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(RESEARCH ARTICLE)

The phytochemical screening, total phenolic and photoprotective potential of date palm seeds (*Phoenix dactylifera* l.)

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Abstract

Date palm seeds (*Phoenix dactylifera* L.) are known to contain phenolic compounds that have the potential as natural photoprotective agents. Phenolic compounds extracted by solvent are suitable for the level of polarity. This study aims to determine the compounds, the value of Sun Protecting Factor (SPF), and the difference in the SPF value of ethanol extract of date palm seeds and their fractions (n-hexane fraction, ethyl acetate fraction, and residue fraction). This research is in two stages. It's the screening stage and the photoprotective test with the spectrophotometric method. This data was analyzed to descriptive qualitative and photoprotective data with graphed prism 8. In this study, the ethanol extract, ethyl acetate fraction, and residue fraction contained phenolic and terpenoid compounds, and the n-hexane fraction contained steroid compounds. Ethanol extract, ethyl fraction, and residue have sunscreen activity, with SPF values of 3.376±0.15; 6, 13±1, 2; and 3.14±0.08. While the n-hexane fraction with an SPF value of 0.512 ± 0.09. The highest SPF value in the ethyl acetate and n-hexane fractions did not meet the photoprotective SPF range. Phenolic compounds as sunscreens. The data analysis showed a significant difference between the ethanol extract, ethyl acetate fraction, residue fraction, and n-hexane fraction of date palm seeds.

Keywords: *Phoenix dactylifera* L; Date seed; Photoprotection activities; Screening phytochemistry; SPF

1 Introduction

Sunlight is a source of energy that important in life. However, excessive sun exposure can cause health problems to the skin. The three types of sunlight reach the earth's surface, namely ultraviolet A (320-400 nm), ultraviolet B (230-320 nm), and ultraviolet C (200-290 nm). Ultraviolet affects the skin, such as erythema, pigmentation, photosensitivity, skin cancer, oxidative stress, premature aging, the eyes, and the immune system. UV-A is considered less harmful but can cause skin cancer through indirect pathways of DNA damage by free radicals and reactive oxygen species[1]. Exposure to UVB rays can cause burns and skin cancer. The light excites the t DNA molecule so that the covalent bond deviation occurs, forming a cytosine base and producing a dimer. UV-B light (290nm-320nm), t can stimulate the occurrence of reactive oxygen species (ROS) and trigger oxidative stress in intracellular and extracellular [2]. The mutation causes cancer [3]. Phenolics are good antioxidants, proven to neutralize free radicals and reactive oxygen species by donating one of their electrons to prevent cell and tissue damage. In the last decade, several researchers have revealed that medicinal plants have antioxidant and photoprotective activities [1, 4, 5]. The plants contain phenolic compounds such as phenolic acids, flavonoids, tannins, lignans, and non-phenolic compounds (carotenoids and vitamin C). The function of antioxidants has anticarcinogenic, antimutagenic, and antiproliferative properties [6,7]. Phenolics are an alternative source of antioxidants for diseases mediated by ultraviolet light exposure [,9,4,10,11]. The Plants produce secondary metabolites of alkaloids, flavonoids, saponins, steroids, and tannins [12]. The constituents of medicinal plants have pharmacological activities and can promote health. The High plants use 14-28% of extracts as medicinal ingredients, and 74% have other functional or traditional medicines [13]. Of the 250,000 species of medicinal plants, only 5-15%

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have their bioactive compounds. The *Phoenix dactylifera* L. contains phenolic compounds and antioxidant activity more than the pulp [14]. Phenolic of Date seeds, such as gallic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, m-coumaric acid, and o-coumaric acid, act as photoprotective agents [15,16]. The process uses a solvent by the polarity level and produces extracts with bioactive compounds of the potential photoprotective agents [17]. The study is to determine the extract and fraction of date palm seeds containing phenolic compounds that have photoprotective power.

2 Material and methods

2.1 Materials

The materials include date palm seeds, 96% ethanol, n-hexane, ethyl acetate, distilled water, Folin Ciocalteu reagent, and FeCl3 solution.

2.2 Methods

2.2.1 Sample preparation

Date palm seeds were cleaned and washed, dried in an oven at 50 0C overnight, and crushed into 40 mesh size powder using a mill machine. Put in polyethylene plastic, and store at 40C until analyzed.

2.2.2 Extraction-Fractionation

Ten grams of date seed powder was extracted with Soxhlet using ethanol (1:10) for ± 7 hours. The filtrate was filtered and concentrated with a vacuum evaporator at 40 0C. In the ethanol extract of the fractionation with the liquid-liquid partition method. Five grams of the ethanol extract were dissolved with 50 mL of water and added 50 mL of n-hexane. Extracted on a separating to two fractions, the lower fraction as the water fraction and the lower fraction as the water fraction. The n-hexane fraction was collected by Erlenmeyer. The residues were added to 50 mL ethyl acetate. This fraction was collected by Erlenmeyer, and each fraction was accommodated in an Erlenmeyer. All fractions were concentrated with a vacuum evaporator at 40 0C.

2.2.3 *Phytochemical screening* [18]

Phenol test

The 0.5 g of the extract with 3-4 drops of FeCl3. Bluish black to dark black indicated the presence of phenol content

Tannin test

The 0.5 grams of the extract were added to 10 mL of hot water and dropped with 1% FeCl3, forming a blackish green color indicating the presence of tannins

Flavonoid test.

0.5 g of extract dissolved in 5 mL of ethanol, heated for ± 5 minutes, and ten drops of HCl and 0.2 grams of magnesium powder were. The color of a reddish black, yellow, or orange color indicates a positive of flavonoids.

Saponin test.

A total of 0.5 grams of the extract was dissolved in 10 mL of preheated distilled water. The mixture of the 1 minute. It is allowed to stand for 10 minutes and observed for foam or foam indicated a positive result of saponins.

Alkaloids test.

The 0.5 g of the extract, was added to 2 ml of chloroform, 10 ml of ammonia, and 10 drops of H2SO4. The mixture form two layers. Three tubes with H2SO4 2.5 ml. All tubes with Mayer, Dragendorf, and Wagner reagents. Mayer's reagent by white precipitate, Dragendorph's reagent is a red or orange precipitate, and Wagner's reagent is a brown

Test for steroids and terpenoids.

A total of 0.5 g of the extract, is added to 2 ml of H2SO4 and shaken slowly for a few minutes. A Blue to green indicates a positive of steroids, and brownish red to purple indicates a positive of the terpenoid.

2.2.4 *Phenol quantitative test* [19]

Ten mg of extract dissolved in 10 mL of ethanol and 0.5 mL of Folin-Ciocalteau reagent, and 2.5 mL of 7.5% Na2CO3 solution. The tube was vortexed for 1 minute and incubated at room temperature for 15 minutes. The spectrophotometry at a wavelength of 756 nm. The total phenolic content is expressed in mg Gallic Acid Equivalent/g

2.2.5 Photoprotection test

The photoprotection with the spectrophotometry, wavelength 290-320 nm, every 5 nm, and five times replication.

$$\text{SPF} = \text{CFx} \sum_{290}^{320} \text{EE}(\lambda)\text{xI}(\lambda)\text{xAbs}(\lambda)$$

Photoprotective power of the SPF value calculates, as follows: SPF= CFx.

Information:

CF (Correction factor) = (CF=10),
EE (λ) = erythema effect of solar radiation at each wavelength,
I (λ) = intensity of sunlight at wavelength
Abs (λ) = sample absorption reading on UV spectrophotometer

The value of EE x I is a predetermined constant (table 1).

Table 1 Value EE X I [20]

Wavelength (ƛ nm)	EE x I
290	0,0150
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0839
320	0,0180

2.3 Analyzed data

Data of screening analyzed with descriptive qualitative and SPF were analyzed on graphed prism 8.

3 Results and discussion

The extraction of the maceration method is simple, and the procedure immerses a solvent and damage thermolabile. The choice of extraction method at the yield produced ethanol extract is 19.60%, the n-hexane fraction is 5.39%, the ethyl acetate fraction is 4.54%, and a residual fraction is 17.6%. The yields because each solvent used will dissolve compounds. Phytochemical screening to determine the class of compounds in date seeds. The data showed that these compounds were in the solvent (Table 2). The ethanol extract, Ethyl acetate fraction, and residual fraction contain phenolic groups, such as tannins, flavonoids, and terpenoids. The ethyl acetate fraction also contains alkaloids, and the n-hexane fraction is steroids.

Testing on an ethanol extract of date palm seeds, ethyl acetate fraction, and the residue fraction contained phenolic compounds. Phenolic compounds have a dark blue after being added with 1% FeCl3. Phenolic compounds are declared positive if s a bluish black to dark black when 1% FeCl3 is added [21], and FeCl3 can react with aromatic –OH groups. Similarly, in the tannin test, a blue color occurs. The flavonoid test is red-orange color, to the instructions that a few drops of HCl solution and Mg powder could reduce the benzopyran core in the flavonoid structure to form red or orange flavilium salts [22]. Steroids are blue, and terpenoids are red-brown. The color change is due to the reaction between

steroids or terpenoids with H2SO4. The color by the group on the C-4 atom [23]. In the alkaloid test on the ethanol extract, a precipitate formed probably because nitrogen (alkaloid) reacted with the potassium metal ion (K+) of Potassium tetraiodomercurate (II) to produce a potassium-alkaloid precipitate complex. Alkaloids consist of nitrogen atoms of the lone pair electrons that form coordinate covalent bonds with metal ions [24]. Polar compounds are phenolic compounds (flavonoids, tannins, saponins, terpenoids), while alkaloids are semipolar and non-polar steroid compounds [25].

Table 2 The result of phytochemical screening of Date seed extract

Compounds	Reagent	Material			
		Ethanol extract	n-Heksan fraction	Ethyl acetate fraction	Residue fraction
Phenolic	$FeCl_3$	Blue (+)	-	Blue(+)	Blue (+)
Tannin	$FeCl_3$	Blue (+)	-	-	Turquoise (+)
Flavonoid	Etanol+HCl+Mg	Orange-red (+)	-	Orange-red (+)	Orange-red (+)
Terpenoid	H_2SO_4	Brownish red (+)	-	Brownish red (+)	Brownish red (+)
Steroid	H_2SO_4	Blue (+)	Blue (+)	-	-
Alkaloids	Mayer Dragendorf Wagner	The white precipitate (+)	-	-	-
		orange precipitate (+)	-	-	-
		The chocolate precipitate (+)	-	The chocolate precipitate(+)	-
saponin	shake vigorously	Foam(+)	-	-	Foam(+)

3.1 Total phenolic content.

The curve for gallic acid with Folin-Ciocalteu reagent using a UV-Vis spectrophotometer (figure 1). Linear equation curve of total phenolic in ethyl acetate and ethanol extract of date palm seeds.

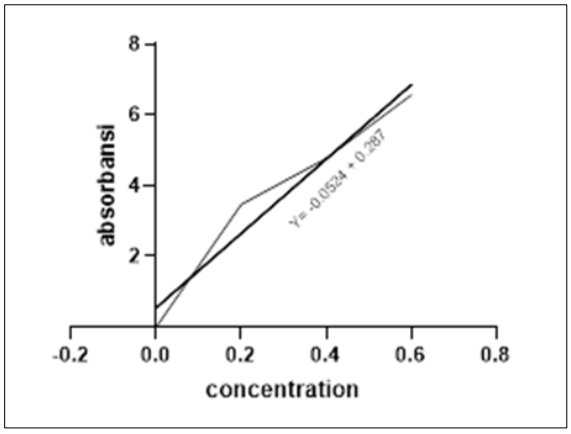

Figure 1 The gallic acid standard curve

Based on calculated of the standard solution regression equation, the total phenolic content of the extracted ethanol of date seed was 0.29%, the ethyl acetate fraction was 0.28%, and the residual fraction was 0.057%. The phenolic in the ethyl acetate fraction than the residue. The phenolic molecular weight may be close to the molecular weight of ethyl acetate so that it is more soluble in ethyl acetate. The total polyphenols on the ethyl acetate fractions are higher. The tannins and flavanols of the polyphenol group were of the same molecular weight as the ethyl acetate [26]. The Phenolic

are generally more soluble in semi-polar solvents [27]. The Gallic acid using a standard solution is a stable phenol. It is a triphenyl or phenolic derived from hydroxybenzoic acid, and a simple phenolic acid group with antioxidant effects [19, 24]. The Folin-Ciocalteu reagent added to the sample, oxidizes phenolics (alkali salts), and reduces heteropoly acids to a molybdenum-tungsten (Mo-W) complex. The reaction of the phenolic-hydroxyl group with the Folin-Ciocalteu reagent forms a blue phosphotungstate-phosphomolybdate complex. Dark the color intensity, the higher the phenol content in the extract and the larger the fraction [28].

3.2 Photoprotective power

The ethyl acetate fraction has the highest absorbance at 290-330 nm. The lowest is in the n-hexane fraction (Figure 2). These are supported by phytochemical screening, that the ethyl acetate fraction gives a blue indicates that the content of phenol compounds is greater than that of extracts and other fractions. The phenolic in the n-hexane fraction is less, a blue that is more faded in color. The absorbance by the number of phenolics in the extract and fractions.

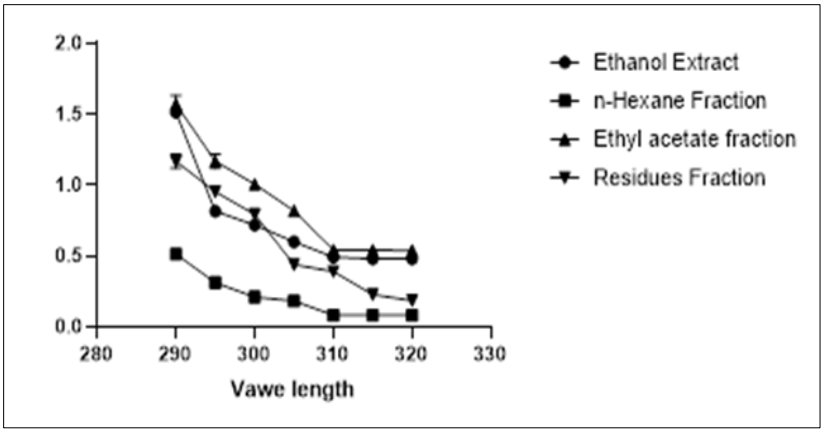

Figure 2 The absorbance of Ethanol Extract, N-Hexane Fraction, Ethyl Acetate Fraction, and Residual Fraction of date seed

Photoprotection power by the value of SPF (Sun Protection Factor). SPF power test to determine the ability of sunscreen compounds to withstand UV rays, and the assessed by Sun Protecting Factor (SPF). The SPF value as protection against UV-B is in the range of 2-100. The results showed that the ethyl acetate and ethanol extract fractions fell into the SPF value range (figure 3).

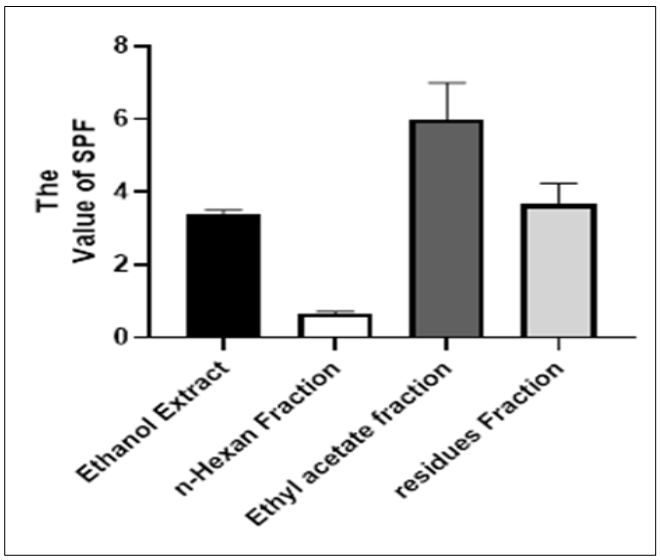

Figure 3 SPF results from ethanol extract, n-hexane, ethyl acetate, and residue fraction

Based on the SPF, indicate of the phenolic compounds that have the potential as photoprotective agents are soluble in ethyl acetate and ethanol. These results are by the literature of Harborne and Dey (1989). The states that phenol

compounds can be soluble in ethyl acetate and ethanol solvents. The SPF value of the n-hexane fraction is the lowest value 0.512±0.09. Because the phenolic in this fraction is smaller amounts based on phytochemical screening tests, the resulting color change is slightly blue. The sunscreen activity of date palm seeds of the phenolic compounds. The compound is gallic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, m-coumaric acid, and o-acidic acid-kumarat. The double bond system in phenolic compounds will experience resonance when exposed to UV rays so that they can be efficacious as sunscreens [29]. Based on the analysis of the results with the One Way ANOVA test showed a significance of 0.00 ($P<0.05$), there is a significant difference between the ethanol extract of date palm seeds and the n-hexane fraction, ethyl acetate fraction, and water fraction. Therefore, fractionation affects the sunscreen activity of date seeds, Ethyl acetate fraction is the sunscreen activity with the highest SPF value.

4 Conclusion

The ethanol extract, ethyl acetate fraction, and residue fraction contained phenolic, terpenoid, and the n-hexane fraction contained steroid. Ethanol extract, ethyl fraction, and residue have sunscreen activity, with SPF values of 3.376±0.15; 6, 13±1, 2; and 3.14±0.08. While the n-hexane fraction with an SPF value of 0.512 ± 0.09. The highest SPF value in the ethyl acetate and n-hexane fraction did not meet the photoprotective SPF range. Phenolic compounds are recommended for sunscreens.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

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