (g)	Solvent Volume (mL)	Concentration (ppm)
24	6,70	50	134000
48	7,72	50	154000
72	6,63	50	132600

Toxicity Test

The results of the probit analysis to determine the LC_{50} value of the Ajwa Nabeez water that can be seen in the following table:

Table 2. Value of LC_{50}

Soaking Time (hour)	LC_{50} (ppm)
24	676,39
48	720,84
72	903,20

Based on the data analysis, it is concluded that Nabeez water with immersion for 24 hours, 48 hours, and 72 hours has the potential as anti-tumor and anti-cancer because it has an LC50 value of less than 1000 ppm, namely 676.39; 720.84 ppm and 903.20 ppm.

Although Nabeez water has anti-tumor and anti-cancer potential because the LC50 value is less than 1000 ppm, this value is still far different from the LC50 value in the research conducted by Marlinda, et al (2012) where the LC_{50} value of the ethanol extract of dry avocado seeds is 34,302 ppm. This is because the content of several compounds that have pharmacological effects as anti-tumor and anti-cancer in Ajwa dates is somewhat smaller when compared to other plants. Research of Putri et al (2020) has shown that the levels of flavonoids contained in Goji Berries infused water, Nabeez water immersed for 12 hours, and Nabeez water immersed for 24 consecutive hours are 40.44 mg / L; 6.83 mg / L; and 8.08 mg / L.

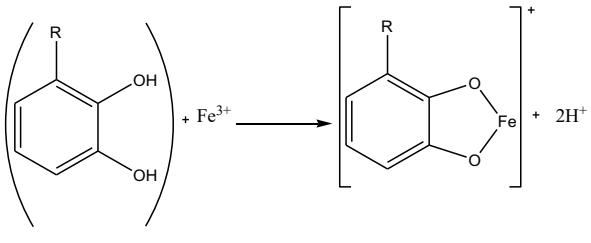
Phytochemical Test

Table 3. Phytochemical Test Results

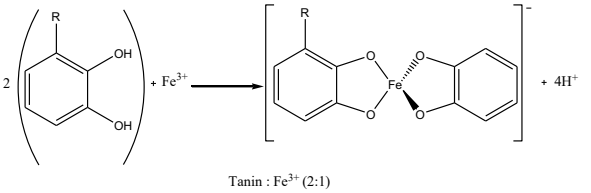
Compound Group	Response	Information
Total of tannins	+	
Catechol tannins	+	
Galat tannins	-	
Phenolic	+	
Steroid and Triterpenoid Test	-	
Flavonoids	+	
Anthraquinones	-	
Saponins	+	
Alkaloids Dragendroff test	+	
Mayer Test	+	
Wagner's test	+	

Based on the toxicity test, Nabeez water had the optimum LC_{50} value at immersion for 24 hours. Therefore, the Nabeez water sample used for the phytochemical test that Nabeez water under immersion for 24 hours. The results of phytochemical tests on water nabeez dates ajwa at a concentration of 174,000 ppm showed compounds of the tannin, flavonoid, phenolic, saponin and alkaloid groups. The results of the phytochemical test are shown in the following table 3.

Research by Fibonacci (2019) has also proven the presence of tannins and phenolics in the water of Ajwa dates. Tannins are polyphenol compounds, so the color chance to blackish green due to the presence of phenol groups in tannins. The phenol group is reacted with $FeCl_3$ to form Fe^{3+} ions along with complex colored compounds [13]. In this study, in addition to know the presence of tannin compounds in Nabeez water, but also to determine the tannin groups contained in the sample. The resulting color change indicates the presence of oxidized phenolic compounds. The phenolic compounds in the sample are thought to be compounds of the tannin group. The central metal ion Fe^{3+} is more stable if it has a coordination number of 6 (octahedral). Therefore polyphenolic compounds such as catechol tannins bind iron in a 3: 1 ratio (1 Fe atom binds 3 catechol tannin bidentate ligands) [22].



Tanin : Fe^{3+} (1:1)



Tanin : Fe^{3+} (2:1)

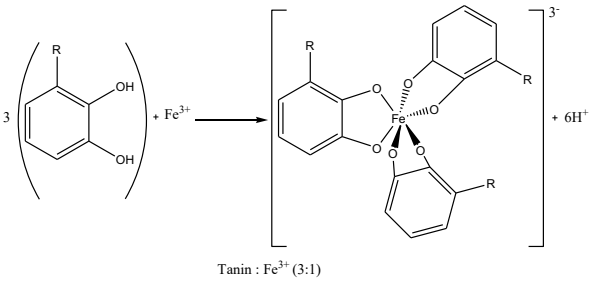


Figure 1. Reaction of Tannins with Fe^{3+}

The two test methods for steroid and triterpenoid compounds show that the water of nabeez dates does not contain triterpenoids or steroids. This result is linear with the research that has been done by Febrianti (2018). The positive test results for triterpenoids show the formation of red cicin at the meeting of the solution phase while the positive test results for steroids form reddish brown rings, Therefore, the addition of concentrated H_2SO_4 must pass through the tube wall so that the two solutions are not mixed. The oxidation of triterpenoids and steroids causes the formation of color changes which resulted from the formation of the conjugated double bonds [6].

Nabeez water of dates ajwa shows positive results when reacted with Shinoda's reagent by giving the solution a change in color from pink to orange. The resulting color is due to the formation of the complex $[Mg(OAr)_6]^{-4}$. This is also relevant to the research of Fibonacci (2019) which shows positive test results for flavonoid compounds in the water of nabeez dates. The reaction that occurs in the shinoda test is as follows [15]:

$$Mg_{(s)} + 2HCl_{(l)} \rightarrow MgCl_{2(aq)} + H_{2(g)}$$
$$MgCl_{2(aq)} + 6ArOH_{(s)} \rightarrow [Mg(OAr)_6]^{4}_{(aq)} + 6H^{+}_{(aq)} + 2Cl^{-}$$

Based on the above reaction, the reaction between solid Mg and concentrated HCl produces an exothermic reaction in which the reaction produces heat. In addition, this reaction will also form H_2 gas bubbles.

The results of the anthraquinone test on the nabeez water of dates ajwa were negative. This is in accordance with research by Fibonacci (2019). Anthraquinone is a compound with a three ringed anthracene structure. These compounds have derivatives, namely hydroxylated, methylated, and carboxylated anthraquinones, whose structures are usually linked to form anthraquinone compounds. Anthraquinones which can be o-glycosides or c-glycosides can bind to sugars. This compound can dissolve in hot water and dilute alcohol. The following is the structure of the anthraquinone compound [2] at Figure 2.

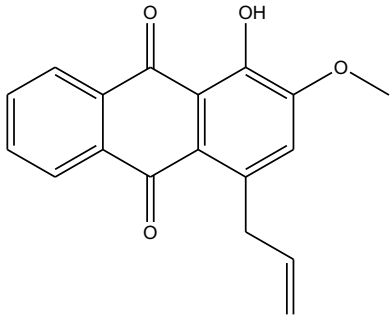


Figure 2. Chemical Structure of Anthraquinone Compounds

Nabeez water gives positive results for the saponin test because it produces foam that does not disappear until 4 minutes after reacting the sample with $NaHCO_3$ 2 N then shaked and heated. The reaction in the saponin test is as follows [21] at Figure 3.

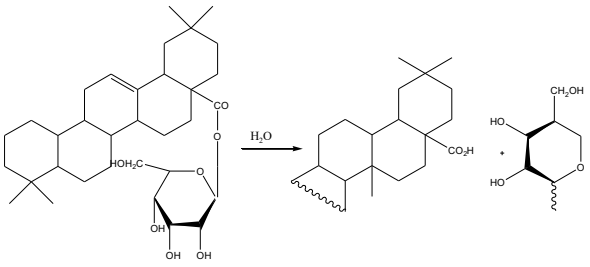


Figure 3. Saponin Test Reaction

Saponins are glycoxides with a hydroxyl group on the molecule, formula $C_{32}H_{18}O_7$. The properties of saponin compounds are like soap, where when they are dissolved in water it will form foam or foam [30]. The purpose of heating the extract and water mixture is to increase the solubility of saponins in water. The mixture is shaken until foam forms. This saponin compound has hydrophobic and hydrophilic

groups which can act as an active surface in foam formation.

Alkaloid compounds can have toxic and pharmacological effects as anti-tumor and anti-cancer. This compound has the ability to bind to tubulin proteins that make up microtubules. How it works by inhibiting or blocking protein polymerization into these microtubules. The microtubules are eventually destroyed and the telomerase enzym disrupted so that mitosis stops at metaphase. The presence of this disorder causes the telomere size at the end of the chromosome to be untenable and cell death (apoptosis)[4]. This research has proven that the water contains alkaloid compounds through three test methods, namely Dragendroff Test, Mayer Test, and Wagner Test.

Characterization Test of Ajwa Dates

The sample characterization test of ajwa dates used the FTIR spectophotometry instrument. The use of FTIR spectophotometry aims to determine the functional groups of compounds present in date samples. Dates sample preparation is dried the crushed dates with a stamper and mortar. Drying is carried out for 24 hours at this temperature 50°C. This method was adopted from previous research with several modifications [8]. Here are the results of the ajwa date characterization test at Figure 4.

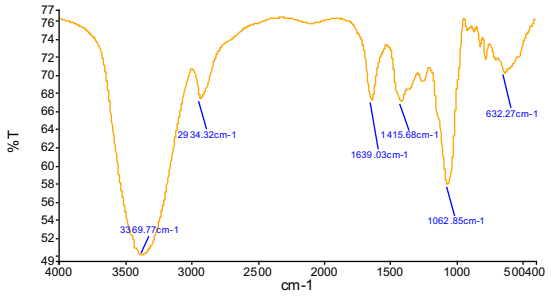


Figure 4. Characterization Results of Ajwa Dates

Several absorption peaks appear on the spectrum above. The peak at wave number 3369.77 / cm can be indicated as a type of vibration -OH because the wave number in the –OH group has a range of 3500 / cm-3200/cm [29]. The –OH group is derived from saponins, tannins, and phenols.

Wave number 2934.32/cm is the absorption stretch specifically owned by the vibration of the aliphatic C-H functional group on methyl or methylene. The wave number 1639.03/cm is a special absorption vibration owned by the functional carbonyl group C=O from saponin compounds. While the wave number 1415.68/cm is the C-C absorption functional group (sp^3) which can be confirmed by the emergence of the C-H aliphatic functional group..

The wave number 1062.85/cm is indicated as the vibration produced by the C-O group which has a vibration range of 1300/cm-1000/cm [29]. the C-O bond comes from saponins, tannins, and flavonoids. Meanwhile, the smallest wave number is 632.27/cm, that probably the fingerprint area of the C-H bending vibration.

4. Conclusion

The conclusion of this study with water of Ajwa dates for 24 hours, 48 hours, and 72 hours of immersion has the potential as anti-tumor and anti-cancer because it has an LC50 value of 676.39 ppm; 720.84 ppm; and 903.20 ppm. This means that the water of Ajwa Nabeez dates can be used as an alternative to natural anti-tumor and anti-cancer drugs.

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