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Decoction and Subcritical Water Extraction of Date Palm Seed: Comparison of Bioactive Compound Analysis

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ABSTRACT

Date palm is one of the oldest cultivated plants and there are several thousand cultivars and varieties around the world. Date seed is a byproduct and a waste of date fruit. It is usually discarded and utilized as animal fodder. Studies have shown that date seeds are rich in dietary fibre, phenolic and antioxidant which can be extracted for industrial purpose. The purpose of this study was to conduct extraction of Safawy date palm seed using decoction and subcritical water extraction (SWE) followed by bioactive compound analysis. The reason for conducting this study is because there are only a few sources that reports the information for the bioactive compound analysis of this particular date palm variety. Extraction of date seed was conducted using decoction and SWE with extraction of 15 minutes and a ratio of sample to water of 1:20. Moreover, the bioactive compound analysis conducted was for the total phenolic and flavonoid content, and antioxidant activity using the Folin-Ciocalteu, aluminium colorimetric and DPPH method respectively and was measured using spectrophotometer. The findings have shown that Safawy date seed contains high phenolic and flavonoid content. SWE extract has shown the highest amount of phenolic and flavonoid content at 602.04 $\pm$ 3.28 mg GAE/g and 81.75 $\pm$ 2.84 mg QE/g respectively. Furthermore, date seed was reported to have at least 90 % antioxidant activity at 1000 ppm. However, extracts of decoction and SWE resulted in reduction of antioxidant activity at 87.29 and 86.50 % respectively. Overall, SWE offers a better extraction result than decoction with a slight reduction in antioxidant activity. In addition, the results showed that date seed potentially can be used for the production of functional foods and pharmaceuticals.

1. Introduction

Date palm, also known as Phoenix dactylifera L., is one of the earliest and the oldest cultivated plant and it plays an important role in economic, political, nutritional, environmental and social in the perspective of the people living in the arid and semi-arid regions of the world [1-3]. Date palm is

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related to the Arecaceae (Palmea) family and there are more than 2,500 varieties of date palm known in the world. Date fruit is considered as berry which consist of a single seed and fibrous flesh of endocarp, mesocarp (the pulp) and epicarp (the skin) surrounding it [4]. As in 2010, five dominant countries of date producers (Iran, United Arab Emirates (UAE), Algeria, Saudi Arabia and Egypt) reported an increase in date fruit production from 710,000 to 1,352,950 metric tonnes [1]. In 2016, it was estimated that the global production of dates is at 8.5 million tonnes. Therefore, it can approximately produce 1 million tonnes if date seeds per year due to the huge global production which can cause concern and threat to the environment [5].

Waste products from fruits that are the result from industrial and agricultural processing can be seen as an economic source for antioxidants [6]. Many studies have proven that fruit waste products or residues can present higher bioactive compounds than the edible part or pulp of the fruit [7]. In the case of date palm, date seed is the byproduct of the date fruit that is generated during consumption or from the date processing industries. Ten percent of the weight of date fruit is attributed to the date seed. It is rich in oils, proteins, minerals, dietary fibre, phenolic compound and antioxidants. These components can be extracted and utilized for industrial purposes such as therapeutic components [2,8].

When the extracted natural products are meant for the use of food industry and pharmaceutical application, non-toxic solvents are selected [9]. Thus, water-based extraction methods are suitable for these industrial applications. Water-based extraction methods are green extraction techniques that uses water as a solvent to obtain bioactive compounds from natural products such as medicinal plants. Water is a great solvent choice for extraction as it is safe, low cost and it is available in huge quantity compared to alcohol and/or organic solvents [5,10]. In the perspective of the food industry, water is a more adequate solvent as it has GRAS (generally recognized as safe) status [11]. Some examples of extraction methods that uses water as a solvent are decoction and subcritical water extraction.

Decoction is a traditional extraction method that uses water as extraction solvent by boiling the plant materials. It is suitable for the extraction of thermostable compounds such as saponin, tannins and flavonoids from tough and fibrous lants e.g. barks, roots and stems [12]. Therefore, in this technique, plant samples are boiled using water followed by cooling and straining to acquire the extract [13]. Moreover, subcritical water extraction (SWE) is an innovative techniques for the extraction of bioactive compounds. It has been referred to as pressurized liquid extraction (PLE), accelerated solvent extraction (ASE) and high-pressure solvent extraction (HPSE) [9,14]. However, when the extraction solvent use is water, this extraction method is referred to as SWE, hot water extraction (HWE) and pressurized hot water extraction (PHWE) [14]. Therefore, SWE uses water as a solvent to extract plant materials while being under pressure high pressure and high temperature [13]. SWE extracts compounds in subcritical condition at temperature between the boiling point (100 ℃) and critical point (374 ℃) of water while maintaining the solvent in liquid state through high pressure [10]. When water is within the subcritical condition, the value of dielectric constant of water is reduced to almost similar value as organic solvents [14].Thus, this causes water in subcritical condition to extract compounds more efficiently.

Works about the extraction of various date palms and its constituents are an abundance. Most works for extraction of date palms are conducted using maceration extraction technique through the use of organic solvents such as methanol and/or ethanol [3,15,16]. In addition, these extracts would undergo extra steps for the removal of organic solvent for the safety of consumers if these extracts were to be used for food and pharmaceutical applications [17]. Therefore, water-based extraction such as decoction and SWE are suitable to prevent these concerns. Literature survey found that studies about a particular variety of date palm, namely the Safawy variety is scarce and lacking.

Furthermore, the extraction of this date variety using SWE is also minimal. Hence, a study about SWE and Safawy date palm would contribute to the lack of studies about Safawy date palm under the application of water-based extraction. In addition, water extracts of natural products are safe for consumer consumption. Thus, the objective of the study is to perform extraction of Safawy date seed through decoction and subcritical water extraction method. This is followed by bioactive compound analysis for phenolic and flavonoid content and antioxidant activity.

2. Methodology

2.1 Materials

Phoenix dactylifera (Green Diamond International, Selangor), Folin-Ciocalteu reagent (R&M Chemicals, Malaysia), sodium carbonate (Na_2CO_3) solution (ChemAR, Malaysia), gallic acid solution (Bio Basic Canada Inc), DPPH reagent (Sigma Aldrich, Germany), L-ascorbic acid (Sigma Aldrich, Germany) were applied.

2.2 Sample Preparation

Twenty kilograms (20 kg) of date palm at tamr stage, namely the Safawy variety, was purchased from the Green Diamond International Sdn. Bhd., Selangor. The seeds were taken out from the date by hand. The seeds were then soaked in saltwater for 5 minutes and then rinsed with distilled water. Afterwards, it was dried at room temperature and then roasted in a convection oven using the conditions conducted by Fikry *et al.*, [8] with some adjustments, where the temperature and time was set to 200℃ and 22 minutes respectively. The seeds were then ground into coarse powder using food grinder. The prepared ground date seed will be known as fresh sample onwards.

2.3 Extraction of Date Seed

The extraction of date seed was done through decoction and SWE. Both extraction method uses water as extraction solvent. For a fair comparison between the extracts, each extraction method uses the same parameter for the ratio of sample to water and extraction time. Thus, the ratio of sample to water was set to 1:20 and the extraction time was set to 15 minutes. The only variables between each extraction methods are pressure and temperature. Decoction uses atmospheric pressure and boiling point (100 ℃) of water. While SWE adds 15 bar of N_2 gas and uses temperature above the boiling point (100 ℃) and under the critical point (374 ℃) of water.

2.3.1 Decoction

Decoction of date seed was based on the work conducted by Basri *et al.,* [10]. It was simply done using heating plate and beaker in the extraction process. Ground date seeds were weighed first and then added into the beaker, followed by the addition of water according to the ratio of sample to water that was set for this study. The extraction temperature was fixed at boiling point (100 ℃) and was left in that condition for 15 minutes. Once the extract cooled down, it was strained and collected into food grade container. The extract was stored in the freezer in the freezer at -20 ℃ until further analysis.

2.3.2 Subcritical water extraction (SWE)

The extraction of date seed was done using the industrial scale subcritical water extractor that was modified by AM Zaideen Ventures Sdn. Bhd. as shown in Figure 1. This extractor has been used by Mansor *et al.*, [17] in their extraction of Zingiberaceae family Rhizomes and thus, the extraction procedure was based on their work with some adjustments.

Briefly, the ground date seeds were weighed and then added into the extraction tank after preparation. Water was then added according to the set amount of sample to water ratio. The extraction tank was then closed and injected with N_2 gas until it reaches 15 bar. Once the temperature within the extraction tank reaches above 100 ℃, the sample was left in subcritical condition for 15 minutes where the temperature within continues to rise. Pressure was then released and the extract was transferred into the cooling tank where it will be collected once the extract cooled down. The extract was stored in the freezer at -20 ℃ until further analysis.

Fig. 1. Industrial scale subcritical water extractor by AM Zaideen ventures Sdn. Bhd.

2.4 Bioactive Compound Analysis
2.4.1 Total phenolic content (TPC)

The total phenolic content was analysed using the Folin-Ciocalteu method based on the works of Shiban *et al.,* [6] and Matshediso *et al.,* [18] with some adjustments. Briefly, 0.5 ml of diluted sample extracts were added into test tubes and was mixed with 2.5 ml diluted Folin-Ciocalteu reagent in distilled water. The mixture was vortexed and rest for 5 minutes 2 ml of 7.5 % (w/v) of sodium carbonate were then added, shaken and incubated in the dark for 2 hours. Absorbance was measured at 750 nm against reagent blank using UV-Vis Spectrometry. The total phenolic compounds were calculated from standard gallic acid solution and was expressed as mg gallic acid equivalent per gram extract (mg GAE/g).

2.4.2 Total flavonoid content (TFC)

The analysis of the total flavonoid content was conducted using the aluminium chloride method. This method was based on the work of Aboulghazi *et al.,* [19]. Stock solution was prepared by diluting fresh sample and each extract in methanol, sonicate and vortexed for 10 minutes. Stock solutions were then mixed with 3 ml of 5 % aluminum chloride and incubated for 30 minutes. Absorbance was measured at 437 nm against a methanol blank using UV-Vis Spectrometry. The total flavonoid

contents were calculated from standard curve of quercetin and were expressed as mg quercetin equivalent per gram extract (mg QE/g).

2.4.3 Antioxidant activity

The analysis of antioxidant activity was done through DPPH following the method conducted by Etim *et al.,* [20] with some adjustments. Briefly, 3.94 mg of DPPH was dissolved in 50 ml of methanol to create 0.2 mM DPPH methanolic solution. Extract solutions with different concentrations of 10 to 10000 ppm was prepared. 2 ml of methanolic DPPH solution was mixed in each diluted extract solution concentrations and incubated in the dark at room temperature for 30 min. The absorbance of each extract solution concentrations was recorded against blank at 517 nm using UV-Vis Spectrometry. The percentage of antioxidant activity was calculated from Eq. (1).

$$\text{Antioxidant activity (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \quad (1)$$

where A_{blank} is the absorbance of the control (methanolic DPPH without crude extract), and A_{sample} is the absorbance of the sample (methanolic DPPH with the crude extract).

2.5 Statistical Analysis

Experiments of bioactive compound analysis are done two or more times for each sample. The results were calculated using the Microsoft Excel 2021 and were reported in the form of mean, average and percentage value. In addition, the standard curve and R^2 of gallic acid and quercetin concentration are also gained from the same application.

3. Results and Discussion

3.1 Total Phenolic content (TPC)

The results of the total phenolic content analysis are as shown in Figure 2 below. The TPC of each sample of each sample was calculated from the standard curve of gallic acid as stated in Eq. (2) with $R^2 = 0.9963$, and was expressed as mg gallic acid equivalent per gram extract (mg GAE/g).

$$y = 0.0008x + 0.0927 \quad (2)$$

where y is the absorbance of the sample and x is the total phenolic content.

The analysis showed that Safawy date seed (after preparation) contains TPC of 104.13 ± 1.02 mg GAE/g. Furthermore, extracts of decoction and SWE showed increase in TPC resulting in 383.71 ± 1.88 and 602.04 ± 3.28 mg GAE/g respectively. These results are aligned with the statement of Ashraf and Hamidi-Esfahani., [2] where the concentration of phenolic acids in date seed increases after extraction. In comparison, Gökşen *et al.,* [15] reported in their work that methanolic extract of Safawy seed contains TPC at 49.66 mg GAE/g. In another work, Li *et al.,* [5] reported the TPC of Hallawi date seed extract obtained from SWE was valued at 997 mg GAE/100g. These varying values may be due to the type of extraction technique, solvents, date cultivars and also analytical techniques used [21].

In addition, SWE extract has the highest amount of TPC can be attributed to the condition of the extraction vessel which is done in closed space. This helps to prevent the loss of bioactive compounds

that is done through evaporation by trapping the steam and condensed it back to the bulk extract. Solid matrix that is heated by water releases volatile compounds which are evaporated and turns into steam [22]. Therefore, the closed system extraction tank in SWE helps in preventing further losses in volatile bioactive compounds. However, in the case of decoction, the extraction process was done in an open space which may resulted in reduction of phenolic content due to evaporation.

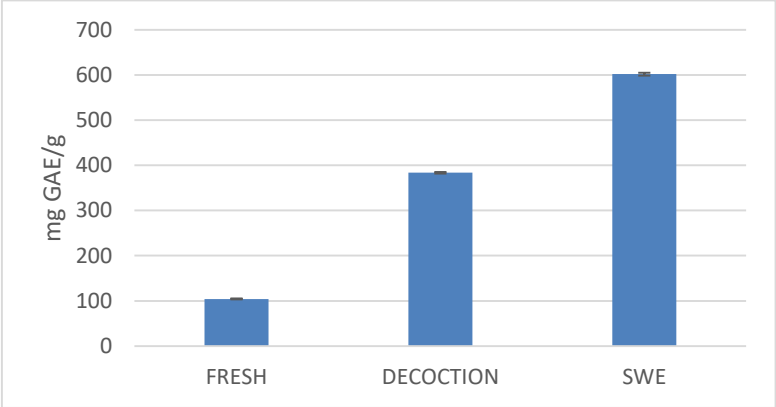

Fig. 2. TPC of Safawy date seed and its extracts

3.2 Total Flavonoid Content (TFC)

The results of the total flavonoid content analysis are as shown in Figure 3 below. The TPC of each sample of each sample was calculated from the standard curve of quercetin concentration as stated in Eq. (3) with $R^2 = 0.9998$, and was expressed as mg quercetin equivalent per gram extract (mg QE/g).

$$y = 0.0016x + 0.0712 \quad (3)$$

where y is the absorbance of the sample and x is the total flavonoid content.

The results showed that fresh Safawy date seed contains TFC of 69.88 $\pm$ 3.19 mg QE/g. However, unlike the previous results, date seed showed different results after extraction where the TFC of decoction extract decreases while SWE extract increases. Decoction and SWE produced extracts that contains TFC of 67.38 $\pm$ 3.57 and 81.75 $\pm$ 2.84 mg QE/g respectively. Thus, SWE extract has the highest TFC followed by fresh sample and decoction extract which is the lowest. The factors contributing to these results may be related to the previous explanation where the condition of the extraction process affects the results where the reduction of TFC in decoction extract is due to evaporation in open space.

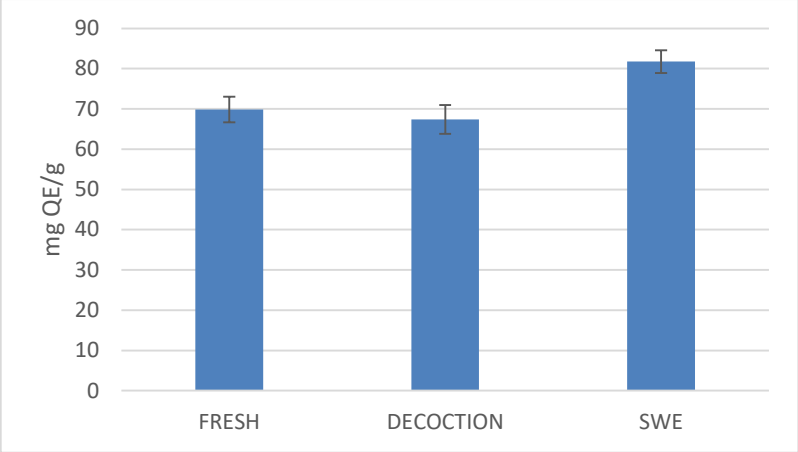

Fig. 3. TFC of Safawy date seed and its extracts

3.3 Antioxidant Activity

The results of antioxidant activity of date seed are as shown in Figure 4. The antioxidant activity of each sample was calculated using the Eq. (1) stated before. The results showed that Safawy date seed has high content of antioxidant activity. At 250 ppm, both fresh sample and SWE extract reached their highest point of antioxidant activity which is above 90 %. However, concentrations after 250 ppm showed reduction in antioxidant activity. In addition, decoction extract at 250 ppm reached above 80 % antioxidant activity and is increasing afterwards. Furthermore, at 1000 ppm, the antioxidant activity calculated for fresh sample, decoction and SWE extracts resulted at 89.24, 87.29 and 86.50 % respectively. Therefore, fresh sample has the highest antioxidant activity followed by decoction and SWE extract which has the lowest at this concentration.

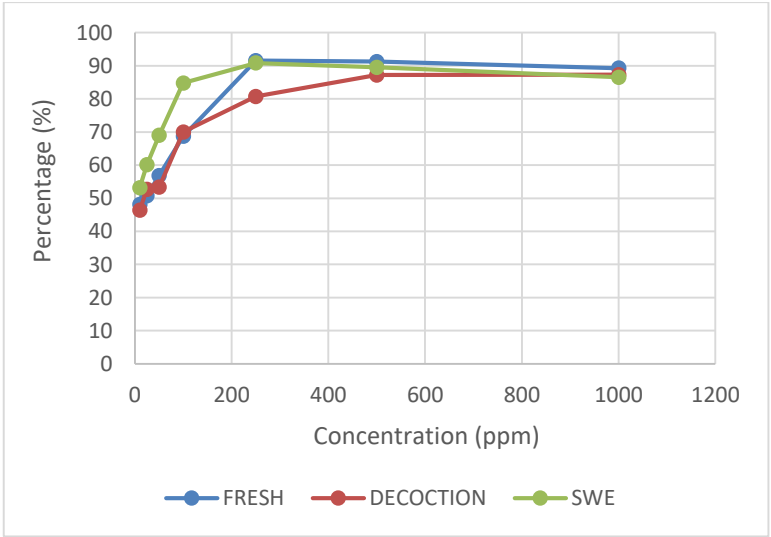

Fig. 4. Antioxidant activity of Safawy date seed and its extract at 1000 ppm

The reduction of antioxidant activity after extraction may be due to the factor of temperature. Based on the work conducted by Li *et al.,* [5] stated that antioxidant activity corresponds with phenolic compounds, which in their report, did not show degradation under 150 ℃. This indicates that the phenolic compounds within the date seed are made up of components with low antioxidant activity that can withstand high temperatures. Therefore, the reduction in both seed extracts was

due to some high antioxidant components were thermosensitive which led to degradation due to heat. Referring back to Figure 2, SWE extract showed the highest TPC but resulting in a lower antioxidant activity due to high temperature above the boiling point of water. Whereas decoction extract presented a lower TPC but higher antioxidant activity than SWE extract. In the same situation, fresh sample presented the lowest TPC among other samples. However, it showed the highest antioxidant activity due to the lack exposure to heat except during the roasting of date seeds in sample preparation.

Recent studies concluded that antioxidants are linked with the phenolic and flavonoid compounds [2] and according to Al-Alawi *et al.,* [4] the antioxidant activity of phenolic compounds is comparable to the standard antioxidants such as vitamin C, vitamin E and β-carotene. With all the result presented, Figure 5 has been prepared to summarize the overall result. In summary, Safawy date seed has a higher phenolic content than flavonoid. Flavonoid content showed consistent result with a slight increase and decrease in SWE and decoction extract respectively. Each sample is within the 85-90 % range of antioxidant activity where date seed after preparation has the highest antioxidant activity among the extracts.

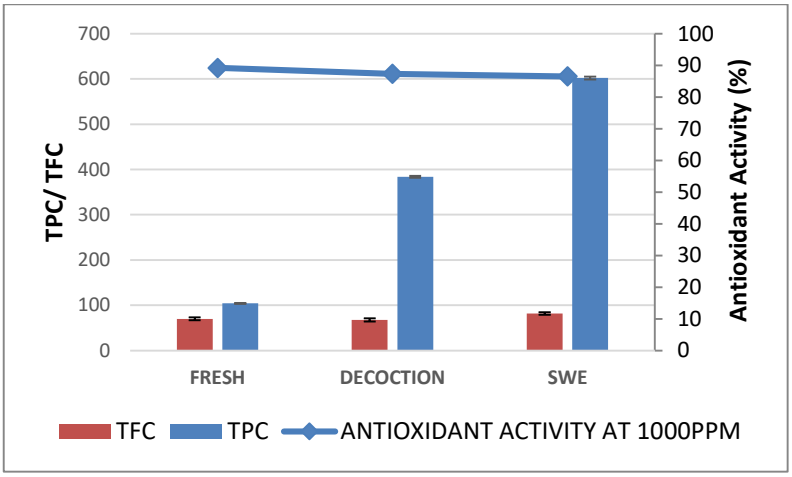

Fig. 5. Comparison of TPC, TFC and antioxidant activity of date seed and its extracts

4. Conclusion

This study reported the bioactive compound analysis of the Safawy date seed and its extracts which includes the total phenolic and flavonoid content, and the antioxidant activity. The results showed that the phenolic and flavonoid content of Safawy date seed increases after extraction. Subcritical water extraction (SWE) extract showed the highest content in both phenolic and flavonoid analysis. This can be attributed to the extraction condition such as temperature and closed vessel. However, antioxidant activity analysis showed slight reduction after extraction where SWE extract is the lowest among other samples followed by decoction and fresh sample which is the highest. In this case, temperature is the contributing factor to these results. Overall, SWE offers a better extraction result than decoction with a slight reduction in antioxidant activity. Nevertheless, the bioactive compound analysis of Safawy date seed showed high content of phenolic, flavonoid and antioxidant activity. Thus, extracts of date seed can be a seen as a functional by-product for food, beverages and even pharmaceuticals.

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